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GUIDELINES FOR THE HOUSING OF MICE IN SCIENTIFIC INSTITUTIONS

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Guideline 22

Guidelines for the Housing of Mice in Scientific Institutions

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Recommendations

The following recommendations appear in the body of the text:

Responsibilities of Institutions

1.2.1 Institutions using mice for scientific purposes are responsible for meeting recommendations of the institution's Animal Ethics Committee to ensure that facilities for the housing and care of mice are appropriate to the maintenance of their well-being and health.

Responsibilities of Chief Investigators/Teachers

1.3.1 The chief investigator/teacher (person in charge of a research/teaching project) has direct and ultimate responsibility for all matters related to the welfare of mice under his or her control, which includes their housing and care. (As per the principle contained in Clause 3.1.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes).

1.3.2 The chief investigator/teacher should ensure that the extent of personnel / staff supervision is compatible with the level of competence of each person and the responsibilities they are given in relation to mouse care and management - Personnel training and competencies should be documented. (As per the principle contained in Clause 3.1.3 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes).

Aspects of Mouse Biology, Physiology and Behaviour Relevant to Housing

1.5.1 To meet the requirements of the Code of Practice (that is to provide accommodation that meets the species specific needs of mice), housing should allow mice the opportunity for social interaction, the opportunity to carry out normal behaviours and the opportunity to rest and withdraw from each other. Normal behaviours of mice include eating, drinking, urinating, defecating, foraging, exploring, gnawing, hiding, climbing, playing, nesting, digging and engaging in a range of social activities.

1.5.2 Housing requirements for individual mice may vary according to strain, age, physiology, stocking density, the purpose and the length of time for which animals are used (for example breeding or experiments)[1].

1.5.3 The code of practice recognises that there may be circumstances where the requirements of experimental procedures will preclude meeting some species specific needs (Clause 4.4.19). Variations to these requirements as part of a project must endeavour to meet the physiological and psychological needs of mice as closely as possible, and must receive prior Animal Ethics Committee approval.

Genetically Modified, Transgenic and Knockout Mice

1.6.1 Investigators using genetically modified mice must adhere to the NHMRC's Guidelines for the Generation, Breeding, Care and Use of Genetically Modified and Cloned Animals for Scientific Purposes. Genetically modified and cloned animals are subject to State and Territory animal welfare legislation. Investigators must make enquiries to determine whether they are

subject to requirements of the Office of the Gene Technology Regulator under the Gene Technology Act.

1.6.2 Mouse behaviour may vary between different strains or stock. Researchers should ensure they understand behaviour and needs of their particular experimental animals and incorporate this knowledge into husbandry and experimental design.

1.6.3 Structured welfare assessments should be performed for newly bred and maintained genetically modified mice and any genetically modified lines introduced into the establishment.

1.6.4 Investigators should evaluate the impact of housing, husbandry and environmental enrichment on welfare and experimental variability for each strain used

1.6.5 For specific husbandry advice for each individual strain, investigators should seek strain-specific information and recommendations from the supplier, institution of origin and murine databases such as the Mouse Genome Informatics Database (www.informatics.jax.org) and Eumorphia (www.eumorphia.org).

1.6.6 When using animals including genetically modified, transgenic or knockout mice, investigators should carefully weigh the value of the experiment against welfare issues related to the particular strain or strains used. Investigators should be mindful that welfare concerns arise not only during the study, but in the development and production of particular strains.

Cage Design – Living Area

2.1.1 The living area for mice must allow them to satisfy their basic physiological and behavioural needs including the ability to eat, drink, urinate, defecate, forage, explore, gnaw, hide, climb, play, nest, dig and engage in a range of social activities.

2.1.2 The living area should be constructed and arranged in such a way to allow mice to compartmentalise their space, so that different areas can be used for urination, defecation, eating and resting.

Cage Design – Floor Area

2.2.1 As a guide, enclosures should allow for a minimum floor area of 250cm^2 for a single housed mouse, a minimum floor area of 500cm^2 for two mice and ensuring a minimum floor area of 60cm^2 per additional adult mouse when mice are housed in larger groups.

2.2.2 As a guide, a breeding pair or female with pups requires a minimum total cage floor area of 500cm^2, with an additional 100cm^2 for each additional adult female.

2.2.3 To reduce anxiety and aggression, larger cages should be designed in such a way as to avoid large open spaces.

2.2.4 Because of the wide variation in conclusions drawn from studies designed to determine optimum cage floor area, it is necessary for researchers to assess whether a particular strain is coping with a particular living area. Parameters assessed may include tendency to perform normal behaviours, aggressive encounters or fight wounds, weight changes, incidence of illness, reproductive performance, use of space, use of enrichment and amount of thigmotaxis observed.

Cage Design – Height and Lid

2.3.1 The height of cages should allow mice to stand on their hind legs, stretch up fully and climb on the bars of the cage lid. This height does not need to be provided over the entire area of the cage.

2.3.2 The cage lid should incorporate a grid section which will allow the animals to climb. The cage height should allow for provision of enrichment.

2.3.3 Where cages are fitted with platforms or in-cage shelters, the distance between the top of the platform or in-cage shelter and the top of the cage should be sufficient to allow mice to climb on top of the platform or in-cage shelter.

2.3.4 While cage height (over part of the cage) should allow for upright standing behaviour, food and water should be accessible at a level that allows mice to sit while eating and drinking

2.3.5 Until further evidence relating to the height of the cage becomes available, it is recommended that mouse cages are a minimum of 12cm high.

2.3.6 The design of the cage lid should facilitate climbing.

Cage Design - Shape

2.4.1 There is no clear evidence of preference among mice for a particular cage shape. Evidence indicates the contents of the cage is more important than cage shape.

2.4.2 Until further evidence comes to light the use of rectangular or square shaped cages is appropriate for mice.

Cage Design - Materials

2.5.1 Cages should be constructed from non-toxic, non-absorbable material that is easy to clean. Untreated wooden cages should not be used.

2.5.2 Cages should be durable, resistant to heat and chemicals, and escape and predator proof.

2.5.3 Worn or damaged cages and/or water containers should be replaced.

2.5.4 Leaching of bisphenol A from polycarbonate and polysulphone cages and water containers is likely if these are washed with strongly alkaline detergents

or sterilised in the presence of high concentrations of corrosion inhibiting amines in autoclave steam. Exposure of mice to bisphenol A (even at low levels) should be avoided, particularly in reproductive studies.

2.5.5 Colourless, tinted transparent cages or white opaque cages are preferable for mice. Unless required for a study, cage colour should be consistent throughout the facility.

2.5.6 Cages should be handled and maintained to minimise damage. For example, cages should not be hit or banged against hard surfaces or stacked more than 15 cages high. Plastic cages and bottles should be washed in hot (60-66°C), soft water with a manufacturer-recommended detergent solution. All residue must be removed prior to autoclaving as this may be baked onto the cage except where sterilisation is required to ensure decontamination of waste and prevent zoonosis.

Cage Design - Flooring

2.6.1 Solid floors are recommended for mouse caging.

2.6.2 Wire mesh floors should not be used for mouse caging without express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such flooring. In such cases, a solid floor section sufficient to accommodate all of the mice and nesting material should be provided. The size of the mesh gaps should not exceed 8mm x 8mm (See also 3.3 Metabolism Cages).

Cage Design - Bedding

2.7.1 Bedding should be provided in mouse cages and should be present in sufficient quantity to cover the entire floor. The depth of bedding required will vary with the type of bedding used, the number of mice in the cage and frequency of cleaning. Ideally mice should be able to dig, if not burrow. As a guide, the depth of the bedding should be a minimum of 2cm.

2.7.2 Bedding should produce a minimal amount of dust and consist of particles that lend themselves to manipulation by mice.

2.7.3 To reduce experimental variability, particularly where pharmacological experiments are concerned, the use of a single type of bedding is recommended.

2.7.4 Autoclaving of bedding is recommended to reduce the potential for microbial contamination. It should be ensured (for example by consulting the manufacturer) that toxic compounds are not formed during treatment of bedding.

2.7.5 Softwood-derived bedding should be avoided. Paper, grass-based or hardwood material should be utilised instead.

2.7.6 Vermiculite bedding or other bedding with small particles should not be used due to the potential for irritation of the mucosal membranes and other health problems.

Cage Design – Nesting material

2.8.1 All mice including males should be provided with nesting material in addition to bedding material.

2.8.2 Nesting material should be non-toxic, non-irritant, atraumatic, loose, manipulable and light enough to be carried. Suitable materials include shredded paper with non-toxic ink and tissues.

2.8.3 To minimise aggression, at least some nesting material should be transferred during cage cleaning.

2.8.4 Depending on the strain of mice used, nesting material may be placed on top of the cage to allow mice to pull the material through the bars.

Cage Design – In-cage Shelters

2.9.1 Mice should be provided with an in-cage shelter within their cage. Shelters should be provided in addition to, not as a substitute for, nesting material.

2.9.2 In-cage shelters should have solid or grid sides with more than one exit to allow subordinate animals to escape entrapment by dominant individuals and a non-slippery roof that allows withdrawal from light (and from other mice) and should be constructed so that mice can climb onto the roof.

2.9.3 Where in-cage shelters are made of chewable material such as paper or cardboard, it should be ensured the material is non-toxic to mice nor prone to cause gastrointestinal obstructions.

2.9.4 There should be enough space between the roof of the shelter and the cage lid to allow for mice climbing onto the roof of the shelter.

Cage Design - Dividers

2.10.1 Cage-dividers, labyrinths and mazes should not be used in the housing of aggressive strains, particularly for male mice.

2.10.2 Cage dividers, if used, should be arranged in a way that provides an escape-route from other mice.

2.10.3 Where cage dividers, labyrinths and mazes are used, there must be sufficient space in the cage to accommodate them and mice should be monitored for fight wounds and/or aggressive behaviour, as this will impact on the welfare of the mice in addition to being a source of experimental variability.

Mouse Care and Management – The Social Environment

3.1.1 Mice are social animals and should, wherever possible, be maintained in stable, harmonious social groups.

3.1.2 Groups of mice should be monitored to ensure social stability as well as the detection of behavioural and physiological abnormalities. There are situations, for example studies involving highly aggressive strains, where group housing is not suitable.

3.1.3 Pair housing of male mice is not recommended due to a high probability of aggression.

3.1.4 Ideally mouse groups should consist of littermates of the same sex.

3.1.5 Mice should be grouped with each other before they reach puberty to minimise aggression between unfamiliar individuals.

3.1.6 As a guide, the optimal size for a group of adult mice is three to five for females and three for males. However, in determining group size, factors such as differences between individual animals, strain, sex, cage size and experimental design should be taken into account. Therefore the scientific literature should be consulted when determining the optimal housing for particular strains and animals must be monitored.

3.1.7 The disruption of established social groups can cause aggression and should be avoided unless it is absolutely essential.

3.1.8 Separation of cage mates should be limited to less than 24 hours.

3.1.9 Mixing adult males from different groups in the same cage should be avoided.

3.1.10 Where it is necessary to mix unfamiliar adult males, they should be exposed to each other before they are mixed together. This can be achieved by placing the newcomer into an adjoining cage to allow visual, auditory and olfactory contact with the other male. They should also be closely monitored after mixing to check for aggression.

3.1.11 Nesting material should be provided to minimise conflict. Following cage cleaning, for sentinel or breeding cages, nesting material should be transferred from the old to the new cage to minimise aggression (see Section 4.7 Cleaning).

3.1.12 Mice should not be housed in the same room, or within auditory, olfactory or visual contact, with predatory species including rats and cats and staff should take care not to transfer scents from predatory species into the mouse room.

Mouse Care and Management – Isolation / Individual Housing

3.2.1 Ideally mice should not be housed individually, however there are some circumstances (for example with highly aggressive individuals or strains) where individual housing may be more conducive to mouse welfare.

3.2.2 Except in cases where immediate isolation of an individual is required to prevent injury, investigators must seek Animal Ethics Committee approval prior to housing mice individually.

3.2.3 Where mice are housed individually due to aggression, for some highly aggressive individuals visual, auditory and olfactory contact with other mice should be limited as far as possible to reduce stress caused by the presence of other mice.

3.2.4 Where mice are housed individually for reasons other than aggression, such as experimental requirements, this should only be with the express permission of the Animal Ethics Committee and they should be housed in visual, auditory and olfactory contact with other mice.

3.2.5 Environmental enrichment is essential for all mice - In cases where individual housing is required, environmental enrichment should be provided to ameliorate the impact of individual housing (see Section 3.5 Environmental Enrichment).

Mouse Care and Management – Metabolism Cages

3.3.1 Mice should not be housed in metabolism cages without the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house mice in this way. In such cases, mice should be able to be in visual, auditory and olfactory contact with other mice as far as possible. The size of the mesh gaps in the floor should not exceed 8mm x 8mm (See also 2.6 Cage Flooring.

3.3.2 Mice should be acclimatised to the metabolism cage before studies commence.

3.3.3 Where metabolism cages have to be used, consideration should be given to enriching the cages (for example by providing an area of solid floor and/or a nest box).

Mouse Care and Management – Effects of Handling, Routine Husbandry Procedures and Transport

Handling - General

3.4.1.1 Animal handlers should wash their hands, change gloves and wear clean coats before handling mice.

3.4.1.2 Steps should be taken to familiarise mice with handlers so as to reduce the stress of handling.

3.4.1.3 Mice should be handled quietly and gently.

3.4.1.4 Periods of restraint should be kept to a minimum.

3.4.1.5 Handling mice for routine husbandry procedures such as cleaning should not follow, nor be associated with, procedures that may cause distress in mice.

3.4.1.6 Chasing mice around their cage should be avoided. Mice that prove difficult to catch by hand should be directed into a plastic tube or similar structure and thence lifted from the enclosure and coaxed from the tube.

Handling - Neonates

3.4.2.1 Investigators must be aware that handling of neonates can have a long term impact on the welfare of animals that persists throughout their lives.

3.4.2.2 Handling of neonates should only be performed where necessary and must be performed consistently across a subpopulation or population of mice to minimise experimental variability.

3.4.2.3 Where neonates are handled, handling must be performed quietly and gently.

3.4.2.4 Early weaning of mice (prior to 21 days of age) should only be performed with permission from the Animal Ethics Committee.

Routine Husbandry Procedures

3.4.3.1 To minimise the impact of disruptions, mice should be allowed a conditioning period to ensure that disturbances such as laboratory animal personnel entering the room do not cause undue stress. A period of at least seven days is recommended.

3.4.3.2 Persons entering the mouse holding room should follow a routine as much as possible.

3.4.3.3 Stressful procedures should be conducted in isolation from other mice in an appropriately equipped procedures room.

Transport

3.4.4.1 Transportation times should be kept to a minimum. Effort should be taken to contain mice in such a way to minimise noise, vibration and extreme variation in temperature.

3.4.4.2 Where possible, mice should be transported in their home cage to minimise stress.

3.4.4.3 Mice should have access to food and water during transport. Precautions should be taken to prevent water spillage, for example by providing an alternate source of water such as a sterile water gel.

3.4.4.4 Following on-site transport, a minimum of 24 hours should be allowed for acclimation.

3.4.4.5 Following off-site transport, a minimum acclimation period of 3-7 days is recommended, although longer may be required for stabilisation of behavioural and reproductive parameters.

3.4.4.6 Mice deemed to be unwell or injured should not be transported, unless it can be established that transport does not result in additional pain or distress.

Mouse Care and Management – Environmental Enrichment

3.5.1 Mice should be provided with environmental enrichment in addition to the necessary nesting material and an in-cage shelter.

3.5.2 Depending on the type of enrichment and how it is implemented, environmental enrichment may be a significant source of experimental variability. It is therefore critical that environmental enrichment is applied consistently to groups of mice.

3.5.3 Items that allow mice to perform each of the five following categories of behaviour should be provided:

(i) social interaction (see Section 3.1 The Social Environment)
(ii) chewing/gnawing
(iii) locomotion (including climbing, exploring, playing)
(iv) nest building, nesting, resting/hiding
(v) manipulating, carrying and hoarding food and objects

3.5.4 Enrichment items can be provided on a rotating basis to increase their novelty value

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3.5.5 When techniques are used in an effort to provide environmental enrichment for mice it is important that the success of the techniques, in terms of improving mouse welfare, is evaluated. In particular, male mice should be monitored for increased aggression.

3.5.6 Spatial conditions should be generous enough to allow coping with any increased aggression that may result from environmental enrichment.

Mouse Care and Management – Food and Water

3.6.1 Food and fresh water should be provided ad libitum *unless special permission has been obtained from the Animal Ethics Committee of the institution to vary this regime*

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3.6.2 A nutritionally adequate diet should be provided for mice.

3.6.3 Where treats are fed, these should be accounted for in the overall ration of mice to avoid obesity. Grain and maize are good enrichment as they are small and easy to disperse in the bedding encouraging foraging.

3.6.4 Food and water should be free of contaminants unless these are part of the study. Autoclaved or irradiated pellets should be used for immunodeficient or barrier-maintained mice.

3.6.5 Food must be stored in a clean, dry, vermin-free, well-ventilated area to reduce the risk of post-purchase contamination.

3.6.6 Water delivery systems should be checked daily to ensure proper function. Care must be taken to ensure water delivery systems do not leak, particularly when cages are moved during cleaning or transport. Where practical, mice

should be provided with an elevated or suspended dry refuge area in case of flooding.

3.6.7 To minimise the risk of cross-contamination, it should be ensured that water bottles are not interchanged between groups of mice.

3.6.8 It should be ensured that mice are able to use water delivery systems.

3.6.9 Food may be scattered throughout the cage as a form of environmental enrichment (see section 3.5 Environmental Enrichment).

Mouse Care and Management – Monitoring of Mice

3.7.1 Welfare monitoring of mice via behavioural observation should be carried out in addition to monitoring for physical health. Investigators should be familiar with strain and/or transgene-mediated health conditions and behavioural problems so that they can be diagnosed and treated in a timely manner.

3.7.2 Monitoring should be carried out when a person with whom the mice are familiar is present. It should be ensured that there are sufficient, properly trained staff and resources including staff time to monitor mice effectively.

3.7.3 In the monitoring and investigation of health issues (such as growth rate, reproductive performance and disease) the effects of housing conditions should be taken into account.

3.7.4 Animal carers should be familiar with the normal physical appearance and behaviour of mice and of the individuals within a group and note any deviations from the norm, including animals that do not move around the cage normally. Mice that give cause for concern may need to be removed from the group but only if absolutely necessary as aggression may occur subsequently to regrouping.

3.7.5 In particular, mice should be monitored for signs of bullying including fight wounds, barbering or loss of body condition secondary to denial of access to food or water.

3.7.6 Mice that become sick unexpectedly should be examined and diagnosed by a veterinarian and any animals that die unexpectedly should routinely be submitted for post-mortem and diagnosis.

3.7.7 Records and score sheets should be kept and reviewed regularly to detect trends and subtle changes.

Environmental Variables - Light

4.2.1.1 Lighting within cages during the light phase should be maintained at a luminance below the threshold of aversion for mice. For most pigmented strains this is below 60lux and for albino strains it is below 25lux. To enable staff to perform tasks in mouse rooms it may be necessary to increase the lighting to 210lux at working height for the period while workers are in the room.

4.2.1.2 Light intensity can be reduced by using recessed lighting consoles in the ceiling with fluorescent lights of about 25-36 watt and a low spectral intensity (wavelength). This can be achieved by using a low colour number, e.g. colour 33 tubes.

4.2.1.3 Shading should be provided over the top shelves of racks and cages and racks should be positioned in a way that protects mice in the top cages from overhead lights and provides more uniform light levels between cages on different shelves.

4.2.1.4 Lighting should be diffuse and uniform to avoid glare, heat clusters and fluctuating lighting conditions for individual cages.

4.2.1.5 If halogen lighting is used, a silica glass cover must be interposed between the bulb and mice to minimise genotoxic and carcinogenic effects.

4.2.1.6 If mice are observed during the dark phase red or sodium lamps should be used to minimise any disruption to their nocturnal activities.

4.2.2.1 A semi-natural light cycle of 12:12 or 10:14 hours light:dark is suggested. Variations in the light:dark cycle to mimic seasonal change could be considered.

4.2.2.2 The use of dimmers in mouse rooms is suggested to allow the creation of twilight periods between the light and dark cycles.

4.2.2.3 Cycles may be disturbed if lighting clocks or timers malfunction. Clocks and timers should be checked regularly. In the event of a disturbance mice should be allowed an additional acclimation/habituation period, as disruption to the light cycle is a source of experimental variability.

4.2.2.4 A change in light cycle should be followed by an acclimatization period before commencing a study.

4.2.2.5 Care should be taken to prevent light leaks in animal rooms during the dark phase.

4.2.2.6 Lights should be checked for flickering and any flickering rectified. Light intensity should also be monitored.

Environmental variables - Temperature

4.3.1 A room temperature range for mouse housing between 20 and 26°C is recommended. Consideration of the strain of mice used (for example hairless or obese strains) and procedures that may disrupt thermoregulatory ability (for example anaesthesia, viral inoculation) should be taken into account.

4.3.2 Significant fluctuations in temperature should be avoided. In particular, ambient temperature must be carefully controlled where cardiovascular parameters and sleep are assessed.

4.3.3 Mice should be provided with nesting and bedding materials, an in-cage shelter and compatible cage companions to allow them to select an appropriate microclimate, particularly for sleeping.

4.3.4 Special attention should be given to those circumstances where the mouse's thermoregulatory ability is altered or compromised. Cage temperature for lactating mice and pups up to three weeks of age should be at the higher end of the recommended range (24-26°C).

4.3.5 Ambient temperature should be monitored within the cage and at various points within the room to monitor variation so as to optimally manage the microenvironment.

4.3.6 Adjusting the ambient temperature may be a potential approach to promoting recuperation following sleep deprivation and mitigating the effects of viral infection. For more information see Jhaveri et al[2].

Environmental Variables - Humidity

4.4.1 A relative ambient humidity at the level of mouse cages of 55 per cent +/-15 per cent (40-70) is recommended for adult mice.

4.4.2 A relative ambient humidity at the level of mouse cages of 50-70% is recommended for young mice prior to weaning.

Environmental Variables – Air Quality and Ventilation

4.5.1 The number of room air changes per hour needs to be adjusted to keep air quality and humidity at acceptable levels within cages. Room ventilation rates of 15-20 ACH may be needed depending on stocking densities.

4.5.2 Racks should be positioned in a room so as to optimise air exchange and avoid animals being exposed to draughts.

4.5.3 Air quality, air flow, temperature and humidity should be measured both in the room and within cages.

4.5.4 Exhausts should be installed close to ground level when cages are placed parallel to walls.

4.5.5 Intra-cage ammonia levels should be kept at 25ppm or below.

Static Isolator Cages and Filter Tops

4.5.2.1 Static isolator cages must be cleaned once a week to avoid excessive ammonia and carbon dioxide levels.

4.5.2.2 Supply air temperature should be maintained at 22 degrees, and room ventilation at 15ACH, to minimise ammonia concentration.

4.5.2.3 The population density of mice in static isolator cages should be kept to a minimum.

Individually Ventilated Cages

4.5.3.1 A minimum of 5 ACH may be sufficient to maintain room air quality but should be determined on engineering advice and in accord with expected workflows in the room.

4.5.3.2 The choice between positive and negative pressure in ventilated cages should depend on study requirements and the protection of animals and personnel - Ideally ventilated systems should be set up so that individual cages are under negative pressure with all air exhaust entering out of the room via a heating, ventilation and air-conditioning system.

4.5.3.3 For individually ventilated cages housing non-gravid adult mice, a ventilation rate of 60 ACH is recommended if cages are changed fortnightly - rigorous testing may show good air quality results for some systems at lower flow rates.

4.5.3.4 For individually ventilated cages housing breeding trios and/or pups, a ventilation rate of 60-100 ACH is recommended - rigorous testing may show good air quality results for some systems at lower flow rates. Cages should be changed fortnightly.

4.5.3.5 It is imperative that nesting material and an in-cage shelter are provided in ventilated cages.

4.5.3.6 In individually ventilated cages cleaning regimes should be managed to maintain ammonia levels within a cage below 25ppm.

4.5.3.7 Investigators should be aware of the potential impact of individually ventilated cages on the emotionality and behaviour of particular mouse strains. For example, different systems may produce different levels of noise and draught, some of which may be aversive or harmful to mice.

4.5.3.8 As air supply can be interrupted by power failure, instillation of an air-flow controller in the supply air duct (positive pressure) or exhaust duct (negative pressure), which is connected to an alarm system, is recommended[3].

Environmental variables – Sound and Vibration

4.6.1 Investigators should familiarise themselves with the hearing range and any potential auditory dysfunction of the strain of mice being used.

4.6.2 Sources of sound including ultrasound should be considered when assessing sound levels to which mice are exposed. Environmental noise may be a source of variance which may confound results, necessitating the use of additional experimental animals. Computers, or any other equipment likely to emit high-frequency ultrasound, should not be used in rooms where mice are housed. If the use of such equipment is unavoidable then measures, such as packing the equipment in polystyrene foam plating, should be taken to dampen ultrasonic noises.

4.6.3 Sound measuring equipment including sound-level meters, condenser microphones, attenuators, amplifiers, weighting and filter networks must be

capable of detecting sounds in the range of frequencies appropriate to the species/strain being used.

4.6.4 Because of the potential for adverse effects, unnecessary sounds or noise should be eliminated from facilities in which mice are kept. In particular, avoid sudden, loud sounds.

4.6.5 Individually ventilated cages and racks should be checked for vibration and vibration in animal rooms, especially of cages, should be eliminated.

4.6.6 Due to the vibrations created, placing motorised equipment on bench tops with cages should be avoided.

Environmental Variables - Cleaning

4.7.1 The need for changing bedding depends on the type and amount of bedding used and air quality. Frequency of bedding changes will also be influenced by stocking rates, ventilation system, strains of mice used and particular disease conditions (for example, diabetes). As a guide, bedding is commonly replaced weekly or fortnightly.

4.7.2 Nesting material should be transferred from the old to the new cage during cleaning to minimise aggression. Note, bedding material soiled with urine and faeces should not be transferred to clean cages as this may exacerbate aggression.

4.7.3 Care should be taken to avoid contamination of cages with scents from different mouse strains. Cages should be cleaned thoroughly and steps taken to ensure soiled bedding or nesting material cannot fall into other cages. In addition, steps should be taken to ensure that male mice are not exposed to the urine of other male or female mice when temporarily removed from their social groups.

4.7.4 Plastic cages and bottles should be washed in hot (62-82°C) soft water with a manufacturer-recommended detergent solution. All residue must be removed prior to autoclaving as this may be baked onto the cage.

Monitoring of Environmental Variables

4.8.1 Mouse rooms should have temperature and humidity readings displayed in a position where staff can easily see them.

4.8.2 Regardless of centralised computer systems regulating the general environmental conditions, it is still essential to check these variables regularly in the room to indicate conditions at the cage level.

4.8.3 Sensors should be fitted to monitor and report malfunctions in ventilation, temperature and humidity control on a 24 hour basis, with automatic alarm activation and alerting of appropriate staff so that any unexpected variations can be identified and corrected.

4.8.4 On a larger scale, facilities must be equipped to detect hazards such as fire or entry of unauthorised persons.

4.8.5 Care should be taken that the operation of an alarm causes minimal disturbance to mice[1] (see Section 4.6 Sound and Vibration*)*

Identification and Records - Identification

5.1.1 Where it is necessary to individually identify mice, the least invasive method that is compatible with the use of mice should be used.

5.1.2 Non-toxic dyes and permanent markers may be used on the fur and tail. These methods of identification usually need to be replaced every two to ten days. Swabbing the tail with 70 per cent isopropyl alcohol prior to marking is recommended to extend the life of marker identification.

5.1.3 Fur clipping may be used but needs to be carried out frequently.

5.1.4 Subcutaneous microchipping, tattooing and ear notching may be used where permanent identification is necessary. Note there is some transitory pain associated with applying these forms of identification. Anaesthesia or sedation and analgesia should be used in applying tattoos and ear notches. The method used for identification must be approved by the AEC.

5.1.5 Toe tip amputation is a painful procedure and should not be used – tail tip amputation is similarly painful and should not be used without the express permission of the AEC and with specific justification in each case.

Identification and Records – Cage Labels

5.2.1 All cages should have labels attached to them that provide the following information, or cross reference to a central record in the same room containing this information:

** Mouse identification (strain, sex, number of mice);*
** Age (date of birth) of litters or of individual mice;*
** Approval number of project in which mice are being used;*
** Name, location and contact numbers of the chief investigator/teacher and, if applicable, other investigators/teachers using mice;*
** Name, location and contact details of staff associated with the housing and care of the mice;*
** Treatments / procedures;*
** Date arrived.*

Identification and Records – Breeding Records

5.3.1 To assist in the monitoring and management of mouse breeding colonies, regular reports must be made to the Animal Ethics Committee, for review, on the fertility, fecundity, morbidity and mortality of all mouse breeding colonies. Reports should be submitted every six months, but may be required more frequently if deemed necessary by the Animal Ethics Committee. For further information refer to ARRP Guideline 16: Supervision of Animal Supply by Animal Ethics Committees.

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5.3.2 Section 4.5.8 Australian Code of Practice for the Care and Use of Animals For Scientific Purposes states that the person in charge must maintain adequate records to allow effective management of the breeding stock including the detection of the origin and spread of disease. Records should include:

(i) the source, care, allocation, movement between locations, use and fate of all animals;

(ii) details of any diseases;

(iii) the fertility, fecundity, morbidity and mortality in breeding colonies; and

(iv) the health status, genetic constitution and physical environment of the animals.

1.0 General

1.1 Introduction

(i) These guidelines are intended for use by people involved in the housing and care of mice in scientific institutions. The guidelines are not intended to be a complete manual on mouse care and management but rather to provide some key guiding principles on best practice standards in mouse housing. The guidelines will be revised from time to time to take account of advances in the understanding of murine physiology and behaviour, technological advances, and changes in community attitudes and expectations about the welfare of animals.

(ii) The recent explosion of scientific studies on the subject of the housing of mice has facilitated the development of evidence-based guidelines[4]. The housing of mice in particular has been targeted as mice used for scientific purposes spend the majority if not all of their existence in laboratory housing. The nature of that housing therefore has the potential to significantly impact upon the welfare of all laboratory mice[4]. The number of mice used in laboratories or maintained in animal facilities is likely to increase, as the use of genetically modified, transgenic and knockout mice to understand gene function has resulted in an increase in the number of animals used in scientific procedures[5, 6, 7]. The implementation of housing guidelines will therefore have a broad impact.

(iii) Under the Australian Code Of Practice For The Care And Use Of Animals For Scientific Purposes (see below section 1.3 Responsibilities of Chief Investigators/Teachers), investigators and teachers who use animals for scientific purposes have personal responsibility for all matters regarding the welfare of these animals, and are obliged to treat animals with respect and consider their welfare when planning or conducting projects. The Code of Practice is underpinned by the principals of replacement of animals with other methods, reduction of the number of animals used and refinement of techniques used to reduce adverse impact on animals[8].

(iv) It is in the interest of investigators and teachers to promote improved animal welfare. Improved animal welfare may translate into improved research outcomes, as pain, suffering and distress in mice can lead to physiological and behavioural changes that may confound experimental data[9]. To minimise confounding variables, investigators should strive to maintain a stable physiological and behavioural baseline. This necessitates a familiarity with behaviour and biology of experimental species and strain on the part of investigators. Furthermore, investigators and teachers must be aware of the potential impact of husbandry and environmental variables on experimental animals. While the guidelines focus on the welfare of mice, it is implicit that conditions contributing to meeting the physiological and behavioural needs of mice will also contribute to the quality of scientific outcomes through provision of the optimum stable environment for the maintenance and care of the animals. The guidelines contain many examples of the physiological and behavioural responses of mice associated with variables in housing and hence the effects of these variables on mice as research subjects.

(iv) The guidelines are based on principles regarding the care and management of mice taken from scientific literature. These principles are detailed throughout the

document, as are recommendations for the care and management of mice which are derived from these principles. In some areas, conclusions to be drawn from the available literature are not entirely clear, and in such areas recommendations are extrapolated from information available and practices in mouse care and management current at the time of writing.

(v) The principles outlined in the document address requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (as outlined below in Section 1.4). The requirements of the Code of Practice include that animals held for scientific purposes should have their species-specific behavioural and physical needs met, whilst at the same time ensuring that the animals can adequately be monitored and are protected from disease, and taking into account the requirements of the research for which the animals are being used.

(vi) The guidelines outline the requirements for housing to meet the physiological and behavioural needs of mice. Where mice are physiologically or behaviourally abnormal, for example post surgery, acute pain models, or disease models such as diabetics and Parkinsonian mice, modification of housing to meet their specific needs may be required.

1.2 Responsibilities of Institutions

Recommendations

1.2.1 Institutions using mice for scientific purposes are responsible for meeting recommendations of the institution's Animal Ethics Committee to ensure that facilities for the housing and care of mice are appropriate to the maintenance of their well-being and health.

1.3 Responsibilities of Chief Investigators / Teachers

Recommendations

1.3.1 The chief investigator/teacher (person in charge of a research/teaching project) has direct and ultimate responsibility for all matters related to the welfare of mice under his or her control, which includes their housing and care. (As per the principle contained in Clause 3.1.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes).

1.3.2 The chief investigator/teacher should ensure that the extent of personnel / staff supervision is compatible with the level of competence of each person and the responsibilities they are given in relation to mouse care and management - Personnel training and competencies should be documented. (As per the principle contained in Clause 3.1.3 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes).

1.4 The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes

Principles

(i) The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 2004 sections 4.4.19 to 4.4.23 states:

4.4.19 Animal accommodation should be designed and managed to meet species-specific needs. Pens, cages and containers should ensure animal well-being and comfort. The following factors should be taken into account:

(i) Species-specific behavioural requirements, including the availability and design of space to enable free movement and activity, sleeping, privacy, contact with others of the same species and environmental enrichment;

(ii) provision of single housing for animals when appropriate for the species and if necessary for the purpose of the project, (for example during recovery from surgery or collection of samples);

(iii) species-specific environmental requirements, such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations;

(iv) the need to provide ready access to food and water;

(v) the need to clean the pen, cage or container;

(vi) protection from spread of pests and disease;

(vii) requirements of the project; and

(viii) the need to observe the animals readily.

4.4.20 Pens, cages and containers must:

(i) be constructed of safe, durable materials;

(ii) be kept clean;

(iii) be maintained in good repair;

(iv) be secure and escape-proof;

(v) protect animals from climatic extremes;

(vi) not cause injury to animals;

(vii) be large enough to for the species and the number of animals held; and

(viii) be compatible with the behavioural needs of the species.

4.4.21 The number of animals in cages, pens or containers and the placement of these should enable social and environmental conditions for the species to be maintained. Where it is necessary to individually house animals of a species that normally exists in social groups, the impact and time of social isolation should be kept to a minimum.

4.4.22 Bedding and litter must be provided if appropriate to the species, and should be comfortable, absorbent, safe, non-toxic, able to be sterilised if needed, and suitable for the particular scientific or educational aims. Pregnant animals must be provided with nesting materials where appropriate.

4.4.23 The AEC, investigators and teachers should be consulted in advance of planned changes to these conditions, since these may affect the welfare of animals and the results of the scientific and teaching activities.

1.5 Aspects of mouse biology, physiology and behaviour relevant to housing

Principles

(i) Mice are physiologically and behaviourally distinct from rats, from which they diverged over 17.5 million years ago[10]. The laboratory mouse (Order Rodentia, family Muridae, subfamily Murinae, genus *Mus*, subgenus *Mus,* species *musculus*) is descended from the house mouse of North America and Europe, *Mus musculus*[11]. The genome of laboratory mice is derived from *M. musculus* and *M. domesticus* subspecies[11, 12]. It is believed that fancy mice strains from Europe and East Asia contributed to the genome of common laboratory strains including C57BL/6, BALB/c and DBA[12, 13]. Mice used in biomedical research range from captive wild individuals to strains bred hundreds or even thousands of generations in a laboratory setting, often with spontaneous or deliberately induced genetic alterations[14]. Most laboratory strains originate from pet dealers who became suppliers of laboratory mice[15].

(ii) Mice are social animals. In the wild they live in groups which vary extensively in size[16]. The social patterns and behaviour of wild mice by necessity differ significantly from those of laboratory mice[16]. Social organisation of wild mice is dynamic and dependent on environmental variables including resource availability and shelter[17-19]. Complex environments may support a higher density of mice than open areas[19]. Commensal or house mouse territories with stable and abundant food supplies may house up to 10mice/m^2 [18]. The extended family unit, known as a deme, may consist of a single dominant male, several subordinate males and breeding females[16]. Feral or dispersed (non-commensal) mouse populations are typically less dense, and less stable[20].

(iii) From birth to approximately 14 to 21 days of age, pups are dependent on their mother for warmth, food and toileting[16]. While pups begin to explore beyond the nest at around three to four weeks of age, they tend to remain in the nest until they reach sexual maturity (at around 5 to 6 weeks, although this may be as late as 12 weeks depending on genotype and environmental factors[16]).

(iv) Dispersing mice seek out a protected site in which they can build a nest and establish territorial boundaries. Territory size varies, depending on environmental factors including food availability and population density[16]. Where a concentrated food source is available, territory size may range from 2-6m^2, while feral or non-commensal mice may have a home range of up to 80,000m^2 [16].

(v) House mice can be polygamous but may pair-bond[16]. If environmental factors are favourable (ample food and nesting material), a reproductive female can produce up to ten litters a year[16]. Gestation lasts from 18 to 21 days, with the female building a nest in the days preceding parturition[16]. During this time females may exhibit aggression towards non-reproductive mice, although pregnant and/or lactating female mice are known to form

communal nests with close relatives, and may share nursing duties[21]. Mothers are known to destroy their own litters (infanticide), a behaviour which may be attributed to disturbances, overcrowding, dietary restriction or other environmental factors[16]. Adult males are highly infanticidal, although less commonly to their own pups[16]. Adult males exhibit varying degrees of tolerance to one another[22].

(vi) Murine sensory input is dominated by olfactory, auditory and tactile cues, many of which are beyond the range of human sensation[16]. The implication is that aspects of the laboratory environment which are highly relevant to mice may be unnoticed by investigators and laboratory personnel[23].

(vii) Olfactory cues are the primary means of communication between mice[24]. Mice employ a main olfactory system to detect airborne volatile scents, in additional to the vomeronasal system which detects pheromones[24]. They signal individual identity via expression of major urinary proteins predominantly in the high mass fraction of their urine[25, 26]. Urine is deposited in streaks and spots in and around the territory[27], with the dominant owner marking more frequently than subordinates[24]. Dominant males refresh their own marks, and may enter neighbouring territories to over-mark the urine of a competitor[16]. Aside from urine, mice have other sources of secretions which may act as olfactory cues, including salivary glands, plantar glands and the preputial gland[28]. Via odours, mice can recognise kin relationships[29], the social status of male mice[30] and the oestrus status of female mice[31]. Mice use olfaction and olfactory cues to assess territorial boundaries, detect food, identify one another, and to evaluate sexual and social status[16, 25]. Mice commonly sniff the ano-genital region of cohabitants and prospective sexual partners[32]. Scent impacts a wide range of behaviour including competitive and territorial aggression between males[24 28, 33]. In addition, pheromones can prime or inhibit reproduction[24, 34]. For example, male odours induce oestrus and synchronise oestrus in females (the Whitten effect)[35] while unfamiliar male odours can prevent the establishment of pregnancy in females (the Bruce effect)[36]. Inbreeding of mice inhibits their ability to discriminate via olfactory cues because individuals are almost genetically identical[37]. This may alter competitive/aggressive behaviour[24, 37] and therefore experimental outcomes. Olfactory cues should be taken into consideration when devising a cleaning protocol, as inadvertent disruption of chemical signals during cleaning may result in outbreaks of aggression[19, 24, 28] (see Section 4.7 Cleaning). In addition, unfamiliar odours (such as those associated with humans) may cause stress responses in mice.

(viii) Mice have a well developed sense of hearing, and can hear sounds from 2300Hz (23kHz) to over 85000Hz (85.5kHz)[38]. Mice produce ultrasonic (above 20kHz) vocalisations during non-aggressive interactions[39] that are inaudible to the unaided human ear. The function of these vocalisations is yet to be established, but in the laboratory setting they occur more frequently in mice housed in socially and environmentally enriched cages[39], suggesting that they may be a useful indicator of affect or

emotion. The ears of mouse pups are closed for up to 10 days post-natally[40], yet they emit ultrasonic vocalisations when separated from the doe, reliably stimulating the mother to retrieve them[39, 41, 42]. Ultrasonic vocalisations in pups may be context specific[42, 43]. For example, distinctive vocalisations were associated with isolation outside the nest, jostling for the doe's nipples, being handled roughly by adults or the immediate post-partum period[43]. It is possible that mice use ultrasonic calls for the purpose of echolocation and judging distances in the darkness, as may be the case in rats[16, 44], but this is yet to be established. Differences in ultrasound vocalisation rate and acoustic structures have been observed between different strains of mice[41]. Some strains of laboratory mice are genetically predisposed to auditory dysfunction and hearing loss (see section 4.6 Sound and Vibration).

(ix) Mice have dichromatic colour vision, similar to red-green colour blindness in humans[45]. They have a retinal mechanism which is maximally sensitive to ultraviolet light. In humans with normal vision, UV is blocked by the cornea, so artificial lighting has been designed to emit little UV. When housed in laboratories without these UV wavelengths, mice may have distorted or altered colour perception[23].

(x) As with all small mammals, risk of predation is an important factor influencing activity and movement patterns of mice. Mice exhibit thigmotaxis, a tendency to maintain contact with vertical surfaces such as walls, particularly when exploring a new area[46, 47]. When faced with a real or perceived threat, mice may retreat or freeze. Retreating mice have a tendency to run away as well as upwards[32]. In captive animals this often results in the animal landing on the bars of the cage if shelter is not available. Muscle fasiculations or convulsive behaviour may be noted. Alternatively, mice may crouch in one spot. The adoption of a full submissive posture, in which the animal lies on its back, has been reported[32].

(xi) Mice are primarily crepuscular or nocturnal[16, 48], however they may alter their activity patterns depending on food availability and due to light cycle and activities in the laboratory [16].

(xii) Mice are omnivorous, but are known to prefer foods high in fat and protein, and will eat meat and live insects[16]. They may acquire most of the water they need from their food[16]. Mice eat up to 20 per cent of their body weight daily, consumed in small, frequent portions. This occurs mostly during the dark phase[16].

(xiii) Normal behaviours of mice include eating, drinking, urinating, defecating, foraging, exploring, gnawing, hiding, climbing, playing, nesting and digging and engaging in a range of social activities.[49, 50].

(xiv) House mice exhibit developmental plasticity, with aspects of the early environment impacting on adult phenotype[16]. For example maternal stress during gestation can delay post-natal development; prenatal stress can

induce masculinisation of female pups and feminisation of male pups (which impacts in turn on reproductive performance and aggression in later life); and low food availability during gestation can reduce weaning weight and increase aggression[16]. Quality and quantity of maternal care can affect weaning weight, onset of sexual maturity and corticosterone responses to stress in later life[16]. Thus the development of mice varies between sites, depending on local environmental factors. This plasticity may account for differences in phenotype between laboratory mice obtained from different facilities[16]. Some inbred strains display behavioural complexes which are believed to reflect functional adaptations to particular habitats. Thus BALB/c mice, which are adapted to living on the surface, display exploratory behaviour, whereas C57BL/6J mice are traditionally hole-dwellers, with a tendency to dig[13]. This may influence the way each strain interacts with a particular environment.

Recommendations

1.5.1 To meet the requirements of the Code of Practice (that is to provide accommodation that meets the species specific needs of mice), housing should allow mice the opportunity for social interaction, the opportunity to carry out normal behaviours and the opportunity to rest and withdraw from each other. Normal behaviours of mice include eating, drinking, urinating, defecating, foraging, exploring, gnawing, hiding, climbing, playing, nesting, digging and engaging in a range of social activities.

1.5.2 Housing requirements for individual mice may vary according to strain, age, physiology, stocking density, the purpose and the length of time for which animals are used (for example breeding or experiments)[1].

1.5.3 The Code of Practice recognises that there may be circumstances where the requirements of experimental procedures will preclude meeting some species specific needs (Clause 4.4.19). Variations to these requirements as part of a project must endeavour to meet the physiological and psychological needs of mice as closely as possible, and must receive prior Animal Ethics Committee approval.

1.6 Genetically modified, transgenic and knockout mice

Principles

(i) For the purposes of these Guidelines, the term "genetically modified" applies to transgenic mice, knock-out mice, knock-in mice, chimeras, cloned mice and mice genetically modified in any other way.

(ii) In addition to these guidelines, investigators using genetically modified mice must adhere to the NHMRC's Guidelines for the Generation, Breeding, Care and Use of Genetically Modified and Cloned Animals for Scientific Purposes[51]. Genetically modified and cloned animals are subject to State and Territory animal welfare legislation. They may also be subject to requirements of the Office of the Gene Technology Regulator under the Gene Technology Act[51].

(iii) A wide variety of genetically modified, transgenic and knockout strains of mice, both inbred and out-bred, are currently used in laboratories within Australia.

(iv) By targeting a particular condition or aspects of that condition using genetically modified animals, researchers may require fewer animals in a study [52]. However, more animals may be required to create and maintain each genetically modified line and a higher than normal culling rate may apply[6, 51, 53].

(v) The GA (Genetically Altered) Mouse Welfare Assessment Working Group found that while being genetically altered was not in itself a welfare issue, the effects of genetic alteration on mouse phenotype may be[5]. Impacts on mouse welfare may be due to techniques used to produce and monitor the genetic modification or modifications; expression of the modified or deleted genes (GM phenotype); position of the modified gene in the genome; action of unpredicted factors in gene expression and interactions between gene products; disruption of physiological processes of the mouse or poor fit between the new strain and its environment[5, 51]. It should be noted that the majority of genetic mutations give rise to no discernible effect, or lead to death at the embryonic or foetal stage. However, genetic modification or mutation may cause perinatal or neonatal death, or produce animals with compromised welfare. Genetic modification can compromise mouse health and welfare by causing or predisposing the animal to pain, suffering, distress or lasting harm[5]. A Danish survey involving 87 mouse genetically modified strains found that 36 per cent of strains were reported as experiencing discomfort, with 21 per cent experiencing minor discomfort (for example mice with increased aggression, lymphoma or a weakened immune response) while 15 per cent experienced severe discomfort (for example mice with cystic fibrosis, diabetes, seizures, malformation of the skull or rectal prolapse)[54]. In addition, 30 per cent of the strains were reported to suffer increases in mortality, disease incidence and susceptibility to disease[54]. The most frequently reported conditions were increased mortality, decreased fertility and diabetes. It is also possible that genetically modified animals may be more robust in the face of experimental challenge (for example exposure to a specific pathogen) than their non-genetically modified counterparts[53].

(vi) Physiology and behaviour can vary markedly between different strains of mice, and even different subpopulations of the same strain[55]. Many genetic alterations are maintained in mice of mixed genetic backgrounds, the genotype of which depends on the breeding strategy and background strains[5]. Transgenic or knockout mice may have severely disturbed physiology[56, 57]. This may manifest as abnormal immune response, altered lifespan, impaired sensory abilities, gender-influenced survival, altered susceptibility to nociceptive stimuli, and altered reproduction, particularly reduced litter size[52, 55, 57, 58], as well as development of clinical disease. To ensure appropriate action is taken to minimise suffering, it is essential that investigators understand the impact of genetic modification on mice[5].

(vii) It has been argued that murine behaviour is simpler and much less flexible than that of the rat[59]. Investigators conducting a behavioural review hypothesised that this simplification, which may assist mice in adapting to a different ecological niche than the rat, may be mediated by accelerated brain maturation during development, rendering the mouse less dependent on complex social behaviour and plastic nervous system changes. If this is the case, differences in genotype may alter behaviour dramatically between strains.

(viii) Phenotype of new strains may be poorly characterised, making it difficult for investigators to set specific monitoring protocols or define endpoints[52]. Investigators should be familiar with strain-specific and transgene-mediated health conditions including tumour growth, hair loss, degenerative joint disease, diabetes, respiratory tract disorders and intestinal obstruction so that they can be diagnosed and treated in a timely manner[57].

(ix) Genetic modification may lead to changes in emotionality, anxiety and predisposition to psychological stress[60], which may also compromise welfare. For example, genetic modification may lead to an anxious phenotype (in some studies this may be desirable[61, 62]). It may also lead to aggression[63], which is stressful to victims and may necessitate individual housing. Some strains are more predisposed to developing stereotypies than others[64], and may be more likely to do so within a particular environment.

(x) Variations in phenotype of genetically modified mice may lead to profoundly different husbandry requirements. For example, food and water sources may need to be placed at the bottom of the cage for mice which are small, debilitated or with neurological models exhibiting balance problems[52, 65]. Toothless phenotypes may require a powdered diet[52]. Some transgenic mice may need to be maintained on a medicated diet (for example, a diet incorporating doxycycline or tamoxifen) to maintain a gene in an “on” or “off” state[66]. Other characteristics that may alter housing requirements include the propensity of certain strains for aggression or cannibalism[13, 57]. Some phenotypes may have a reduced capacity to thermoregulate. For example, nude strains and those with poor maternal performance may require supplemental nesting, bedding materials or haired companions[52, 58]. Immunocompromised strains such as models for severe combined immunodeficiency (SCID) require a high level of biological containment, including individually ventilated sterile cages as well as sterilised bedding, food and water, to prevent fatal septicaemia[6].These variations should be taken into account in relation to mouse housing and husbandry, as well as experimental design[13].

(xi) Phenotypic expression may be significantly affected by aspects of husbandry and housing, including laboratory conditions[67], investigator[68] and environmental enrichment[5].

(xii) The social consequences of genetic modification and extreme inbreeding are not well characterised, but have the potential to impact on both mouse welfare and experimental variability. For example, fully inbred BALB/c strains failed to discriminate between their own scent marks and those of other males[37]. This could lead to social disruption between dominant and subordinate males, with the possibility of marked urine retention with subsequent nephritis in some strains.

(xiii) Because of the vast number of new strains emerging it is beyond the scope of these guidelines to provide specific husbandry advice for each individual strain. Investigators should seek strain-specific information and recommendations from the supplier, institution of origin and literature databases[52]. Both the Mouse Genome Informatics Database (www.informatics.jax.org) and Eumorphia (www.eumorphia.org) provide information on phenotypes of different strains.

(xiv) As stated in the NHMRC's Guidelines for the Generation, Breeding, Care and Use of Genetically Modified and Cloned Animals for Scientific Purposes:

> *1.3.1 Each project involving genetic modification or cloning will have unique features that require attentive monitoring and assessment of the welfare of the animals across the various stages of the project. The purpose of monitoring is to make and analyse routine observations in order to detect deviations from norms of health and well-being in animals and to signal the need for responses to distress. The purpose of assessment is to characterise the nature of deviations or distress detected by monitoring so that interventions can be appropriate.*

(xv) Non-invasive, structured welfare assessments can be carried out to ensure that the needs of newly bred and maintained genetically altered mice are met and any genetically modified lines introduced into an establishment. A list of standard indicators of welfare is used to analyse the phenotype, as per Wells et al[5]. It is critical to take into account the choice of background strain and/or the breeding experience of the dam when conducting a neonatal welfare assessment, as some strains are negatively affected by disturbance while others tolerate increased intervention[5]. Welfare assessments should take into account factors such as appearance, morphology, coat condition, posture, gait, activity, interaction with the environment, relative size, clinical signs and pre and post weaning losses. Whole of life monitoring is necessary to fully appreciate the physiological, behavioural and welfare implications of a particular modification as the expression of genes can occur at any stage in the mouse's life[51]. However should intervention become necessary to avoid suffering, this should take precedence over whole of life monitoring.

(xvi) When using genetically modified, transgenic or knockout mice, investigators should carefully weigh the value of the experiment against welfare issues related to the particular strain. Welfare concerns arise not

only during the study, but in the development and production of particular strains[52].

Recommendations

1.6.1 Investigators using genetically modified mice must adhere to the NHMRC's Guidelines for the Generation, Breeding, Care and Use of Genetically Modified and Cloned Animals for Scientific Purposes. Genetically modified and cloned animals are subject to State and Territory animal welfare legislation. Investigators must make enquiries to determine whether they are subject to requirements of the Office of the Gene Technology Regulator under the Gene Technology Act.

1.6.2 Mouse behaviour may vary between different strains or stock. Researchers should ensure they understand behaviour and needs of their particular experimental animals and incorporate this knowledge into husbandry and experimental design.

1.6.3 Structured welfare assessments should be performed for newly bred and maintained genetically modified mice and any genetically modified lines introduced into the establishment.

1.6.4 Investigators should evaluate the impact of housing, husbandry and environmental enrichment on welfare and experimental variability for each strain used.

1.6.5 For specific husbandry advice for each individual strain, investigators should seek strain-specific information and recommendations from the supplier, institution of origin and murine databases such as the Mouse Genome Informatics Database (www.informatics.jax.org) and Eumorphia (www.eumorphia.org).

1.6.6 When using animals including genetically modified, transgenic or knockout mice, investigators should carefully weigh the value of the experiment against welfare issues related to the particular strain or strains used. Investigators should be mindful that welfare concerns arise not only during the study, but in the development and production of particular strains.

2.0 Cage Design

2.1 Living area

Principles

(i) The living area for mice is three dimensional, comprised of the floor area as well as the vertical space. These parameters need to be considered together, rather than in isolation, including the postulates that the quality of the space is more important than the quantity but there is a minimum quantity that is necessary to ensure quality. Despite numerous experimental studies, there is no consensus on minimal or optimal cage space for mice[69]. The Council of Europe revised housing guidelines in 2006 to regulate for increased minimum space of laboratory mice[70]. The rationale behind the change was to allow the incorporation of environmental enrichment to facilitate expression of species-typical behaviour. At the same time, several US-based groups published studies suggesting that less space may be advantageous for mice[69, 71-73].

(ii) There is no single definition of crowding or excessive population density for mice[18]. Given that free-living male mice establish territories ranging from $1m^2$ to $80,000m^2$ [16], it is possible that even housing mice in the same room as other mice in separate cages within visual, auditory and/or olfactory range may be experienced as crowding[18] (see Section 3.1 The Social Environment for further information on population density). Thus if a mouse shows the same behaviour when allowed a floor area of $56.25cm^2$ and $225cm^2$, it is possible that both cage areas are too large or too small or acceptable to the mouse[69].

(iii) In addition to three dimensional space, the shape of the living area needs to be taken into account in determining optimal living area.

(iv) The living area for mice must allow them to satisfy their basic physiological and behavioural needs including the ability to eat, drink, play, rest, groom, forage for food, explore, gnaw, hide, reproduce, engage in a range of social activities, urinate and defaecate[49]. If given the opportunity, mice tend to compartmentalise their living areas for these different activities, for example feeding, resting, urination and defaecation[28, 50, 74, 75]. These divisions allow mice to control and predict their environment, including light levels and temperature[50]. An example of a cage that promotes compartmentalisation is the Cambridge cage[76].

(v) The design, construction and management of a mouse's immediate enclosure will determine to a large extent how environmental factors, such as temperature, light levels, humidity and air quality impact on the mouse[77].

(vi) Living area or cage size affects feeding and energy expenditure of mice, at least in wild species. Thus wild-type *Peromyscus californicus* housed in smaller cages (L29cm x W19cm x H13cm: $7163cm^3$) had lower daily energy expenditure and lower food intake than their counterparts housed in larger (L48cm x W27cm x H20cm: $25,920cm^3$) cages[78]. In contrast,

energy expenditure and food intake of smaller *Peromyscus eremicus* mice were not affected by cage size. Thus the impact of living area varies with species and may also vary with strain of laboratory mice.

(vii) When attempting to determine optimal living area, different studies assess different parameters including cage microenvironment (ammonia, relative humidity, temperature, noise, airflow), reproduction (fertility, litter size), physical parameters (growth, survival), mouse physiology (stress hormones, immune function) behaviour and preference tests[69]. Recommendations are frequently based on the weight of individual mice and number of animals per cage[79]. Many studies which assess living area requirements for mice do not address potential confounding variables such as group size, strain effects, sex, age of mice and stage in breeding cycle, type of space (vertical vs horizontal) or enrichment methods[79]. Due to the complexity and variability of experimental design, it is difficult to extrapolate optimal cage size and population density.

(viii) The cage lid, insofar as it allows mice to climb, is an important consideration in determining living area (see Section 2.3 Cage Height and Cage Lid).

Recommendations

2.1.1 The living area for mice must allow them to satisfy their basic physiological and behavioural needs including the ability to eat, drink, urinate, defecate, forage, explore, gnaw, hide, climb, play, nest, dig and engage in a range of social activities.

2.1.2 The living area should be constructed and arranged in such a way to allow mice to compartmentalise their space, so that different areas can be used for urination, defecation, eating and resting.

2.2 Cage floor area

Principles

(i) There is no consensus in the scientific literature about the minimum cage floor area or maximum stocking density for housing laboratory mice. Different strains may have significantly different space requirements, which may be altered by in-cage furnishings or enrichment items. Table 1 provides a summary of some of the major studies evaluating cage floor area in different strains of mice.

(ii) As discussed in Section 2.1 Living Area, the living area should be large enough to allow mice to compartmentalise their space. At the same time, cages with large quantities of open, empty space without hiding places should be avoided as these may be stressful to mice.

(iii) In terms of physical movements, mice should be able to turn freely without twisting their heads and bodies, walk at least a few steps, stand on their hind limbs and stretch up. They should also have room to shelter and rest. The floor area should ensure that no part of a mouse's body is unavoidably distorted by contact with the cage in any of the postures that mice may

adopt. However, this does not imply that a larger cage is necessarily better. Mice exhibit thigmotaxis, and may therefore not respond to an increase in living area in the same way as other species[80].

Table 1: Studies evaluating cage floor area (listed alphabetically)

Author	Strain	Sex	No. mice per cage	Cage dimensions, total floor area or floor area per mouse	Parameters measured	Key results	Conclusion/ Comments
Benhar E[81]	C57BL/6JWn and SWR	M,F	Breeding pairs with litters of 9	29cm x 14cm (406cm^2) or 29cm x 17.5cm (507.5cm^2)	Number of litters, number of weaned mice	C57BL/6JWn mice in the larger cage produced 19 per cent fewer weaned mice. SWR mice in the larger cage produced 15 per cent fewer weaned mice.	Increased cage floor area may reduce reproductive performance.
Davidson LP et al[82]	Cr:SW	M,F	Breeding pairs with litters of ten	429cm^2, 505cm^2 or 729cm^2	Open field, light-dark exploration, elevated plus maze, weaning weight, locomotor skills of pups	No differences in weaning weight between cage size. Mice reared in 505 and 729cm^2 cages explored a significantly larger area; mice in 505cm^2 cages spent more time in the centre than those in the larger cages; failed to establish consistent link between decreased floor space and increased anxiety like behaviour. No consistent association between available floor space and development of locomotor skills in pups.	Increased cage floor area may be associated with increased anxiety.
Forsyth NY et al[83]	C57BL/6NCrl, Crl:CD-1, BALB/cAnNCrl	F	4	15.2cm x 15.9cm (58cm^2/mouse); 15.2cm x 26cm (96.8cm^2/mouse); 43.2cm x 20.3cm (219.4cm^2/mouse)	Organ weight, white blood cell counts	C57BL/6 and CD-1 mice had the lowest total white cell counts in medium cages; BALB/c mice had lowest total white cell counts in small cages. All strains had the highest total white cell counts in the large cages. Cage size did not affect body or organ weight.	Increased cage floor area may be stressful for mice, with some strains more vulnerable than others.
Fullwood et al[84]	C57BL/6	M	3	32.2cm^2/mouse; 64.5cm^2/mouse; 96.8cm^2/mouse; 129cm2/mouse	Body weight, food and water consumption, immunological parameters, mortality,	Cage size did not influence body weight. Mice in smaller cages consumed or wasted more food and water than those in larger cages.	The findings are difficult to interpret as increased plasma glucocorticoid

					adrenal weight, plasma glucocorticoid concentrations	Mice in the smallest cages had greater lymphocyte proliferation, but mice given 64.5cm^2 each had greater natural killer cytotoxicity than those given greater or less space. Mortality increased as more space was provided. In the larger cages mortality was due to bite wounds. In contrast, adrenal weights and plasma glucocorticoid concentrations were progressively greater with less space.	concentrations and adrenal weights are typically considered indicators of stress.
McGlone JJ et al[80]	BALB/cJ	M, F	3 litter mates of same sex	32.2cm^2/mouse; 96.8cm^2/mouse; 129.0cm^2/mouse	Growth rates, mortality, weight, food and water consumption, immunologic parameters, grooming, behavioural parameters	Increased weight gain, sitting behaviours, grooming behaviours and T-lymphocyte proliferative response in females in smallest cages; no mortalities of mice in smallest cages. Necropsy of mice which died in larger cages revealed emaciation, barbering and bite wounds suggesting increased aggression.	Reduced cage floor area (32.2cm^2/mouse) did not adversely affect behaviour, health, immunocompetence or performance in this strain.
McMahon K et al[85]	C57BL/6	F	1 female with pups	20.3cm x 40.2cm (816.06cm^2 total cage size) vs 15.2cm x 25.4cm (386.08cm^2 total cage)	Reproductive performance, microenvironment	Mice housed in larger cages had higher birth rates (9.8pups/female) than those in smaller cages (7.2pups/female). Larger cages had lower ammonia (17ppm) than smaller cages (24ppm), as measured on day of cage change.	Increased cage floor area may increase reproductive performance. The authors suggest that differences in reproductive performance may be due to differing ammonia levels.
Manosevitz M and Pryor[86]	C57BL/6	M,F	1 female with 4-8 pups	Approx 26.7cm x 16.5cm (440.55cm^2) vs	Weight, open-field activity and defacation, running wheel activity,	Males reared in large cages weighed 12% more than those housed in small cages at 38 days. Animals	Increased cage floor area may be associated with

				75.6cm x 70.8cm (5352.48cm^2)	exploration, water consumption	reared in large cages were 16% more active in an open field. Those reared in small cages defecated 2.2 times more, and had lower water consumption.	increased body weight, exploration and water consumption. Cage size confounded with cage texture (wire mesh vs plexiglass) and environmental enrichment.
O'Malley J et al[87]	ICR	F	1 female with 5-16 pups or 1 female with litter culled to 6 pups	419.25cm^2 total cage floor area	Faecal corticosterone levels; growth; weaning weights; reproductive performance of progeny.	Growth rates of pups from culled litters (smaller litters) was significantly greater, however when corrected for litter size to account for competition to nurse, growth rate did not differ between pups from intact versus culled litters. Corticosterone levels did not differ significantly between groups nor did reproductive performance of progeny.	Reduced cage floor space per pup is not stressful
Peters A and Festing M[88]	BALB/c, MF1	M,F	6, 10, 35, 36	33cm x 15cm (495cm^2) or 45cm x 28cm (1260cm^2); 33cm^2/mouse; 55cm^2/mouse; 27cm^2/mouse; 37cm^2/mouse	Aggressive encounters, mortality, weight, growth rate, adrenal weight	BALB/c mice gained more weight and had significantly smaller adrenal weights in higher density housing (groups of 35 vs 26).	Reduced cage floor area may reduce anxiety and aggression. Cage size confounded with population density. The difference between 27cm^2 and 37cm^2 may be too small to reveal any adverse effects[69].
Sherwin CM[89]	CB57	F	4	37cm x 21cm (777cm^2) + additional space	Preference for additional space	Mice worked to gain access to additional space, despite increasing costs.	Mice did not show a preference for a particular amount of

				(29x11cm or 319cm^2; 37x21cm or 777cm^2; 50x32cm or 1600cm^2)			additional space over another, thus additional space was an important resource but quantity was not. Findings may indicate true preference for additional space for its own sake. May also be a refuge from other mice or territorial monitoring.
Sherwin CM[90]	TO	M	1	27cm x 10cm (270cm^2) with a range of additional space available (196cm^2 to 1600cm^2)	Preference for additional space	Mice worked to gain access to additional space, despite increasing costs.	As above.
Sherwin, CM[91]	C57BL/6	F	4	Enriched cage 50cm x 32cm (1600cm^2) + additional space 37cm x 21cm (777cm^2)	Preference for additional space	Mice worked to gain access to an empty cage despite being housed in an enriched cage containing cagemates, food, water, nesting material, shelter, cardboard tube, chew sticks and running wheel	As above.
Smith A et al[71]	C57BL/6J	M,F	4-20	Cage size 333cm^2 or 728cm^2 with 20.6cm^2 per mouse – 77.4cm^2 per mouse	Injury, hair loss, aggressive behaviour, survival, body weight, food and water consumption, cage microenvironment, urine testosterone concentration	Ammonia concentrations exceeded limits at 20.6cm^2 although mice had microscopically normal nasal passages and eyeballs. All parameters within normal limits when mice housed at 36.1cm^2 or above.	Reduced cage floor area not associated with adverse effects.
Smith A et al[72]	BALB/cJ, NOD/LtJ, FVB/NJ	M,F	4-20	Cage size 333cm^2 or 728cm^2 with	Injury, hair loss, aggressive behaviour,	FVB/NJ displayed early onset aggression with reduced floor space;	Reduced cage floor area not associated

				36.1cm^2 per mouse – 83.2cm^2 per mouse	survival, body weight, food and water consumption, cage microenvironment, urine testosterone concentration	no apparent deleterious effects on BALB/cJ or NOD/LtJ strains.	with adverse effects in 2/3 strains, but associated with increased aggression in one strain. Early onset aggression may be an age effect as investigators were unable to source sufficient numbers of 3 week old mice so ages ranged from 3-5 weeks; alternatively this strain may be highly sensitive to variation in cage floor area.
Van Loo PLP et al[92]	BALB/cAnNCrlBr	M	3, 5 and 8	80cm^2/mouse or 125cm^2/mouse	Frequency of attack, latency to attack, urine corticosterone levels, food and water intake, weight, number of wounds, tyrosine hydroxylase, organ weight	Larger cages associated with moderate increase in aggression, with aggression considerably higher in groups of 8 animals compared with groups of 3. Dominant and subordinate mice demonstrated different stress levels.	Increased cage floor area may be associated with increased aggression. Aggression may be increased at lower population densities where available space can be defended. Decreasing floor size may be used as a temporary measure to reduce high levels of aggression in an existing group of male mice, but group size should be kept to 3-5 animals.

Whitaker J et al[93]	C57BL/6Tac	M,F	3 adults plus 1-20 pups	208.3cm^2 or 315cm^2	Litter size, litter survival to weaning age, average pup weight at 7, 14 and 21 days, and number of days between litter births. Male and female performance in elevated plus maze test, open field assay and acoustic startle test before and after an intraperitoneal saline injection.	Cage size had no significant impact on reproductive parameters and inconsistent effects on behaviour in weaned pups.	No significant difference between mice housed in standard cages and cages that are 50 per cent larger. Enrichment provided in this study (nestlet and PVC tunnel in all cages) may have masked effects of cage size on reproduction and behaviour[69].

(iv) A number of studies have challenged the generosity of cage floor area recommendations published in overseas guidelines, on the grounds that they appear to be based on current practice rather than evidence. In addition, there is evidence that increased cage size may be associated with increased mortality, in particular due to fighting between male mice. However, investigators should keep in mind that most studies confound effects of cage floor area with effects of group size when reviewing the literature. Furthermore, many do not factor in the presence of nesting and bedding material and an in-cage shelter.

(v) In-cage shelters (see section 2.9 In-Cage Shelters) are desirable additions to mouse housing, however dimensions of the floor area must be sufficient to accommodate such furnishings without negatively impacting on mouse behaviour by reducing floor space or restricting access to areas of the cage. Negative effects of increased floor area may be offset by the provision of cage furnishings, dividers or other structural elements. One study reports increased aggression between males with a cage shelter[460].

(vi) A 2008 survey of animal units in the United Kingdom found that the floor area per mouse ranged from 22cm^2 to 960cm^2 [94]. In the majority of cases (95.9 per cent), mice were housed in cages which allowed for 60cm^2 per mouse. Only one per cent of cages allowed for less than 30cm^2 per mouse, which was acceptable only for short term housing of recently weaned animals.

(vii) The Cambridge cage, one of the few examples of an environment designed to allow mice to compartmentalise their living area, measured 27cmx22cm, yielding a total floor area of 594cm^2 [76].

(viii) Optimal cage floor area and housing density will facilitate normal behaviour and support physiologically normal mice, but it is impossible to determine exactly what that will be based on the current literature alone. Given significant strain variation, a single set of recommendations is unlikely to be appropriate[79].

(ix) Factors other than floor area may influence how mice use floor space – for example, brightly lit open space is more likely to be avoided[95].

(x) The recommendations below represent a best estimation, based on the scientific literature on minimum cage size (see Table 1), as well as literature on the needs of mice. The recommendations are consistent with the dimensions of common commercially available mouse cages in Australia at the time of publication.

Recommendations

2.2.1 As a guide, enclosures should allow for a minimum floor area of 250cm^2 for a single housed mouse, a minimum floor area of 500cm^2 for two mice and ensuring a minimum floor area of 60cm^2 per additional adult mouse when mice are housed in larger groups.

2.2.2 As a guide, a breeding pair or female with pups requires a minimum total cage floor area of 500cm^2, with an additional 100cm^2 for each additional adult female.

2.2.3 To reduce anxiety and aggression, larger cages should be designed in such a way as to avoid large open spaces.

2.2.4 Because of the wide variation in conclusions drawn from studies designed to determine optimum cage floor area, it is necessary for researchers to assess whether a particular strain is coping with a particular living area. Parameters assessed may include tendency to perform normal behaviours, aggressive encounters or fight wounds, weight changes, incidence of illness, reproductive performance, use of space, use of enrichment and amount of thigmotaxis observed.

2.3 Cage Height and Cage Lid

Principles

(i) Normal behaviours of mice include standing on their hind limbs and stretching, sitting on their haunches and grooming, and climbing. Where cage design permits, climbing is a regular component of locomotor activity. Buttner[96] found that mice invested more time in climbing on the cage lid than locomotion on the ground. Climbing onto an in-cage shelter is also an important locomotor activity in mice[97, 98].

Figure 2.3.1 Traditional wire-topped cages facilitate climbing behaviour in mice.

(ii) Evidence suggests that climbing on the cage lid is important for mice, as it may be a positive natural behaviour associated with exploration of the home environment. However, this is controversial, as one survey found a correlation between increased levels of stereotypy and increased levels of climbing[94]. The authors noted that this is unsurprising as many documented mouse stereotypies, such as gnawing, circling and somersaulting, tend to occur at or on the bars of the cage lid. They conclude that high levels of climbing may form an integral part of certain stereotypies. In the same study, climbing was associated with increased physical injuries, yet it was also associated with a reduction in the incidence of obesity. An earlier study found that wire-gnawing and jumping (both stereotypies) developed from climbing behaviours[99]. The same authors found that preventing stereotypic wire-gnawing had no significant effects on chronic measures of stress, therefore they concluded that this behaviour did not reduce stress[100]. However, another study found that C57BL/6 mice that were prevented from climbing on bars (those housed in a cage with a plexiglass lid) from age three to seven weeks exhibited altered fear responses and impaired fear-motivated associative learning in behavioural tests compared with controls[101]. Females were particularly sensitive to thwarting of lid-climbing behaviour, demonstrating increasing anxiety levels in an elevated plus maze, hyperactivity in an open field, reduced condition freezing and reduced prepulse inhibition. The authors described this as a "complex syndrome of anxiety and psychotic-like symptoms." Therefore, based on current knowledge, cage height should not be increased to such an extent that it prevents access to bars from which mice frequently hang, as this would thwart normal behaviour[4]. Male and female mice were able to reach and climb on bars that were 19cm off the cage floor[101].

(iii) Mice housed in individually ventilated cages have limited climbing opportunities compared with mice housed in conventional wire grid-topped cages. While the provision of a wire grid in individually ventilated cages did not appear to compensate for housing effects on spontaneous behaviour, sensorimotor behaviour and fear learning, it did improve response in fear-potentiated startle tests in singly housed B6J males[102].

(iv) Where mice are provided with a food-hopper built into the cage lid, they may utilise this to nest beneath.

(v) Information on the height requirements of mouse caging is scarce and many recommendations are based on available products. According to Article A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123)[1], the minimum height of cages in which mice are housed should be 12cm. This is slightly lower than the US National Research Council Guidelines for the Use of Laboratory Animals, which stipulate that cages have a minimum height of 12.7cm[73].

Recommendations

2.3.1 The height of cages should allow mice to stand on their hind legs, stretch up fully and climb on the bars of the cage lid. This height does not need to be provided over the entire area of the cage.

2.3.2 The cage lid should incorporate a grid section which will allow the animals to climb. The cage height should allow for provision of enrichment.

2.3.3 Where cages are fitted with platforms or in-cage shelters, the distance between the top of the platform or in-cage shelter and the top of the cage should be sufficient to allow mice to climb on top of the platform or in-cage shelter.

2.3.4 While cage height (over part of the cage) should allow for upright standing behaviour, food and water should be accessible at a level that allows mice to sit while eating and drinking.

2.3.5 Until further evidence relating to the height of the cage becomes available, it is recommended that mouse cages are a minimum of 12cm high.

2.3.6 The design of the cage lid should facilitate climbing.

2.4 Cage shape

Principles

(i) To date there are no studies investigating the impact of cage shape alone in mice. For example, in one study mice preferred the more square-shaped enclosure but this differed from the other enclosures in its height, opacity and the presence of a shelter[103]. In another study, mice of different strains housed in the square-shaped Cambridge cage produced more young per female than their standard-housed counterparts[104]. It should be noted, however, that aside from a different cage-shape, the Cambridge cage incorporates other forms of environmental enrichment including shelter material which may be perceived to be more important to mice than the shape of the cage per se.

(ii) Mice exhibit thigmotaxis and may spend much of their time in contact with the wall of a cage. Thigmotaxis is used as a measure of emotionality (anxiety and fear) in mice, and some strains are more thigmotactic than others[105]. This should be taken into account when selecting cage shape.

Recommendations

2.4.1 There is no clear evidence of preference among mice for a particular cage shape. Evidence indicates the contents of the cage is more important than cage shape.

2.4.2 Until further evidence comes to light the use of rectangular or square shaped cages is appropriate for mice.

2.5 Cage materials

Principles

(i) Cages must be constructed from non-toxic, non-absorbable material that can be cleaned (autoclaved). They must be escape and predator proof.

(ii) Ideally, caging material should be resistant to heat and chemicals, inexpensive and durable[106]. Most mouse cages today are solid tubs made from plastics such as polypropylene (opaque), polycarbonate, polysulphone and polyetherimide (transparent). Other cage materials include polystyrene and polyphenylsulfone. Wood is not a suitable material as rodents tend to chew it. Unless coated with an impervious finish, wood also tends to soak up urine and is extremely difficult to clean.

(iii) Recent studies have shown that some synthetic materials release bioactive substances that may affect mice. For example, high temperature polycarbonate (polyphthalate carbonate) cages and water bottles damaged by one-off washing in a harsh alkaline quarternary ammonium detergent released bisphenol A (BPA), an oestrogenic compound that led to increases in meiotic disturbances, including an 8.3 fold increase in aneuploidy and a 20-fold increase in chromosome misalignment in mice [106, 107]. In addition, there was an increased frequency of mortality in young (one- to four-month-old) mice during the period of maximal exposure. In the months following exposure, investigators noted an increase in reproductive tract tumours in exposed mice. In this report the detergent caused visible, progressive damage to the cages including change in colour from yellow, becoming initially slightly crazed, before turning opaque, then whitish and rough, and finally sticky and bubbly[106]. Water bottles were slower to deteriorate, possibly due a protective effect of water. However, visible damage is not an accurate indicator of the amount of BPA leaching from exposed materials[107].

(iv) Laboratory mice housed in polycarbonate and polysulfone cages are exposed to BPA via leaching, with exposure levels highest in older cages[108]. Bisphenol A hydrolyses and leaches from polycarbonate products under heat and alkaline conditions, with the amount of leaching increasing with use. Significant levels of BPA (up to 310µg/L) were leached from used polycarbonate cages placed in water (neutral pH) at room temperature[108]. In addition, detectable levels of BPA were released from new polycarbonate cages (up to 0.3µg /L) as well as new polysulphone cages (1.5 µg/L), while no BPA was detected in water incubated in glass or used polypropylene cages. Pre-pubescent female CD-1 mice subjected to BPA by being housed in polycarbonate cages had a 16 per cent increase in uterine weight compared with mice housed in used polypropylene cages, although the difference was not statistically significant.

(v) While normal care and use of some synthetic cages can result in leaching of BPA, exposure to a basic detergent, continued use of cages and/or water bottles beyond the manufacturer's recommended shelf life or high-concentration of corrosion-controlling amines in autoclave steam are events which may exacerbate cage and/or water bottle damage and

increase the risk of BPA leaching[106]. Cages may also be damaged by banging the plastic against a hard surface (for example when removing soiled, stuck bedding); over-stacking (mouse cages stacked more than 15 high); washing in hard as opposed to soft water; heating or autoclaving cages that contain debris or disinfectant residue; and use of amine corrosion inhibitors in steam sterilisation systems[109].

(vi) Cage materials can affect the microclimate by modifying light and heat exchange. Opaque cages have the advantage of filtering out harmful glare and allowing mice to hide from humans. They have the disadvantages of impeding the observation of mice from outside the cage (thereby necessitating more disruption to check mice), restricting mice's vision of activities outside the cage (including humans and other mice), and blocking the passage of light, resulting in different light levels in boxes at different levels on cage racks. Transparent cages have the advantage of allowing observation of mice from outside the cage. They have the disadvantage of not allowing mice to hide as effectively from humans and high light intensities. Heat is well preserved in solid plastic tubs, such as polypropylene and polycarbonate.

(vii) A change in cage materials may affect the breeding performance of mice. For example, the number of young weaned by CBA does transferred from opaque to transparent cages was lower[110]. These changes may be transient, as the number slightly increased in the second generation. In another study, inbred BALB/cW, DBA/2W, RIII/W, C3H/A, C57BL/W, BN/a and BN/b mice transferred from wooden to plastic cages showed a decrease in productivity for one to two years, followed by a gradual increase[111]. Q values (number of young weaned/prenatal days x 100) for most inbred strains were in fact higher at the end of the study period. For this reason it is important to be consistent in the cage materials used throughout a study.

(viii) Cage materials may impact on mouse body composition. For example, male mice transferred from aluminium to other identical metal cages had a body fat percentage of 21.8 per cent at fourteen weeks of age, compared to 13.6 per cent in male siblings transferred into polypropylene cages[112]. However, the study confounded cage type with cage volume, light penetrance and the presence of aluminium sulphate, each of which independently varied body fat to some extent. In another study, male C3H/HeJ mice housed in polycarbonate cages showed a consistent trend to higher body weights than those kept in stainless-steel wire mesh cages[113]. This may have been due to variation in temperature between the cages.

(ix) Behaviour and physiology of mice may be affected by cage colour. Female CBA mice consistently showed a significant preference for white cages over black, green and red cages[114]. The colour of the home cage strongly influenced behaviour, with mice from white home cages having the highest food consumption, lowest body weight and least anxiety (as evaluated in the Elevated Plus Maze test) than those originally housed in black, green and red cages. The colour of the cage that the animals were

born in may have influenced their later behaviour (C Sherwin pers. comm.).

Recommendations

2.5.1 Cages should be constructed from non-toxic, non-absorbable material that is easy to clean. Untreated wooden cages should not be used.

2.5.2 Cages should be durable, resistant to heat and chemicals, and escape and predator proof.

2.5.3 Worn or damaged cages and/or water containers should be replaced.

2.5.4 Leaching of bisphenol A from polycarbonate and polysulphone cages and water containers is likely if these are washed with strongly alkaline detergents or sterilised in the presence of high concentrations of corrosion inhibiting amines in autoclave steam. Exposure of mice to bisphenol A (even at low levels) should be avoided, particularly in reproductive studies.

2.5.5 Colourless, tinted transparent cages or white opaque cages are preferable for mice. Unless required for a study, cage colour should be consistent throughout the facility.

2.5.6 Cages should be handled and maintained to minimise damage. For example, cages should not be hit or banged against hard surfaces or stacked more than 15 cages high. Plastic cages and bottles should be washed in hot (60-66°C), soft water with a manufacturer-recommended detergent solution. All residue must be removed prior to autoclaving as this may be baked onto the cage except where sterilisation is required to ensure decontamination of waste and prevent zoonosis.

2.6 Cage flooring

Principles

(i) When given a choice between bedding material on a solid floor and a wire mesh floor, mice preferred the former[115], however preference was affected by ambient temperature[75] (see Section 4.3 Temperature). Similarly, when provided with synthetic gauze pads, group-housed male and female B6C3F1 and individually-housed male CD-1 mice in stainless-steel ventilated cages with wire mesh floors preferred to rest on the pads[116 117].

(ii) Housing mice on wire mesh floors can be detrimental to their health and well-being. In a 2 year feeding study, significantly fewer BC63F1 mice housed on wire mesh floors survived to the end of the study compared with those housed in solid floored polycarbonate cages, irrespective of diet, sex and whether they were individually housed[118].

(iii) While female B6C3F1 mice housed in suspended wire cages with a flooring grid of 2mm round intersecting stainless steel wires with mesh gaps measuring 8mm x 8mm did not show cage associated differences in clinical signs, body temperature, grasping power of fore and hind-limbs, tail flick latency or motor nerve conduction velocity than their solid-floor

housed counterparts, they exhibited a significant decrease in body weight and serum triglycerides than their solid-floor housed counterparts[119].

(iv) Housing mice on wire mesh floors is associated with mouse urological syndrome (MUS), a potentially fatal inflammatory condition of the urinary tract. In one study, all entire male AKR/NCrIBR mice housed in suspended wire cages or raised wire floors for a period of sixteen weeks developed MUS, compared with none of their solid-floor housed counterparts[120]. While the researchers did note that this strain was highly susceptible to MUS, they found that MUS occurred in B6C3FI/CRlBR and NIH Swiss strains, albeit at a lower incidence (6 per cent and 21 per cent respectively) housed in suspended wire cages. None of the mice kept on a solid floor with bedding got MUS.

(v) Neonatal pups may slip through large-spaced mesh (1cm x 1cm)[121].

(vi) Because wire mesh floors are open they allow dissipation of heat from the bodies of mice and may thus influence thermoregulation. When given a choice, mice housed on a wire mesh floor chose an ambient temperature of 28°C[75]. Thus the cage temperature for mice housed on a wire mesh floor may need to be higher than for mice housed on a solid floor (see Section 4.3 Temperature).

Recommendations

2.6.1 Solid floors are recommended for mouse caging.

2.6.2 Wire mesh floors should not be used for mouse caging without express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such flooring. In such cases, a solid floor section sufficient to accommodate all of the mice and nesting material should be provided. The size of the mesh gaps should not exceed 8mm x 8mm (See also 3.3 Metabolism Cages).

2.7 Bedding

Principles

(i) Mice have a behavioural need to burrow and are highly motivated to do so [122,123]. Burrowing behaviour persists even in the presence of a previously built system of burrows or shelters[123-125]. Furthermore, the number of burrowing bouts increased, rather than decreased, as burrows were constructed[123]. Young male TO mice preferred to sleep in sawdust than make use of a selection of pre-fabricated shelters (tubes) to sleep in[125].

(ii) Deep bedding provides opportunities for digging and burrowing behaviours and mice spend a significant amount of time digging in such bedding when provided[126]. When digging, mice tend to alternate between digging with their forepaws and kicking back with their hindpaws[32].

(iii) Bedding material may allow mice to perform selective soiling behaviour. Male TO mice preferred to defaecate on floors covered with sawdust bedding than on a bare plastic floor[74].

(iv) Ideally bedding should be non-toxic, free of dust, microbial, parasitic or chemical contaminants, atraumatic, moisture absorbent and ammonia-binding[127]. In addition it is desirable for bedding to be inexpensive, readily available, easy to store, easy to use and easy to dispose of[11].

(v) There is evidence that the size and manipulability of bedding material are the main determinants of selection by mice. When given a choice between ten commercially available bedding products, pregnant ARS Ha (ICR) Swiss mice most commonly chose a combination of materials, with products of wood origin overwhelmingly preferred[128]. When offered a choice, male ICR mice chose soft bedding that allowed them to hide and build nests[129]. In another study, male ICR mice preferred cloth bedding over recycled paper, wood shavings and paper[130]. Female C57BL/6JIco and BALB/cBYJIco mice prefer bedding consisting of relatively large, rough, fibrous particles over sawdust[115].

(vi) Pregnant CF-1 mice preferred sawdust bedding over commercial, deodorised cellulose[131].

(vii) Bedding may impact on aggression. Lawton *et al* found that adult MF1 Nu-Nu males housed on thick corn cob bedding were less aggressive than those housed on fine grade corn cob, hemp, sawdust or aspen chip[132]. Aspen chip appeared to increase aggression in this strain.

(viii) Small bedding particles (<300µm) may irritate or damage the airways of mice[127]. For example, vermiculite bedding was reported to cause histological changes in the lungs of mice, reduced body weight and lead to fewer litters[133]. Small particles may also irritate and traumatise the vaginal or preputial mucosa[115].

(ix) Variations in absorbency of bedding can affect in-cage humidity, temperature and ammonia levels via concentration of urease-producing bacteria which convert urea into ammonia. For example, relative humidity in cages containing male NOD/LrJ mice varied significantly depending on the type of bedding used[134]. This is a potential source of experimental variability. A comparative study of absorbency of six commonly used bedding materials found that a product derived from corn cob had higher absorbency per unit volume than one made from wood pulp[461].

(x) Some bedding materials contain chemical compounds which can impact on mouse physiology and the response of mice to pharmacological agents. These compounds can enter the experimental model via direct ingestion of bedding, inhalation of volatiles or inhalation of dust particles[135]. Bedding made from hard and softwoods contains organic compounds such as tannins, alkaloids, lignins and resins that may impact on experimental results and even constitute a health hazard to mice and those working with

them[135]. For example, some softwood products derived from Red cedar (*Juniperus virginiana*), Ponderosa pine (*Pinus ponderosa*), White pine (*Pinus strobus*), Scots pine (*Pinus silvestris*) and Douglas fir (*Pseudotsuga sp.*) induced changes in hepatic enzymes involved in drug metabolism in both mice and rats [136-142] resulting in altered drug metabolism and increasing the incidence of spontaneous tumours[139]. Some of these also altered barbiturate-induced sleep times[139, 140, 142-144] and/or demonstrated cytotoxicity[145]. In addition, the liver-to-body-weight ratio of mice exposed to red cedar bedding was significantly increased compared to mice exposed to the other beddings[140]. It should be noted that not all strains were affected to the same extent[142]. Industrial-derived wood contains anti-fungal and insecticidal agents which are also potentially toxic[145].

(xi) Pelkonen and Hanninen[146] examined the cytotoxic and enzyme-inducing effects of a variety of types of bedding from different parts of the world, finding a 200-fold variability in the hepatocyte toxicity of commonly used bedding materials. Pine shavings were generally found to be highly cytotoxic (although the least cytotoxic of these was from Australia) (see also [145]). Extracts of corn-cob, rice hulls and straw were found to be minimally toxic. Corn-cob extracts were practically devoid of inducers, whereas straw, rice-hulls and sugar cane based beddings had enzyme inducer activity comparable to the hardwoods. The authors recommend avoidance of softwood bedding, concluding that hardwoods were less problematic than softwoods, and grass-based bedding was better still.

(xii) Where wood-derived bedding is utilised, investigators should be familiar with the species of tree from which it is sourced and the manufacturing process, as well as its potential impact on the biological system and experimental outcomes[135]. This requires knowledge about naturally occurring compounds present in the bedding that may impact on mouse physiology as well as likelihood of treatment-induced compounds in the bedding that may impact on mouse physiology.

(xiii) Paper typically has a low cytotoxicity and inducer activity[141, 145]. Recycled or bleached paper products including paper towel had a higher cytotoxicity[145, 147] and enzyme inducing activity[147] than unbleached pulp. Analysis of telephone book strips found that while this bedding was minimally cytotoxic compared to other wood-derived bedding materials, it had quite high enzyme-inducer activity (comparable to pine)[146]. The latter may have been due to the presence of ink or the use of polyhalogenated compounds during manufacture.

(xiv) Treatment of bedding may alter properties of bedding including toxicity. Autoclaving bedding material did not alter barbiturate-induced sleep times or liver:body weight ratios[140] nor did it appear to impact on the enzyme-induction properties of bedding[145]. In fact, potentially toxic compounds could form during treatment (eg heat treating or steam sterilisation) of bedding[135].

Recommendations

2.7.1 Bedding should be provided in mouse cages and should be present in sufficient quantity to cover the entire floor. The depth of bedding required will vary with the type of bedding used, the number of mice in the cage and frequency of cleaning. Ideally mice should be able to dig, if not burrow. As a guide, the depth of the bedding should be a minimum of 2cm.

2.7.2 Bedding should produce a minimal amount of dust and consist of particles that lend themselves to manipulation by mice.

2.7.3 To reduce experimental variability, particularly where pharmacological experiments are concerned, the use of a single type of bedding is recommended.

2.7.4 Autoclaving of bedding is recommended to reduce the potential for microbial contamination. It should be ensured (for example by consulting the manufacturer) that toxic compounds are not formed during treatment of bedding.

2.7.5 Softwood-derived bedding should be avoided. Paper, grass-based or hardwood material should be utilised instead.

2.7.6 Vermiculite bedding or other bedding with small particles should not be used due to the potential for irritation of the mucosal membranes and other health problems.

2.8 Nesting material

Principles

(i) Mice of most strains, whether wild or captive, build nests when materials are available[148]. Nesting behaviour has been observed in young and old, male and female, pregnant and non-pregnant mice (see [149] and [150] for reviews of nest building by mice of different gender, reproductive status and strain), and is thus not exclusively relevant to adult females or dependent on pregnancy.

(ii) Mice are highly motivated to build nests and if nests are removed daily they will repeatedly rebuild them[149]. Mice will work (by pressing a lever) for access to nesting material[151-154] and will endure an aversive experience such as traversing shallow water[155, 156] or living on grid floor[98] for access to nesting material.

(iii) The provision of nesting material enables mice to control their microenvironment[148, 149] (see also section 4.3 on Temperature), avoid aggressive cohabitants[149, 157, 158] and shelter from light or external disturbance[149, 159].

Figure 2.8.1 Mice are highly motivated to build nests. This single-housed female mouse has shred--ded tissue to create a nest.

(iv) The use of nesting material reduces pre-weaning mortality and enhances the number of litters in both mice[160] and rats[463].

(v) Mice provided with nesting material spent 10 to 20 per cent of their time (day and night) manipulating that nesting material[157]. Mice provided with a cellulose nestlet spent significantly more time interacting with this form of enrichment than did mice provided with other enrichment items (a marble and a split polyvinyl chloride pipe)[126]. Materials such as this encourage activity as they require mice to tease them apart in order to build them into a nest[161]. Similarly, mice provided with gauze pads spent time pulling at the gauze threads[116].

(vi) Provision of nesting material influences stress related parameters. In one study, male BALB/c and CD-1 mice provided with nesting material in the form of two tissues, which were transferred to a new cage during cleaning, had lower urine cortisone levels and heavier thymuses than controls[162].

(vii) Provision of nesting material resulted in reduced food consumption yet equal or higher weight gain than controls[116, 157, 162, 163]. Possible explanations for this trend include that a) nesting material enables mice to better thermoregulate, reducing metabolic demand for food and water[157, 162]; b) mice consume more food and water in the absence of nesting material due to a boredom effect[164] and/or c) mice housed without nesting material exhibit a stress effect[162].

(viii) As with bedding the size and manipulability/structure of nesting material may be a stronger determinant of preference than the material itself[159]. For example, mice preferred cages containing tissues or paper towels over

those containing shredded paper, and cages with cotton or wood wool were preferred over wood shavings[159].

(ix) When given a choice mice show a strong preference for nesting material over rigid structures such as commercially available nesting boxes[98, 149, 165, 166].

(x) When offered a selection of nesting and/or bedding materials mice tend to use a combination to construct their nests[128, 149, 159, 167]. Quality and quantity of nesting material interact to determine nest quality and nesting material preferences. C57BL/6J mice provided with shredded paper built better nests than cohorts supplied with tissue paper alone or cotton squares alone. When both tissue paper and shredded paper were provided, the tissue was used to line the nests indicating that mice will select from available materials for different purposes[462].

(xi) The provision of nesting material in male mice may reduce aggression in the short term by allowing subordinate males to avoid their cohabitants. Group-housed male BALB/c mice provided with corn-husk nesting material had significantly fewer wounds than controls four days after the nesting material was introduced, but the difference between the groups was not significant on day seven[158]. Aggression in group-housed male BALB/c and CD-1 mice was reduced if nesting material (tissues) was transferred at cage cleaning[28, 162]. Furthermore, male BALB/c and CD-1 mice housed in cages enriched with nesting material (tissues) had lower urinary corticosterone levels than standard-housed mice[168].

(xii) Nesting material appeared to increase aggression in highly aggressive male NIH/S mice[169] however this was completely replaced (and not transferred) during cage cleaning. The mice were housed in cages 42cm (l) x 25cm (w) x 15cm (h) with 4 mice per cage. The authors found that provision of an in-cage shelter (a tube or box) in conjunction with nesting material prevented fighting.

(xiii) Nesting material should be carefully selected as it has the potential to act as a foreign body or irritant. For example, female athymic nude Cby.Cg-*Foxn* mice provided with a commercial cotton nesting material invariably developed conjunctivitis, with fragments of the material found trapped in the conjunctival sac[170]. The authors suggest that these mice were predisposed to conjunctival foreign bodies due to the strain's absence of eyelashes. In another study, post-mortem examination of the gastrointestinal tracts of mice provided with gauze pads found no evidence of foreign material or lesions referrable to the pads[116] suggesting that these are safe to use in mice.

(xiv) The provision of nesting material is not likely to jeopardise experimental outcomes[150].

Recommendations

2.8.1 All mice including males should be provided with nesting material in addition to bedding material.

2.8.2 Nesting material should be non-toxic, non-irritant, atraumatic, loose, manipulable and light enough to be carried. Suitable materials include shredded paper with non-toxic ink and tissues.

2.8.3 To minimise aggression, at least some nesting material should be transferred during cage cleaning.

2.8.4 Depending on the strain of mice used, nesting material may be placed on top of the cage to allow mice to pull the material through the bars.

2.9 In-cage shelters

Principles

(i) For the purposes of these guidelines, the term shelter refers to a rigid structure within the cage, also known as an in-cage shelter or nest box.

(ii) In the wild, mice live in complex burrows consisting of tunnels and nest chambers in which they build their nests[122]. Mice reared for generations in a laboratory will build tunnels within hours of being given a suitable substrate[123]. When given a choice mice prefer cages containing an in-cage shelter and avoid cages without one [97].

(iii) The provision of a shelter may improve mouse welfare. In a study across 46 facilities across the UK, the frequency of stereotypic behaviour was lower in cages containing a shelter[94].

(iv) Female BALB/c mice provided with a shelter and nesting material had a comparatively lower food and water intake than those housed in standard or nest-only enriched conditions[163]. Both BALB/c and C57BL female mice enriched with this combination of an in-cage shelter and nesting material were more active, spent less time eating and drinking and weighed less than control and nest-material only groups. They had the highest urinary corticosterone/creatinine ratio of all groups, possibly due to greater level of physical activity. The provision of shelters with nesting material, or nesting material alone, had no effect on organ weights, adrenal histology, lymphocyte proliferation or plasma corticosterone concentration[163]. Male ICR mice provided with a red-tinted polycarbonate hemisphere (Mouse Igloo) showed no change in food intake or weight gain when compared with controls[171].

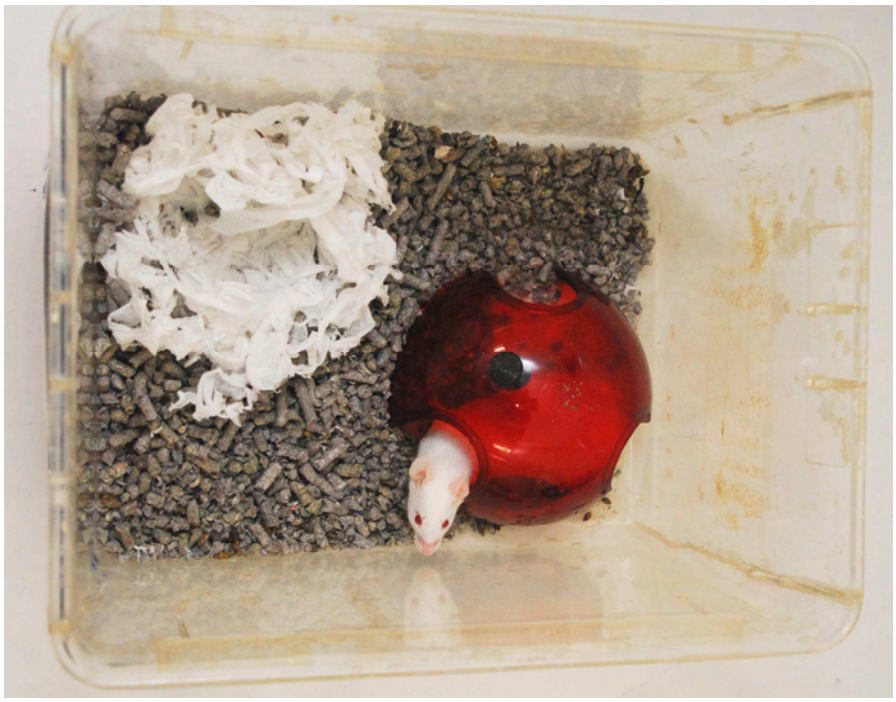

Figure 2.9.1: Mice make use of in-cage shelters. The model in this picture is an igloo-style shelter.

(v) When shelters were provided in combination with cage-dividers, male CFLP mice displayed an increase in aggression and reduced resistance to experimental infection with *Babesia microti* when compared with controls[172]. However, resistance to infection increased as time spent in the shelters increased, suggesting that the shelters offset the damaging effects of aggression on immunocompetence by providing refuge from attacks.

(vi) When given a choice between different shelters, mice preferred small, angular structures over larger, circular shelters[173]. They preferred shelters made of grid metal over clear or white perspex boxes or no box, and boxes made out of perforated metal over those made from grey PVC or sheet metal[97]. Grid or perforated shelters may be preferred because they allow for passage of olfactory cues[97]. When offered a shelter with one open side or two, the majority of mice preferred a box with one open side and typically slept with their head towards that side.

(vii) Given the opportunity, mice will make use of space in the vertical as well as horizontal plane. In one study, wild-caught mice spent a significant amount of time sitting on top of a shelter[47]. Climbing onto a shelter may be an important component of locomotor activity in mice[97]. Slippery or steep-sloped shelters may prevent mice from using additional space by climbing onto the roof of the shelter. Shelters that facilitate climbing may be preferred.

(viii) Mice may retreat into shelters when disturbed or threatened[97], however provision of shelters does not complicate catching or handling of mice.

Male FVB (inbred) and NMRI (outbred) were no more difficult to catch or handle when provided with two 15cm long, 5cm diameter PVC tubes than those in standard cages[174]. Furthermore, the tubes facilitated catching of NMRI mice, evidenced by reduced catching times, and did not significantly affect food or water intake in either strain.

(ix) Mice are highly motivated to nest even when a shelter is provided. When nesting material, for example tissues, is available mice will drag it into a shelter to build elaborate nests[166]. The performance of nesting behaviour may be just as important as its outcome, therefore mice should be provided with nesting material in addition to in-cage shelters[156].

(x) The use and benefits of shelters may vary significantly between strains, and may be gender dependent.

(xi) In summary, in-cage shelters have multiple functions and can be used for a variety of activities that are part of the natural behavioural repertoire of the mouse:

- They provide a microclimate which may aid in thermoregulation (temperature and relatively humidity are usually higher within the shelter);
- They facilitate the use of nesting material;
- They allow withdrawal from light;
- They provide a means of escape from aggressive social interactions;
- They better satisfy the thigmotactic (wall-hugging) aspects of mouse behaviour than a single large cage;
- They may expand functional living area by providing a structure that mice can climb and interact with - Disposable cardboard shelters also provide an opportunity for mice to chew.

Recommendations

2.9.1 Mice should be provided with an in-cage shelter within their cage. Shelters should be provided in addition to, not as a substitute for, nesting material.

2.9.2 In-cage shelters should have solid or grid sides with more than one exit to allow subordinate animals to escape entrapment by dominant individuals and a non-slippery roof that allows withdrawal from light (and from other mice) and should be constructed so that mice can climb onto the roof.

2.9.3 Where in-cage shelters are made of chewable material such as paper or cardboard, it should be ensured the material is non-toxic to mice nor prone to cause gastrointestinal obstructions.

2.9.4 There should be enough space between the roof of the shelter and the cage lid to allow for mice climbing onto the roof of the shelter.

2.10 Cage dividers

Principles

(i) Cage dividers including in-cage mazes can be used to increase environmental complexity. The use of vertical and horizontal in-cage dividers may increase living area but also be more reflective of the natural environment, facilitate increased exercise/activity, and reduce perceived animal density[175].

(ii) Cage dividers may reduce stress and emotionality in mice. Male and female CLFP mice reared in complex cages (with multiple vertical dividers and a horizontal platform) gained more weight, demonstrated increased activity levels, defaecated less, performed better in box emergence and open field tests, and had smaller adrenal weights, than those kept in simple cages with no dividers[175]. In addition, regardless of complexity of home cage, all mice demonstrated a strong preference for more complex cages when given the choice.

(iii) Male BALB/c mice provided with a cage divider comprised of two platforms and an in-built shelter exhibited a significantly higher frequency of exploratory behaviour and significantly lower frequencies of bar gnawing, wood gnawing and drinking compared to counterparts in standard laboratory cages[176].

(iv) Increased cage-complexity has been associated with increased aggression among male mice. Male CFLP mice housed in cages furnished with shelves and in-cage shelters (nest boxes) displayed increased aggression and reduced resistance to experimental infection with *Babesia microti*[172]. Male DBA/2J mice housed in cages furnished with a platform based on vertical dividers showed a marked increase in territorial aggression, with increased aggression toward intruders, a less stable dominance hierarchy and higher plasma cortisone levels than controls[177]. Both DBA/2J and CBA/J male mice showed an increase in aggression and delayed body weight gain when housed in cages furnished with a horizontal labyrinth[178]. Attack frequencies of males housed in these cages progressively increased during the course of the study. Strain differences were reported in the way in which the presence of in-cage dividers affected social organisation and endocrine parameters. Contrasting study results may reflect strain differences, as some studies employed highly aggressive strains while others did not[179]. Furthermore, properties of cage dividers/labyrinths/mazes, including the number of openings in walls and their interaction with cage size and remaining floor space, may trigger aggression in mice.

(v) In one study examining the impact of structural complexity of territory in captive-born, wild male house mice, almost all encounters with intruders ended because the resident lost track of the intruder[19]. In another study comparing aggressive behaviour in environments enriched with dividers, the number of bite wounds was 45 times higher in inbred male HLG/Zte mice in cages with a closed passageway which limited escape than those housed with parallel open corridors or controls with no dividers[180].

(vi) Although the differences between structures can be subtle, territorial aggression may be more of a problem when in-cage dividers or mazes/labyrinths are used than when in-cage shelters are provided. Thus time spent in shelters offset negative effects of co-habiting in a complex environment[172] (see Section 2.9 In-Cage Shelters).

Recommendations

2.10.1 Cage-dividers, labyrinths and mazes should not be used in the housing of aggressive strains, particularly for male mice.

2.10.2 Cage dividers, if used, should be arranged in a way that provides an escape-route from other mice.

2.10.3 Where cage dividers, labyrinths and mazes are used, there must be sufficient space in the cage to accommodate them and mice should be monitored for fight wounds and/or aggressive behaviour, as this will impact on the welfare of the mice in addition to being a source of experimental variability.

3.0 Mouse care and management

3.1 The social environment

Principles

(i) Mice are social animals and should, wherever possible, be maintained in stable, harmonious social groups[181]. Mice have a strong preference for each other's company. When given a preference, male BALB/cAnNCrlBR mice preferred each other's company to individual housing, irrespective of social status or kinship[182, 183].

(ii) Strains may differ in their degree of social affiliation. For example, one study found that DBA mice were more likely to stay close to cage-mates than C57 mice[184]. This impacted on behaviour in open field tests: C57 mice exhibited increased exploratory behaviour when alone, whereas DBA mice showed increased exploratory behaviour when in groups.

(iii) Aggression between male mice is a well-recognised problem in laboratories world wide[185] . Aggressive behaviour may be due to offensive, defensive or predatory motivation, competition for resources, or a mixture of these[17]. Aggression levels vary markedly with strain. For example, outbred Swiss CD-1 mice exhibited higher levels of inter-male aggression, inter-female aggression, maternal aggression and infanticide than other strains[186]. Environmental and husbandry factors may exacerbate agression[185] (see especially Sections 2.10 Cage Dividers, 3.5 Environmental Enrichment, 4.7 Cleaning).

(iv) Isolation may exacerbate aggression. Individual housing of male mice followed by group housing reliably induces aggression in many strains[33, 63, 187]. For example, individually-housed male DD/S mice changed to group-housing showed an increased tendency to fight, when compared with their permanently group-housed counterparts[188].

(v) Pair-housing of male mice is not recommended, as the subordinate mouse may be frequently exposed to attacks and suffer subsequent stress and injuries[181].

(vi) Behaviour exhibited by dominant mice includes attacking, tail rattling, chasing, biting and adopting a side-on offensive posture[181]. Conversely, subordinate mice exhibit behaviours such as flight, hiding and freezing. Subordinate mice do not initiate attacks. Where conflict leads to injury, it may be necessary to remove the dominant mouse[181]. Identification and removal of dominant male Crl:CD-1 mice lead to a 57 per cent reduction in the number of mice reported for clinical signs, euthanasia and death[181]. When removed dominant mice should be housed in another room as their urine may stimulate aggressive behaviour in dominants housed in the same room[181]. Nesting material should be supplied as this may partly compensate for deprivation of social contact[165].

(vii) For males unable to be housed with other male mice, ovarectomised females may be suitable companions as they do not induce male behavioural change through copulation or courting behaviour[189] and production of unwanted progeny can be prevented[190]. However, the welfare of the male must be weighed against the welfare of the female, who must undergo major abdominal surgery.

(viii) Female mice may exhibit maternal aggression, attacking both defensively and offensively[17]. Interfemale aggression may also be stimulated by male urinary odour. For example, virgin Swiss albino females individually housed for 24 hours in a cage previously inhabited by a male showed increased levels of attack and mounting of same-sex intruders[17].

(ix) Previous social experiences influence aggressive behaviour. Thus Swiss mice that had repeatedly defeated conspecifics showed increased offensive aggression towards intruders than those without positive fighting experience[191].

(x) There is evidence that keeping siblings together may reduce aggression and improve well-being. There were no physiological or behavioural differences detected in dominant or subordinate male Swiss CD-1 mice grouped in same-sex sibling groups from birth[192]. The authors argue that what is stressful for the mice is not group housing in itself, but a lack of familiarity or relatedness with respect to cage-mates. Therefore group-housing of same-sex siblings from birth may be used to reduce the risk of detrimental aggression between mice. Male and female mice from litters that had been combined with other litters experienced a marked decrease in weight gain in comparison to undisturbed litters, regardless of litter size[193].

(xi) The age at which mice are grouped may impact on behaviour and physiology. For example, 26 to 28 day old male Swiss CD-1 mice mixed into groups of five to six animals from different litters exhibited higher levels of aggression, smaller preputial glands and marked reduction of

neophobia in a free exploratory paradigm than controls which remained in same-sex littermate groups since weaning, and those grouped after puberty[194].

(xii) Brief periods of isolation, such as those that may occur during husbandry or experimental procedures, may not alter dominant/subordinate relationships. For example, dominant/subordinate relationships between pair-housed Swiss male mice remained unchanged following individual housing for periods of 6 to 12 hours[191].

(xiii) Optimal population density depends on a number of factors including strain, age, gender, experimental duration, genotype, degree of inbreeding, previous social experience of mice, familiarity of mice with one another, experimental procedures and the order in which animals are tested[69]. Male BALB/c Crl mice housed in a stainless steel cage with a floor area of 390cm^2 had significantly elevated plasma corticosterone levels and decreased initial peripheral lymphocyte count when housed in pairs or groups of eight, compared to those housed in groups of 4[195].

(xiv) The level of aggression between male mice can be influenced substantially by group size, and cage size with aggression increasing with group size and a cage size allowing 125cm^2 per animal [92]. Aggressive behaviour in group-housed male BALB/c mice was best prevented by housing the animals in groups of three to five with a cage size allowing 80cm^2 per animal.

(xv) Social structure in female laboratory mice depends on the number of animals per cage. Dominant female mice had significantly lower corticosterone plasma levels than subordinates[196]. This study found that groups of three or five females were much more stable than pairs or groups of four. In groups of three or five, all females had a sufficient number of social contacts. The dominant female was able to reduce her contacts (for example pushing other mice away) to a minimum, while subordinate females stayed in close contact with one another.

(xvi) High population density has been associated with deleterious effects, although some strains may be more vulnerable than others. In one study comparing weight gain, plasma corticosterone, behaviour and immune parameters in female BALB/c and C57BL/6 mice housed at population densities ranging from 2-10 mice per cage (484cm^2 total cage floor area, or 48.4-242cm^2/mouse), high density housing had more deleterious effects on BALB/c mice[197]. Thus BALB/c mice housed at ten animals per cage gained less weight, had higher corticosterone levels, spent more time in the outer portion of the open field and had fewer entries into the open field area than those housed at two animals per cage. Furthermore, helper T (CD4+) cells were lower in BALB/c mice housed at ten per cage. C57BL/6 females housed at ten per cage showed less exploratory behaviour than those housed at two per cage, but other parameters were unaffected.

(xvii) Other adverse effects associated with high population density include:

- Suppressive effect on granuloma formation when compared to individually housed controls (albino male mice, strain not specified; population density five adult mice in a single 17.8cm x 25.4cm cage)[198];
- An increase in plasma cholesterol and lipid levels, and increased severity of fatty lesion (atherosclerosis) development, in female C57BL/6 mice[199] (it should be noted that group sizes in the study ranged from one to five mice, but cage size was not specified);
- Reduced brain weight in C57BL/10 male mice[200];
- Tail dermatitis with possible self trauma in male and female C3H/HeJ mice[201]. The incidence of these lesions was 4 per cent in breeding pairs but 21 per cent in weaned mice housed in groups of 40. Incidence was lowered among weaned mice kept in groups of 40 in large cages with sexes separated, but healing of the lesions occurred when mice kept in groups of 40 were separated into groups of five in smaller cages.

(xviii) A number of published studies in mice indicate that housing them within visual, olfactory and/or auditory contact of predators, including rats, is stressful[202, 203]:

- Four strains of mice (BALB/c, C57BL/6, CD-1 and Swiss-Webster) exposed to a rat through a wire screen demonstrated varying degrees of defensive behaviour including freezing and avoidance[204].
- Group-housed BALB/c mice housed in a room containing rats had increased levels of sympathetic neurotransmitters when compared with controls[205]. In the same study investigators found that there was a greater increase in sympathetic nervous system activity in individually-housed mice exposed to rat odour, suggesting the problem was compounded by the stress of isolation.
- Chronic exposure to auditory and olfactory cues from rats affected both sucrose intake and behaviour in an elevated plus maze in male CD1 mice[206]. In this particular study, housing mice in the same room as rats caused such a degree of stress that it reduced their sensitivity to a reward (sucrose) and prevented habituation to the elevated plus-maze.
- In another study, olfactory and visual exposure to rats elicited anxiety responses in male BALB/cByJ and C57BL/6ByJ[207].
- As with other stressors, exposure to rats can alter immune parameters. For example, group housed male CD-1, BALB/cByJ and C57BL/6ByJ mice given visual and olfactory exposure to rats for a fifteen minute period had reduced macrophage activity and natural killer cell cytotoxicity[208].
- Exposure to rats reliably provoked an increase in urination and defaceation, as well as fear-associated behaviour including startle response and freezing. Similarly, exposure of BALB/c mice to cat odour resulted in fearful behaviour including reduced locomotion, reduced rearing behaviour and moving away from the odour[209]. Mice housed in standard (as opposed to enriched) cages also exhibited

higher levels of plasma corticosterone following exposure to cat odour. Male ICR mice exposed to cat urine odour for 58 days failed to habituate to the scent and became more aggressive when compared to mice exposed to rabbit urine or water over the same period of time[210].

One study in female C57BL/6 mice found no long-term changes in physiological parameters when Wistar rats were introduced into the room with the mice however the authors comment that precautions are necessary in drawing conclusions from the results as the stress response in mice to the presence of rats appears to be context dependent and may differ between genders and, or strains[464].

Recommendations

3.1.1 Mice are social animals and should, wherever possible, be maintained in stable, harmonious social groups.

3.1.2 Groups of mice should be monitored to ensure social stability as well as the detection of behavioural and physiological abnormalities. There are situations, for example studies involving highly aggressive strains, where group housing is not suitable.

3.1.3 Pair housing of male mice is not recommended due to a high probability of aggression.

3.1.4 Ideally mouse groups should consist of littermates of the same sex.

3.1.5 Mice should be grouped with each other before they reach puberty to minimise aggression between unfamiliar individuals.

3.1.6 As a guide, the optimal size for a group of adult mice is three to five for females and three for males. However, in determining group size, factors such as differences between individual animals, strain, sex, cage size and experimental design should be taken into account. Therefore the scientific literature should be consulted when determining the optimal housing for particular strains and animals must be monitored.

3.1.7 The disruption of established social groups can cause aggression and should be avoided unless it is absolutely essential.

3.1.8 Separation of cage mates should be limited to less than 24 hours.

3.1.9 Mixing adult males from different groups in the same cage should be avoided.

3.1.10 Where it is necessary to mix unfamiliar adult males, they should be exposed to each other before they are mixed together. This can be achieved by placing the newcomer into an adjoining cage to allow visual, auditory and olfactory contact with the other male. They should also be closely monitored after mixing to check for aggression.

3.1.11 Nesting material should be provided to minimise conflict. Following cage cleaning, for sentinel or breeding cages, nesting material should be transferred from the old to the new cage to minimise aggression (see Section 4.7 Cleaning).

3.1.12 Mice should not be housed in the same room, or within auditory, olfactory or visual contact, with predatory species including rats and cats and staff should take care not to transfer scents from predatory species into the mouse room.

3.2 Isolation and individual housing

Principles

(i) Numerous studies have suggested that individually housed rodents, including mice, may exhibit physiological and behavioural changes when compared with their group-housed counterparts[211, 212]. These changes, which may include alterations in corticosterone levels, neurochemistry, metabolism, growth, reproduction and behaviour, are often collectively referred to as "isolation syndrome"[213, 214]. Reports on isolation syndrome must be interpreted with caution in mice, as the terms single-housing, individual-housing and isolation are frequently used interchangably[213], but may not describe the same circumstances. For example, in some studies, mice are housed individually but are not completely isolated, in that they remain in olfactory, auditory and/or visual contact with conspecifics.

(ii) Although the concept of "isolation stress" has been challenged[211, 215, 216], individual housing of mice is associated with a range of behavioural and physiological changes, some of which may indicate a stress response. Reported behavioural and physiological consequences of individual housing of mice include:

- Increased aggression, particularly in male mice, towards cohabitants[33, 63, 187] or inanimate targets[217] (the effect is well established, such that isolation is commonly used to induce aggressive behaviour in mice);
- Increased risk assessment behaviour, thought to be a manifestation of anxiety, in female CD-1 Swiss albino mice[218];
- Increased irritability[219];
- Altered drug metabolism[220, 221] (for a review see Baer[212]) ;
- Increased faecal corticosterone levels persisting for up to 14 days in outbred male MF1 mice[222];
- Convulsions in male C_3H mice associated with routine husbandry procedures such as cage cleaning and weighing[223];
- A significantly increased heart rate (4 per cent higher than pair-housed counterparts) in outbred adult male HanIbm:NMRI mice[189];
- Disruption of sleeping pattern in outbred adult male HanIbm:NMRI mice, as evidenced by increased frequency and decreased duration of resting[189];
- Increase in consumption of food[224, 225];
- Significant weight gain in B6C3F1 mice, associated with a large increase in the incidence of liver tumours in both sexes and a smaller increase in lung neoplasia in males[226];

- Significant increase in neurotransmitter release in male BALB/c mice after 7-14 days[205];
- Behavioural changes including hyperactivity, increased anxiety behaviour, impaired memory and reduced habituation in male C57BL/6J and DBA/2 mice[227]; increased activity in male NIH Swiss mice[215]; altered performance in consumer demand studies in female CB57 mice[465].
- Increased sensitivity to stressors[211]. For example, exposure of individually housed male Swiss CD-1 mice to a novelty environment led to higher basal corticosterone, reduced splenocyte proliferation and lower type 1 (IL-2) and type 2 (IL-4) cytokines[228];
- Increased susceptibility to experimental infection[228];
- Increased secondary IgM and IgG titres following inoculation challenge in male C57BL/6J and BALB/c mice[229];
- Strain-dependent activation of cytokine production in male C57BL/6 and BALB/c mice[230];
- Transient increase in lymphocytic reactivity and increased resistance to infection in male C_3H/HeJ mice[231] (immune reactivity did not alter with housing conditions in female C_3H/HeJ or male C57BL/6J mice);
- Decreased IgM plaque forming cell response to sheep red blood cells in male CD-1 mice was antagonised with an anxiolytic (diazepam)[232];
- Reduced natural killer cell activity in NC900 and NC100 mice[233] and DD/S mice[234];
- Enhanced tumour growth following tumour cell transplantation[235].

(iii) In a study where individually housed female BALB/c and C57BL/6 mice in unenriched cages were handled irregularly, they had elevated basal heart rate and core body temperature, and a significantly increased relative recovery time following routine husbandry procedures, than mice provided with group-housing, frequent handling and an enriched cage[236].

(iv) Individual housing may affect a range of physiological parameters. Single-housed male C57BL/6J mice were smaller, had less soft-lean tissue with lower bone mineral content and bone mineral density than their group-housed counterparts[237].

(v) For welfare reasons, individual housing may be recommended for known highly aggressive strains or individuals such as FVB or Swiss/CD-1 mice[33, 94, 238] which cannot workably be group-housed with siblings. Alternatively, excessive aggression may be addressed by using females instead of males or a docile strain. Ovarectomised females may be suitable companions for males (see Section 3.1 The Social Environment).

(vi) The effects of individual housing will vary depending on the period of isolation, in addition to the age, sex, strain, and social and housing history of the individual animal. For example, effects of individual housing on exploratory and emotional behaviour were more marked in DBA/2 than C57BL/6J mice[227].

(vii) Separating mice in a cage using a grid partition, so that the animals remain in tactile, visual, olfactory and auditory contact with one another, may be more stressful than isolating mice completely. Adult male Hsd:NMRI mice housed for ten days with sensory contact to an unfamiliar male displayed significant increases in heart rate, core body temperature and motor activity, in addition to impaired nest-building, with almost no habituation, when compared to those housed in sensory contact with a female companion[239]. The authors argued that this degree of stress far exceeded that brought about by complete social isolation. The effects on females were less dramatic. Female C57BL/6JOlaHsd mice separated by a grid partition following abdominal surgery had a significantly higher heart rate (as measured by telemetry) than group-housed or fully isolated counterparts[224]. However, interpretation of these results is difficult as the separated mice had half of the space afforded to their counterparts.

(viii) Change in housing condition, for example from group to individual housing versus individual to group housing, may be particularly stressful. Group-housed male DD/S mice changed to individual housing had markedly increased tumor growth rates than permanently individually housed mice, permanently group-housed mice and individually-housed mice changed to group housing[188]. Furthermore, when changed from group to individual housing, these mice demonstrated a decrease in survival time and an altered response to chemotherapy[240]. Male C3He mice changed from group to individual housing had markedly reduced natural killer cell cytolytic activity[241]. Change from group to individual housing, and vice versa, lead to an exacerbation of tumour growth in male DBA/2 mice[235]. Change from individual housing to group housing and back again reduced antibody production in male CBA/USC mice[242].

(ix) While mirrors may be a useful form of environmental enrichment in some species when individually housed, they do not ameliorate the negative effects of isolation in mice. In preference tests, adult male and female C57BL/6J mice demonstrated a mild aversion to cages containing mirrors, with a strong aversion during feeding[243]. Furthermore, mirrors are unlikely to prevent bar-biting behaviour in mice.

Recommendations

3.2.1 Ideally mice should not be housed individually, however there are some circumstances (for example with highly aggressive individuals or strains) where individual housing may be more conducive to mouse welfare.

3.2.2 Except in cases where immediate isolation of an individual is required to prevent injury, investigators must seek Animal Ethics Committee approval prior to housing mice individually.

3.2.3 Where mice are housed individually due to aggression, for some highly aggressive individuals visual, auditory and olfactory contact with other mice should be limited as far as possible to reduce stress caused by the presence of other mice.

3.2.4 Where mice are housed individually for reasons other than aggression, such as experimental requirements, this should only be with the express permission of the Animal Ethics Committee and they should be housed in visual, auditory and olfactory contact with other mice.

3.2.5 Environmental enrichment is essential for all mice - In cases where individual housing is required, environmental enrichment should be provided to ameliorate the impact of individual housing (see Section 3.5 Environmental Enrichment).

3.3 Metabolism Cages

Principles

(i) When metabolism cages are used to house mice individually the degree of isolation may be greater than from individual housing in standard cages, in that the design of metabolism cages will restrict exposure of mice to olfactory, auditory and visual contact with other mice. Furthermore, metabolism cages have a wire mesh floor (see section 2.6 Cage Flooring). Thus the potential impact on the well-being of mice is greater and there are fewer options to ameliorate these effects. A study using MF1male mice indicated that a period of 14 days acclimatisation would be necessary to ensure that housing conditions did not affect results in individually housed animals[222].

(ii) Limited environmental enrichment of metabolism cages can be introduced without affecting data. Male BALB/c and C57BL mice housed in enriched metabolism cages made extensive use of enriched sections of the cage, including a section of solid floor, a nest box or a nest box with a solid floor, where these were provided[244]. Effects of enrichment on food and water intake, faeces and urine production were minimal. Urine creatinine levels did not differ significantly between mice housed in standard and enriched cages, although mice of both strains housed in enriched cages had higher body weights than those in non-enriched metabolism cages.

Recommendations

3.3.1 Mice should not be housed in metabolism cages without the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house mice in this way. In such cases, mice should be able to be in visual, auditory and olfactory contact with other mice as far as possible. The size of the mesh gaps in the floor should not exceed 8mm x 8mm (See also 2.6 Cage Flooring).

3.3.2 Mice should be acclimatised to the metabolism cage before studies commence.

3.3.3 Where metabolism cages have to be used, consideration should be given to enriching the cages (for example by providing an area of solid floor and/or a nest box).

3.4 Effects of handling, routine husbandry procedures and transport

3.4.1 Handling (general)

Principles

(i) In both animal holding facilities and the laboratory it is inevitable that mice come into contact with humans either directly when they are handled or indirectly when they are exposed to human activity. In both situations interactions with humans elicit physiological and behavioural responses which have implications for animal welfare and the validity of data collection[245].

(ii) As mice have a strong sense of smell, it is important that animal handlers wash their hands, change gloves and wear clean coats when touching them, particularly after handling predator species such as cats, dogs and rats[246]. The use of scented soap and/or perfumes and colognes should be avoided as this may disrupt the behaviour of some mice[148].

(iii) Basic handling procedures have a significant impact on the heart rate and may be associated with stress-induced hyperthermia in mice. Studies using telemetry to record electrocardiograms (ECG) have revealed that in freely moving male BALB/c mice with a normal resting heart rate of 450-500 beats per minute (bpm), weighing mice increased the heart rate to 700-750bpm[247]. Hand restraint or placement of the mouse in another cage increased the heart rate further, to a maximum of 750-800bpm. In another study, male CD-1 mice removed from their cage, picked up and held for 15 seconds had a core body temperature increase of approximately 1.7°C, as measured by telemetry[248]. In the same study, intra-peritoneal injection of saline further increased core body temperature. Body temperature tended to peak 15 minutes post-handling and remain elevated for several hours, although mouse activity in the hours following handling may have contributed to the increase in temperature. Repeated disturbance of individually housed male NMRI mice (being touched gently on the back five times at one minute intervals) lead to hyperthermia lasting for at least 20 minutes[249]. A study in female C57BL/6 mice showed that changes in heart rate, plasma corticosterone and to a lesser extent body temperature, correlated with the method of restraint and the severity of the procedure[466].

(iv) Both manual handling and restraint using various apparatus cause stress in mice. Immobilisation of individually housed male NMRI mice in a cylinder for one minute lead to hyperthermia[249]. Plasma glucose was significantly elevated in B6C3F1 and ICR mice following primary handling and transportation[250]. Additionally, decapitation in mice caused a significant increase in plasma glucose concentrations, probably related to handling techniques with which mice about to be decapitated were unfamiliar[250]. In the same study, blood sampling by venesection of the tail vein resulted in a rapid but transient increase in plasma glucose concentrations, which took about an hour to return to baseline levels. Female CD-1 mice restrained by scruffing for 15 seconds exhibited a

decrease in locomotor activity for 30 minutes following return to home cage.

(v) Different strains may habituate to handling at different rates. For example, the gene response of C57BL/6 mice to repeated saline injections over a period of two weeks was the same as controls, whereas many brain areas of saline-injected DBA/2J mice still showed elevated Fos and Fos-related antigen expression affecting differences in brain biochemistry that may influence responses to experimental manipulations such as performance in cognitive tasks[251].

(vi) Some mice may not habituate to handling. Corticosterone levels rose significantly in both group and individually housed male CD-1 mice which were removed from their home cage and placed for 1.1hour into a black Plexiglass container and bled following decapitation[252]. Corticosterone levels increased with daily handling over a period of 15 days. In another study, female BALB/c mice handled for two minutes daily (by being picked up by the tail and handled without restraint on a gloved palm) for two weeks prior to injection with alveolar carcinoma cells had increased metastases compared with controls[253]. Interestingly, mice handled for one week had decreased metastases when compared with controls, suggesting that the relationship between handling and immune response is not linear in mice. In another study, male BALB/c mice handled for injection for seven days had significantly higher serum corticosterone than those not subjected to handling for the same period[254].

(vii) Handling of mice affects other immune parameters. Both handling alone and handling combined with rectal temperature measurement significantly increased natural killer cell activity in female BALB/c mice[255]. These interventions also had differential effects on mouse hormone and cytokine profiles. In another study, individually housed male C3J/HeJ mice handled daily for two weeks (restrained as if for an injection) had reduced titres of IgM and IgG to an antigen when compared with unhandled controls[256]. In another study by the same authors, group-housed female mice had a reduced primary IgG response to an intraperitoneally injected antigen following once daily handling for two weeks[257].

(viii) Interpretation of studies on the effects of handling is difficult. Handling effects on mouse immunity may not be mediated by glucocorticoids. There was no difference in baseline corticosterone between male C3H/HeJ mice handled daily and unhandled controls[256]. Similarly, there was no increase in glucocorticoid levels in handled BALB/c mice following an intraperitoneal injection of an antigen, while unhandled mice had significantly elevated corticosterone levels in response to the injection[258].

(ix) Restraint stress can impact on reproductive parameters. In one study, female B6D2 mice mated to SW males exposed to five hours of restraint stress on days 1-3, 4-6 or 1-6 of pregnancy had reduced pregnancy rates (52 per cent, as compared with 90 per cent) and reduced litter size compared with controls (8.2 versus 5.2 pups)[259]. In another study, a single four-hour period of restraint in a tubular restraining device led to an increased abortion rate in pregnant C3H/HeJ mice, but not in CBA/J or A/J strains[260].

Figure 3.4.1.1 Animal handlers should wash their hands, change gloves and wear clean clothes before handling mice.

(x) Mice can be easily injured if handled roughly or subjected to procedures by inexperienced personnel. Juvenile male ICR mice administered deionised water via gastric gavage had significantly higher mortality and less efficiency of food utility when the procedure was carried out by personnel with zero to three years experience than those with over fifteen years experience[261]. The study did not determine whether the reduced efficiency of food utility and increased mortality was due to a stress response or sub-clinical injury. In an assay of thermal nociception (tail flick/withdrawal test) in mice of varied strains, investigator identity had the strongest association with tail-withdrawal latency, outweighed by genotype[67]. This may be related to the level of experience or manner of handling employed by particular investigators or their interpretation of the response.

(xi) Different strains may respond differently to handling. For example, wild strains may be much more difficult to catch and handle (thus taking more time and potentially exposing the mouse to injury and/or greater stress) than laboratory strains. However, one study found substantial variation in ease of handling between common laboratory strains[262].

Recommendations

3.4.1.1 Animal handlers should wash their hands, change gloves and wear clean coats before handling mice.

3.4.1.2 Steps should be taken to familiarise mice with handlers so as to reduce the stress of handling.

3.4.1.3 Mice should be handled quietly and gently.

3.4.1.4 Periods of restraint should be kept to a minimum.

3.4.1.5 Handling mice for routine husbandry procedures such as cleaning should not follow, nor be associated with, procedures that may cause distress in mice.

3.4.1.6 Chasing mice around their cage should be avoided. Mice that prove difficult to catch by hand should be directed into a plastic tube or similar structure and thence lifted from the enclosure and coaxed from the tube.

3.4.2 Handling (neonates)

Principles

(i) Handling neonates produces effects that may persist through the animal's life[263]. In rats, the most likely mediator for handling effects is increased maternal care (licking and grooming) following the return of stressed pups to the nest[264, 265]. In a mouse study, maternal care was not affected by neonatal handling in a highly-aggressive strain (NC900), but it was significantly augmented in a low-aggressive strain (NC100)[266].

(ii) The effects of neonatal handling can vary between strains and housing systems. In one study, handling involved placing the entire litter in an opaque plastic beaker for 60 seconds once every 48 hours from day three postpartum until weaning at 21 days[266]. Handled NC100 mice had reduced corticosterone levels compared with handled NC900 mice and non-handled controls. Handled mice of both strains showed an up-regulation in dopamine receptors, with the effect increased in group-housed males. DBA/2 pups removed from their nest, put in a container and replaced back in the nest from day 1 to 24 had significantly reduced survival time following intraperitoneal inoculation of leukaemia cells[267]. However, there were significant differences between handled and non-handled BALB/c pups when a similar protocol was used[268].

(iii) Regular handling of neonates may lead to habituation. Male CD-1 mice removed from their cage, weighed and injected with saline from days 2 to 19 of age showed increased latencies in nociception tests at 35 days of age when compared with non-handled controls[263]. At days 80 and 140, an increase in body weight was noted.

(iv) Handling of mouse pups can influence neural development, immune parameters and behaviour. Neonatal mice removed from their home cage and exposed to clean, unfamiliar bedding in the absence of their mother for

15 minutes daily for the first two weeks of life demonstrated increased exploratory behaviour and less fearfulness compared to control mice exposed to home cage bedding in the absence of their mother[269]. CD-1 mice handled for fifteen minutes daily from post-natal days 2 to 14 inclusive exhibited increased nerve growth factor levels and did not respond to an anxiolytic drug (chlordiazepoxide) when confronted with acute, novel stress[270]. Effects may be delayed. For example, C3H/St pups handled daily from birth to weaning showed no differences in splenic-B and T-cell proliferative mitogen responses at day 21, however they exhibited enhanced humoral and cell-mediated immunity as adults[271].

(v) Early-weaning may lead to increased anxiety and aggression in mice[272]. Male and female BALB/c mice weaned early (at 14 days as opposed to 21 days) had higher levels of anxiety when compared with controls weaned at 21 days[273]. Early weaned males sustained more fight wounds when regrouped after isolation when compared with controls.

Recommendations

3.4.2.1 Investigators must be aware that handling of neonates can have a long term impact on the welfare of animals that persists throughout their lives.

3.4.2.2 Handling of neonates should only be performed where necessary and must be performed consistently across a subpopulation or population of mice to minimise experimental variability.

3.4.2.3 Where neonates are handled, handling must be performed quietly and gently.

3.4.2.4 Early weaning of mice (prior to 21 days of age) should only be performed with permission from the Animal Ethics Committee.

3.4.3 Routine husbandry procedures

Principles

(i) Routine husbandry procedures can impact significantly on welfare and physiological parameters of mice.

(ii) The event of a person entering the room, even without handling animals, increased heart rate and body temperature in individually-housed male C57BL/6N mice, as measured by radio telemetry[274]. After twelve days of habituation, heart rate values were lower, but still significantly increased compared with heart-rate values taken before a person entered the room. The heart rate appeared to rise most when investigators first entered the room each morning at the beginning of the animals' rest period. Conditioning or repeated handling of mice, with or without forewarning via an acoustic stimulus, reduced the increase in heart rate associated with handling over a period of twelve days, particularly when animals were handled in the afternoon.

(iii) Witness effects (stress induced by animals witnessing other animals being subjected to procedures including euthanasia) have been well documented in rats[275], however few studies have documented an affect in mice. Tuli *et*

al[276] found that mice present in the room when other mice were bled and killed did not exhibit a significant difference in corticosterone levels, or spleen or adrenal gland weight than controls. However, mice in this study may have had an adaptive response and investigators conceded there was not enough evidence to conclude the absence of a witness effect. In another study, group-housed female C57BL/6 mice were captured, restrained and decapitated successively at two minute intervals. Corticosterone levels increased by two to three-fold in the fourth and fifth mice in a cage compared to the first mouse, that is from 4-6 minutes and 6-8 minutes after the capture of the first mouse[277], suggesting a witness effect. Chesler *et al*., found that the first mouse tested in a 49°C hot water tail withdrawal/flick test had a higher latency than the others[67]. This may be due to a witness effect, or could be due to extended handling or exposure to a novel environment prior to testing. There is some evidence that mice in a group situation experience anticipatory anxiety. When individual male Swiss mice were removed one-by-one from a group situation, the first animals to be removed did not exhibit hyperthermia while the last animals removed did[278]. This stress-induced hyperthermia was prevented by administration of anxiolytics, but not antidepressants, neuroleptics, antipyretics, muscle relaxants, antihypertensives or naloxone, suggesting that anxiety, possibly secondary to witness effect, is the cause of hyperthermia in these cases[279].

(iv) Stressful procedures performed in close proximity to, but not in view of cage mates, may be stressful to cage mates witnessing the event. Male BALB/c mice whose cage mate was weighed or restrained for one hour had an increased heart rate and core body temperature than controls[280]. Restraint of a cage mate was significantly more stressful than simply handling and weighing the cage mate. Some habituation to stress was observed over 14 days, but the vas deferens of witnesses had a significantly increased response to application of exogenous noradrenaline – thought to be an indicator of chronic stress.

Recommendations

3.4.3.1 To minimise the impact of disruptions, mice should be allowed a conditioning period to ensure that disturbances such as laboratory animal personnel entering the room do not cause undue stress. A period of at least seven days is recommended prior to commencement of research.

3.4.3.2 Persons entering the mouse holding room should follow a routine as much as possible.

3.4.3.3 Stressful procedures should be conducted in isolation from other mice in an appropriately equipped procedures room.

3.4.4 Transport

Principles

(i) Transport, whether to another laboratory or within the same facility, involves multiple stressors and may impact adversely on mice[281]. Aside

from direct handling, which may or may not be involved, potentially stressful aspects of transport include exposure to novel environments, duration and mode of transport, exposure to temperature variation (see section 4.3 Temperature), changes in light:dark cycle (see section 4.2.2 Light Cycles), noise and vibrations (see section 4.6 Sound and Vibration), and reduced availability of food and water (see section 3.6 food and water)[66, 282].

(vi) Adverse effects of transport on mice have been documented. CD-1 mice exposed to 24-36 hours of air transport or 36-38 hours of truck transport exhibited a marked increase in plasma corticosterone, levels of which remained at a high value for 48 hours[283]. In addition, immunologic function as detected by the foot pad test, haemagluttination assay and plaque forming assay, was depressed for 48 hours. In another study, C57BL/6J mice exposed to air and truck transportation demonstrated increased plasma corticosterone and decreased NK cell activity on arrival[284]. These parameters returned to control levels after 24 hours.

(vii) Long-term effects of transport-induced stress are reported. Transportation (via a combination of air and truck) suppressed reproduction in agouti and non-agouti deer mice for several weeks[285].

(viii) Transportation within a facility may result in adverse effects. BALB/Ola mice subjected to a short, local transportation (taken in a cage which was wrapped in a black polythene bag to an experimental room, via a ten minute walk and two minutes in lifts) had an immediate marked increase in corticosterone levels, which returned to baseline within 24 hours[281]. Behaviours including rearing, climbing, grooming, feeding and aggressive sexual behaviour also changed. These behaviours were largely stabilised in 24 hours, but variability in some behaviours continued over four days.

(ix) When transport times are equal, truck and air transport cause a similar magnitude of stress[283, 284]. Lengthy or traumatic transportation events could be expected to increase the magnitude of physiological effects and potentially the time taken for these to return to baseline levels[282].

(x) Transport stress may confound research if mice are used in experiments before homeostasis is restored[282]. In general, primary mediators of the stress response (primarily catecholamines and glucocorticoids) return to normal within 24 hours of arrival, but secondary physiological outcomes (weight loss, changes in immune parameters, endocrine function and changes in behaviour) may take longer to return to baseline levels[282]. Reproductive performance may take significantly longer (weeks to months) to normalise[285].

(xi) Feeding and activity levels were shown to be altered significantly in the first 24 hours after transportation[281]. Mice should be monitored post transport to ensure they are eating and drinking sufficiently, particularly where there is a change in diet, treatment of water or method of water

delivery[66]. Animals should be checked for any clinical manifestation of disease, or signs of trauma including fighting following transport.

(xii) It is likely that the optimal period for acclimation will vary depending on the strain of mouse used, research procedures and organ system or physiological parameters studied[66]. Periods of between 1 and 7 days have been recommended[66].

Recommendations

3.4.4.1 Transportation times should be kept to a minimum. Effort should be taken to contain mice in such a way to minimise noise, vibration and extreme variation in temperature.

3.4.4.2 Where possible, mice should be transported in their home cage to minimise stress.

3.4.4.3 Mice should have access to food and water during transport. Precautions should be taken to prevent water spillage, for example by providing an alternate source of water such as a sterile water gel.

3.4.4.4 Following on-site transport, a minimum of 24 hours should be allowed for acclimation.

3.4.4.5 Following off-site transport, a minimum acclimation period of 3-7 days is recommended, although longer may be required for stabilisation of behavioural and reproductive parameters.

3.4.4.6 Mice deemed to be unwell or injured should not be transported, unless it can be established that transport does not result in additional pain or distress.

3.5 Environmental enrichment

Principles

(i) The term "environmental enrichment" is used inconsistently in scientific literature[286] to describe modifications of captive animal environments. For the purposes of these Guidelines, the term "environmental enrichment" applies to a modification of the cage environment that seeks to enhance murine physical and psychological well-being by providing stimuli to meet the animals' species-specific needs and promote species-specific behaviour[50]. When mice are deprived of the opportunity to perform species-specific behaviour, they may show signs of distress including but not limited to stereotypies, chronic stress or other pathological conditions (see section 3.7 Monitoring of Mice). The aim of environmental enrichment is to provide the animal with choice of activity and control over its social and spatial environment[50]. Enrichments may be classed as Social, Food-related, Exploratory, Security, Physical exercise, Other?

(ii) In some papers, the term environmental enrichment encompasses nesting material and in-cage shelters, while in others these are considered basic husbandry requirements. For the purposes of these Guidelines,

"environmental enrichment" is deemed to encompass environmental modifications additional to the provision of nesting material and an in-cage shelter. These are addressed at length in sections 2.8 Nesting Material and 2.9 In-Cage Shelters respectively.

(iii) It has been widely argued that standard laboratory housing compromises murine welfare[4, 150, 287]. In one study, female C57BL/6 mice housed in standard cages self-administered an anxiolytic significantly more than those housed in enriched cages (supplied with tubes, chew blocks and a running wheel), suggesting that mice housed in standard cages were more anxious and/or fearful than those in an enriched environment[288]. In the same study, mice in enriched cages spent less time resting and performing bar-related behaviours and more time performing exploratory/locomotory behaviours[289].

(iv) Environmental enrichment must be carefully selected so that it does not pose risks to mice (in terms of potential to injure or exacerbate aggression); does not pose risks to staff; and does not adversely interfere with experimental outcomes (for example by increasing experimental variability and the numbers of animals required)[50, 290]. Environmental enrichment (in the form of provision of a nest box, nesting material, gnawing stick and PVC tube) had no effect on the mean of behavioural parameters measured in BALB/c and C57BL/6 mice when compared to their standard-housed counterparts[291].

(v) Environmental enrichment may modulate the reactivity of the mouse immune system, buffering mice against episodes of acute stress. Female C57BL/6 mice exposed to long-term environmental enrichment (provision of a running wheel, nesting material, toys and in-cage shelters) had lower splenic proliferative responses to acute stress than their standard-housed counterparts[292], such that they behaved immunologically like non-stressed mice.

(vi) Different mouse strains respond differently to environmental enrichment protocols[164, 293, 294]. Thus subtle environmental changes (the provision of a cardboard roll as opposed to a plastic shelter) had a significant impact on emotionality and sensory responsiveness in some strains[295]. In another study, a low anxiety, exploratory strain (ICR (CD-1)) made greater use of enrichment objects introduced weekly into the cage than a high anxiety strain (C57BL/6) which exhibited high levels of bar-climbing[293]. The authors suggest that high anxiety strains may benefit from a more stable cage environment.

(vii) Environmental enrichment may lead to increased aggression, particularly between males. For example, enrichment led to an increase in antagonistic encounters between male inbred CS mice[296]. This does not automatically translate into poor mouse welfare, as mice kept in enriched conditions in the same study also displayed increased play behaviour relative to controls.

(viii) Strains may vary in their response to environmental enrichment. Thus while CS mice exhibited increased aggression, ABG mice did not. In a study using a docile strain, male ABG mice exhibited significantly increased play behaviour and activity levels with increasing enrichment[297]. No differences in agonistic behaviour were noted when compared with standard-housed counterparts.

(ix) Because different mouse strains express markedly different behaviour, it is important that environmental enrichment is evaluated in terms of the preference and motivation of mice to use it; the effect on mouse behaviour (notably the absence or reduction of abnormal behaviour); its facilitation of species-specific behaviour; and the effect on physiological parameters (for example body weight, heart rate, stress-related hormones and immune parameters)[50]. Potentially negative effects are reported. For example, DBA/2 mice showed an increase in stereotypy when provided with nesting material and a Perspex tunnel than other strains (C57BL/6, CBA/Ca, BALB/c, ICR (CD-1) and TO) when compared with control mice in a non-enriched environment[298]. In the same study, environmental enrichment was associated with an increase in testosterone in aggressive strains (ICR(CD-1), TO and BALB/c)[298]. Effects of enrichment objects or designs may vary depending on the gender of mice and the variable studied[299].

(x) Environmental enrichment may promote wider use of cage-space. For example, wild-caught mice housed in a cage with increased ground-level complexity (small bricks scattered around the floor) emerged from protected nest sites and moved away from cage walls more often[47].

(xi) Environmental enrichment delayed the onset of Huntington's disease in transgenic Huntington's model R6/1 (slow onset) and R6/2 (rapid onset) mice[300, 301]. In the latter study, even limited environmental enrichment (provision of a cardboard tube, distribution of food pellets on the cage floor, wood shaving bedding, and four mice housed in a 120mm x 30mm cage) slowed decline in Rotarod performance despite rapid disease progression. In addition, enrichment delayed loss of cerebral volume in Huntington's disease model mice. The findings suggest that individual housing of mice in non-enriched conditions may lead to a marked worsening of disease phenotype, at least with neurological disorders.

(xii) There is some evidence that environmental enrichment may delay progressive memory loss and cognitive decline associated with deposition of beta-amyloid peptides and neuronal loss (associated with Alzheimer's disease) in mice. Male APPswe X PS1AlphaE9 mice exposed to an enriched environment (containing running wheels, coloured tunnels, toys and chewable material) from the time of weaning to the age of six months resulted in a marked reduction of beta-amyloid deposition in the central nervous system compared to mice housed in standard conditions[302]. This may have been associated with increased exercise, as the most significant reductions in amyloid burden were seen in enriched mice exhibiting the most physical activity.

(xiii) Female C57BL/6 mice raised in an enriched environment (group-housed in large clear plexiglass containers containing ladders, tunnels, ramps, shelves and toys, as well as visual cues such as posters, shapes and moving patterns, as opposed to those housed in opaque white cages without stimulating objects) had 18 per cent higher visual acuity than their counterparts[303]. The authors argue that rearing animals in the non-enriched cages did not provide sufficient exposure to high spatial frequency information to allow the visual system to achieve maximal acuity.

(xiv) Environmental enrichment may assist mice in coping with experimental procedures. In one study, male and female BALB/c nude mice were subjected to a surgical incision closed with a wound clip. Those provided with enrichment objects (nestlets and cardboard rolls) post surgically had a much lower incidence of clip removal (16 per cent and 0 per cent in two experiments) than their counterparts (50 per cent of whom removed wound clips)[304]. Furthermore, in mice provided with enrichment objects, the lag between surgery and premature clip removal was longer (2-3 days compared with one day), suggesting that environmental enrichment provided a distraction.

(xv) Provision of non-food material such as cardboard or aluminium foil may reduce stress levels in mice. In one study, the presence of cardboard or aluminium foil in a novel environment elicited chewing and reduced the initial corticosterone response to the novel environment when compared with controls[305]. In contrast, provision of highly-palatable edible peanut butter chips evoked little chewing and had no impact on the initial (0-60 minutes) plasma corticosterone response.

(xvi) Other documented effects of environmental enrichment on mice include:

- An increase in brain weight[306];
- Increased cortical depth for longer than isolated mice[307];
- an increase in behavioural repertoire and reduction in stress[286, 308];
- increased reactivity and alertness[164];
- increased sensorimotor skills[309];
- a reduction in fearful or anxious behaviour and increased ease of handling[157, 308, 310];
- reduced incidence of abnormal behaviours, notably stereotypic bar biting and looping from cage floor over cage lid continuously[161];
- a reduction in aggression between male BALB/c mice following cage cleaning[311];
- a reduction in offensive behaviour (for example chasing) in male CD-1 mice[312];
- increased neurogenesis in the hippocampus and dentate gyrus[313], and increased motor function, following stroke[314, 315].

(xvii) Examples of enrichment items include:

- Nesting material (See 2.8 Nesting Material);

- In-cage shelters (See 2.9 In-cage Shelters);
- Social interaction (See 3.1 The Social Environment);
- Structural enrichment – for example climbing apparatus or running wheel;
- Manipulanda – objects that can be moved or altered by a mouse or those which promote fine motor movements, including wooden blocks or chew toys[14];
- Novel foods or novel food locations (See 3.6 Food and Water);
- Sensory enrichment (for example background noise, See 4.6 Sound and Vibration).

Figure 3.5.1 Cardboard rolls are an inexpensive form of environmental enrichment that are used by mice by manipulating, tunnelling and chewing.

(xviii) Provision of running wheels as a form of environmental enrichment is controversial. Mice have a strong preference for wheel running over tunnelling[316]. Furthermore, wheel running appeared to reduce negative changes associated with intermittent individual housing. Female BALB/c mice housed individually every second day with access to a running wheel exhibited increased locomotor activity, reduced nerve growth factor and brain-derived neurotrophic factor levels in the frontal cortex and increased brain-derived neurotrophic levels in the amygdala and hippocampus, as well as increased mRNA in the hippocampus[317]. However, wheel running affects mouse brain development differently to other types of enrichment, potentially skewing experimental data. Thus wheel running increased

proliferation of microglia in a number of superficial areas of the cortex[318], but does not contribute to morphological changes in the hippocampus region CA1 and the dentate gyrus as associated with other types of enrichment[319]. It is difficult to determine what needs are met by running wheels but they are used when provided (see Sherwin[316] for an extensive review on the subject).

Recommendations

3.5.1 Mice should be provided with environmental enrichment in addition to the necessary nesting material and an in-cage shelter.

3.5.2 Depending on the type of enrichment and how it is implemented, environmental enrichment may be a significant source of experimental variability. It is therefore critical that environmental enrichment is applied consistently to groups of mice.

3.5.3 Items that allow mice to perform each of the five following categories of behaviour should be provided:

(i) social interaction (see Section 3.1 The Social Environment)
(ii) chewing/gnawing
(iii) locomotion (including climbing, exploring, playing)
(iv) nest building, nesting, resting, hiding
(v) manipulating, carrying and hoarding food and objects

3.5.4 Enrichment items can be provided on a rotating basis to increase their novelty value. Mice should be observed carefully when new items are provided as strains may react differently to the presence of unfamiliar items.

3.5.5 When techniques are used in an effort to provide environmental enrichment for mice it is important that the success of the techniques, in terms of improving mouse welfare, is evaluated. In particular, male mice should be monitored for increased aggression.

3.5.6 Spatial conditions should be generous enough to allow the inclusion of enrichment objects as well as space for allowing mice to retreat from and cope with any increased aggression that may result from the addition of an environmental enrichment.

3.6 Food and water

Principles

(i) Food and water consumption are affected by the social environment and other environmental variables including light:dark cycles[320], the position of the cage on a rack[321] and temperature[322]. Physiology also influences food and water consumption, with pregnant and lactating mice demonstrating an increased food intake[320]. Mice may avoid novel foods when they are initially offered[323]. Mice ingest most of their food during the dark period[324].

(ii) Mice locate food by sniffing. They typically pick up food with their mouth, move to a preferred eating location and sit on the haunches while they eat, manipulating the food with their front paws[246].

(iii) A review of feeding patterns in mice fed *ad libitum* found that the number of meals taken in a 24 hour period varied from 2 to 50[320], with some animals adopting a grazing pattern while others adopted a gorging pattern of eating, depending on the type of diet available and ease of procurement. As mice are nocturnally active, most meals (75 per cent) were consumed during the dark phase of a 12:12 light:dark cycle[320].

(iv) Food disappearance and body weight changes were found to be correlated with cage shelf level in one study. Female BALB/c mice housed on the top shelf of a rack removed the most food while those on the second shelf removed the least[321]. Food removal increased in a stepwise fashion from the second-down to the sixth (bottom) shelf on the rack. Mice on the top shelf had the lowest weight gain while the bottom two shelves ranked next. Animals on shelves two, three and four gained the most weight. The authors speculated that this may have been due to a wastage or temperature effect.

(v) Over-nutrition may lead to a high incidence of obesity and increase in tumour incidence[325]. Treats, which are primarily used as a motivation or environmental enrichment, should be accounted for in the overall nutrition of the animal[320]. Nonetheless, one study[171] found that male ICR mice offered fruit-flavoured crunchy treats reduced their intake of regular food almost calorie for calorie, suggesting that these may be a useful form of enrichment in some studies.

(vi) Mice require approximately 15ml/100g/day of water (approximately 5-8ml/animal/day) and 15g dry weight of food/100g/day (approximately 4-8g/animal/day)[3]. Food and water intake is influenced by ambient temperature. For example, increasing the ambient temperature to 29-33°C markedly reduced food intake[326].

(vii) Caloric restriction may result in physiological and behavioural changes in mice. For example a single, over-night fast in mice housed at 23°C can induce a state of torpor, in which the core body temperature of the mouse drops below 31°C[326]. Calorie-restricted mice may show a paradoxical increase in wheel-running and cage activity[326]. In another study, restriction of food promoted stereotypical behaviour in male DBA mice, manifested as repetitive, invariant cage lid climbing despite food being available on the bottom of the cage[327]. Interestingly, dietary restriction did not lead to stereotypies in C57BL/6 male mice, suggesting a strain effect.

(viii) Mice on a calorie-restricted diet must be given food of sufficiently high quality to ensure they do not suffer dietary deficiencies, for example protein deficiency or deficiency of one or more micronutrients[320]. Group-housing of mice on calorie-restricted diets may lead to uncontrolled

experimental variability due to in-fighting and/or unequal competition for rationed food[320].

(ix) Food and deprivation results in absolute loss of body weight. Smaller strains may be more vulnerable. Death occurred when the percentage of body weight lost reaches 17-23 per cent over a 28 hour period[328].

(x) In the wild, mice forage for food, and may consume up to 200 small meals a night from 20-30 food sites[16]. Scattering items of food around the cage rather than in fixed dispensers can encourage foraging behaviours and allow mice to adopt normal postures for eating.

(xi) Like all rodents mice have a strong motivation to chew[246]. This behaviour is not restricted to food items, and may extend to objects that can be reached through the cage bars. This may lead to damaged incisors, which can become caught, broken or misaligned[246]. It is important to ensure that no objects are inadvertently left within chewing range of the cage.

(xii) Food or water may contain natural and synthetic chemical compounds that have significant effects on physiologic processes, for example heavy metals, phytoestrogens (such as the isoflavone genistein) or biological contaminants such as aflatoxin[329]. This may lead to disease and compromise animal welfare, and/or be a source of experimental variability. Autoclavable or irradiated pellets should be used for immunodeficient or barrier-maintained mice[330].

(xiii) Mice must have access to potable, uncontaminated drinking water *ad libitum*. Water may be treated to reduce microbial contamination, but any potential physiological effects of treatment should be evaluated by investigators[330].

(xiv) Water delivery systems may leak, particularly when cages are moved during cleaning or transport. This can result in flooding or wet bedding which may in turn alter the microclimate of the cage. Both situations are potentially lethal, particularly for neonates[331]. Similarly, water delivery systems may malfunction, or become plugged with nesting or bedding material[331] preventing access to an adequate supply of water.

(xv) Mice have a high water turnover and small body size. Therefore weight loss is an important sign of dehydration in mice[320].

(xvi) Some strains of mice do not adapt to particular water delivery systems and subsequently become dehydrated[330].

Recommendations

3.6.1 Food and fresh water should be provided ad libitum *unless special permission has been obtained from the Animal Ethics Committee of the institution to vary this regime*

3.6.2 A nutritionally adequate diet should be provided for mice.

3.6.3 Where treats are fed, these should be accounted for in the overall ration of mice to avoid obesity. Grain and maize are good enrichment as they are small and easy to disperse in the bedding encouraging foraging.

3.6.4 Food and water should be free of contaminants unless these are part of the study. Autoclaved or irradiated pellets should be used for immunodeficient or barrier-maintained mice.

3.6.5 Food must be stored in a clean, dry, vermin-free, well-ventilated area to reduce the risk of post-purchase contamination.

3.6.6 Water delivery systems should be checked daily to ensure proper function. Care must be taken to ensure water delivery systems do not leak, particularly when cages are moved during cleaning or transport. Where practical, mice should be provided with an elevated or suspended dry refuge area in case of flooding.

3.6.7 To minimise the risk of cross-contamination, it should be ensured that water bottles are not interchanged between groups of mice.

3.6.8 It should be ensured that mice are able to use water delivery systems.

3.6.9 Food may be scattered throughout the cage as a form of environmental enrichment (see section 3.5 Environmental Enrichment).

3.7 Monitoring of mice

Principles

i) Mice are affected by their living conditions, including their physical environment, their social environment and their interaction with humans. When assessing the responses of mice to their living conditions, assessment of physiological and behavioural parameters are useful. Negative trends in these parameters, such as loss of body weight, failure to reproduce and changed behaviour patterns may indicate that mice are distressed and failing to cope with their environment[467].

ii) The well-being of prey species including mice can be difficult to assess due to instinctive masking of signs of physical compromise or injury[332, 333]. In addition the speed with which mice move, their small body size, propensity for burrowing and nocturnal activity compound this difficulty[333]. Recognition of signs of pain or distress requires sufficient time for observation of an animal or group of animals. As mice are nocturnal the full range of wake-hour behaviours are best observed at night using minimal illumination (see Section 4.2.1 Light intensity). It may be necessary to observe mice in such a way that they are unaware of the presence of an observer, for example by using a camera or recording device[334].

iii) Mice should be monitored for signs of pain. As defined by the International Association for the Study of Pain (IASP), pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[335]. Pain is a subjective experience that we can detect in animals that exhibit a behavioural response to pain. According to the Federation of European Laboratory Animal Science Associations (FELASA), all mammals including mice may be assumed to perceive and experience pain and remember situations associated with pain sensations[336]. Alternatively, pain has been defined as "an aversive sensory experience that elicits protective motor actions, results in learned avoidance, and may modify species-specific traits of behaviour, including social behaviour.[337]" Pain is a source of variance in experimental results due to a range of biochemical responses (e.g. neurotransmitter, hormonal) elicited. Animals in pain are therefore often poor research subjects.

iv) Use of cage side assessments of behaviour, appearance and demeanour may be more useful for immediate identification and treatment of pain than retrospective observations such as body weight change. Behavioural changes may give an early indication of pain or that something is wrong with a mouse's well-being. Changes that are subtle and non-specific should not be overlooked[333]. Signs of pain, discomfort and/or distress in the mouse include but are not limited to: reduction in faecal/urine output; reduction in food/water intake; abnormal gait; vocalisation; rubbing, scratching or chewing at a surgical site or wound; reluctance to move; restlessness; pacing; hunched posture; unusual sleeping position (for example stretched out on one side); social withdrawal; head-pressing; poor grooming/rough hair coat; piloerection; weight loss; increased or laboured respiration (may manifest as open-mouth breathing, pronounced chest movements); porphyrin discharge around eyes and nose[336]; blood or saliva in bedding; or change in behavioural repertoire[246, 330, 333, 338]. Other signs include alterations in core body temperature and heart rate; increased faecal glucocorticoid levels; reduced interaction with conspecifics; reduced exploratory and grooming behaviours[65]; reduced use of nesting material; irritation at injection sites; ptyalism (excessive salivation) and grinding teeth[332, 338]. Mice experiencing pain may attempt to bite when handled[333]. Vocalisation may indicate acute pain, but its absence in the face of a painful stimulus should not be interpreted as absence of pain or distress[339].

v) Changes in nest building behaviour have been reported to be sensitive early indicators of distress or illness in mice. Thus male HsdHan:NMRI mice not treated with analgesia following exploratory laparotomy damaged their nests, and failed to build proper nests for up to two days following surgery[190]. In some instances investigators could not identify a nest, or found several fragmentary nests at different locations in the cage. In contrast, mice treated with analgesics built normal nests within the first day and did not engage in nest destroying behaviour.

vi) Barbering – defined as the plucking of fur or whiskers from cage mates or oneself[340] – is a common form of abnormal repetitive behaviour in mice which may be related to environmental factors such as cage design, cage location, relationships between cage-mates and the presence of other barbers in the cage[341]. Lesions tend to be larger than those inflicted by aggressive encounters (which may be as small as 1-3mm in diameter); non-pruritic (not-itchy); not inflamed; and with no surrounding scarring or scabbing. Barbered mice may be functionally abnormal as whisker trimming can alter anatomy and function of the barrel cortex, reducing the ability of the mouse to discriminate between textures or control balance, and altering whisking patterns[16, 342, 343]. While the underlying reasons for barbering are poorly understood[342], they may be triggered by husbandry factors. Mice housed in steel cages were 1.82 times more likely to barber than mice housed in plastic cages[341], although barbering was more severe overall in plastic cages (3 per cent of body area versus 2.4 per cent in steel cages). Mice housed entirely with siblings were 3.66 times more likely to barber than mice housed entirely with non-siblings. This may signify frustration as mice normally disperse at puberty, the age that barbering behaviour tends to appear. Some strains are more likely to barber than others[340]. Provision of environmental enrichment items such as a nesting box, cylinder or manipulable objects reduced the incidence of barbering in one study[344].

vii) Stereotypic behaviours are repetitive, unvarying actions with no apparent goal or function[100, 345] which may be induced by frustration, attempts to cope and/or central nervous system dysfunction[345]. They may indicate attempts to cope with past challenges rather than current ones, consequently care is required in interpreting the point of origin and cause[344]. In mice, stereotypic behaviours include bar mouthing or gnawing, jumping up and down at the cage wall, back-flipping, somersaulting, circling and cage-top twirling[346]. While one survey found a positive correlation between the incidence of cage climbing and stereotypic behaviour[94], climbing on the cage bars and lid are not stereotypic behaviours per se and thwarting this behaviour may lead to anxiety in some strains[101]. It is estimated that 50 per cent of mice in research and breeding establishments exhibit some form of stereotypic behaviour[347]. Stereotypic behaviours are often associated with environmental restriction and their incidence may be reduced in an enriched environment[348]. They are probable indicators of poor welfare. For example, bar chewing may reflect escape attempts and may provide a behavioural indication of the animal's perception of its cage environment[349]. Some mouse strains are more likely to develop stereotypies than others, with more active strains at a higher risk[94, 346]. Other risk factors include premature or sudden weaning, lack of shelter and inability to explore cues (for example olfactory cues from adjacent cages) in the surrounding environment[346]. Over time, stereotypies tend to increase in frequency and duration while becoming increasingly fixed in form and orientation[346]. Perhaps more of a concern is the fact that these behaviours may persist even in the absence of initiating factors, suggesting changes at a neural level[346]. Mice exhibiting stereotypic behaviours may therefore be poor research subjects.

viii) Investigators should be familiar with strain and/or transgene-mediated health conditions including tumour growth, hair loss, degenerative joint disease, diabetes, respiratory tract disorders and intestinal obstruction so that they can be diagnosed and treated in a timely manner[57].

ix) One of the most useful methods of monitoring experimental mice is the adoption of an objective scoring system[332, 350, 351, 468]. To ensure consistency, score sheets should be filled out by the same staff each time. Scoring parameters should be adjusted to take into account the specific characteristics of a strain, particularly where transgenic mice are concerned[352, 469] and particular clinical signs that may be expected during an experiment[350]. If score sheets are used these should be regularly reviewed to detect subtle changes[332].

x) It is important to bear in mind that individual mice vary in their response to pain or stressors, and that this response is influenced by genetic factors, previous experience, age and physiological state[353].

xi) The NHMRC has produced Guidelines on the Assessment and Alleviation of Pain and Distress in Research Animals which can aid investigators in developing protocols for assessing, minimising and monitoring pain and distress during studies[353].

Recommendations

3.7.1 Welfare monitoring of mice via behavioural observation should be carried out in addition to monitoring for physical health. Investigators should be familiar with strain and/or transgene-mediated health conditions and behavioural problems so that they can be diagnosed and treated in a timely manner.

3.7.2 Monitoring should be carried out when a person with whom the mice are familiar is present. It should be ensured that there are sufficient, properly trained staff and resources including staff time to monitor mice effectively.

3.7.3 In the monitoring and investigation of health issues (such as growth rate, reproductive performance and disease) the effects of housing conditions should be taken into account.

3.7.4 Animal carers should be familiar with the normal physical appearance and behaviour of mice and of the individuals within a group and note any deviations from the norm, including animals that do not move around the cage normally. Mice that give cause for concern may need to be removed from the group but only if absolutely necessary as aggression may occur subsequently to regrouping.

3.7.5 In particular, mice should be monitored for signs of bullying including fight wounds, barbering or loss of body condition secondary to denial of access to food or water.

3.7.6 Mice that become sick unexpectedly should be examined and diagnosed by a veterinarian and any animals that die unexpectedly should routinely be submitted for post-mortem and diagnosis.

3.7.7 Records and score sheets should be kept and reviewed regularly to detect trends and subtle changes.

4.0 Environmental Variables

4.1 General

Principles

(i) Good animal husbandry involves maintaining animal health and welfare by meeting physiological as well as behavioural needs. Management of environmental variables such as light, temperature, humidity, air quality and ventilation and noise levels plays a significant role in achieving these ends. If not controlled, environmental variables may confound and compromise experimental data, resulting in the unnecessary use of more animals.

4.2 Light

Principles

(i) Light intensity, wavelength and periodicity (light:dark cycles) impact on the behaviour, physiology and reproductive parameters of mammalian species[329].

4.2.1 Light intensity and wavelength

Principles

(i) Light intensity can influence the behaviour of mice, as well as progression of eye pathology and reproductive parameters.

(ii) Mice in the wild are typically nocturnal and generally avoid brightly lit areas. Behavioural tests for anxiety, such as open field exploration, the elevated plus maze and light:dark tests are predicated on this aversion of mice to brightly illuminated areas[354, 355]. Thus in one study, 400 lux illumination in a white cage area was aversive to C57BL/6, DBA_2 and albino BKW mice, and inhibited exploratory behaviour[356].

(iii) Light intensity decreases with the square of the distance of its source, hence intra-cage illumination is influenced by the position of a cage within a particular room and rack[329]. Intra-cage light intensity can vary by over 80-fold in transparent plastic cages on racks on shelves (from 3lux at the bottom to 250 lux at the top)[357]. Even within a single cage light intensity can vary as much as 20-fold (7-140lux), with intra-cage variability lowest in cages farthest from the light source.

(iv) Phototoxic retinopathy (progressive loss of the outer retinal layers associated with excessive exposure to light) can occur in a variety of species, but is most commonly reported in laboratory rodents[329]. The extent of photoreceptor damage is affected by light intensity, photoperiod duration, temperature, activity levels during the light phase, light levels under which an animal was raised, age, hormone status and albinism[329].

(v) Albino mice are particularly sensitive to light-induced photoreceptor degeneration, with some albino strains more susceptible than others. Extremely high light exposure of around 2010 lux for 18-24 months caused retinal atrophy in 20 per cent of exposed BALB/c mice[358]. In another study, seven different albino strains were exposed to constant fluorescent light at 1265-1430 lux for three weeks prior to histological examination of the eyes. All exhibited photoreceptor degeneration[359].

(vi) Studies have shown a relationship between cage shelf-level and retinal atrophy, presumably caused by differences in lighting intensity. In one chronic study, 19.7 per cent of mice on the top shelf of a rack had retinal atrophy when sacrificed at 24 months, as compared to 0.2 per cent of animals on lower shelves. By 33 months, retinal atrophy was present in 30.2 per cent of mice on the top shelf, compared with 12 per cent on the shelf immediately below it and 0.7 per cent on lower shelves[358]. Light-induced complications may be reduced by utilising racks with shaded tops[1], or rotating the position of a cage within the rack, shelf and room[357].

(vii) Light intensity influenced the oestrus cycle, including duration of vaginal cornification and time periods between vaginal cornification, in outbred albino (LACA) mice[360], as well as pigmented C57BL/10 and cogenic albino C57BL/10 mice[361].

(viii) Reproductive efficiency of wild mice is reduced under high-intensity lighting. Both laboratory (CF-1) and wild mice bred equally well under a lighting intensity of 10-20lux[362]. However, at a lighting intensity of 1000lux productivity – especially litter size – of wild mice decreased significantly while that of laboratory mice was not affected. In addition, body weight was depressed in wild mice with increasing light intensity.

(ix) Reproductive efficacy of laboratory mice is reduced under high-intensity lighting. In one study, inbred laboratory mice housed at a cage lighting level of 500lux demonstrated a 50 per cent pre-weaning mortality rate, compared with only 5 per cent losses at a level of five lux[363]. Brighter illumination was associated with poor maternal behaviour, inadequate nest building and pups being scattered throughout the cage.

(x) Light intensity influenced wall-leaving behaviour in inbred strains of mice, with significant increases in both wall-leaving and cage-crossing behaviour in C57BL/6J, C3H/HeJ and BALB/cJ mice under low illumination (a 25W clear bulb, shielded by a paper towel and suspended 172.7cm above the centre of the open field) as compared to high illumination (a 100W clear bulb suspended 116.8cm above the centre of the field)[95]. In addition, there was a significant reduction in defecation and urination under low illumination.

(xi) Tests of avoidance behaviour in rats showed that albino rats avoided light intensities as low as 25 lux and pigmented rats as low as 60lux[364]. The authors concluded that because the rats were motivated to leave a warm

nest to avoid these light intensities, exposure to these intensities caused distress. Similar studies are yet to be conducted for mice.

(xii) Uncovered halogen quartz lamps are carcinogenic to mice. Almost 100 per cent of hairless SKH-1 albino, MF-1/Ola/Hsd albino and C3H/Tif-pigmented mice exposed to uncovered halogen lamps for 12 hours a day at an luminance level of 10,000lux developed multiple benign or malignant tumours of various histological types with a short latency period[365]. Groups exposed to lower levels of luminance (e.g. 3,333 and 1000 lux) developed tumours with a longer latency. In contrast, none of the control mice developed spontaneous skin tumours. Additionally, when a silica glass cover was interposed between the lamps and the mice, no exposed mice developed spontaneous skin tumours.

(xiii) Different colours generated by fluorescent lights may have different effects on mice. For example, black UV light was associated with increased body weight in male and female Ha:(ICR) mice, compared to blue, cool and full-spectrum lights[366]. Light colour affected the weight of the pituitary, adrenals, kidneys and prostate in male mice and the adrenals, thyroid and pineal glands in females.

(xiv) The eyes of mice are sensitive to green, blue and near ultraviolet light but have limited ability to detect light in the red range of the spectra[367, 368]. Humans have greater red vision than mice due to fact that two of our three retinal cones are sensitive to red. Red light can therefore be used to observe mice with minimal disturbance during the dark phase.

(xv) Sodium lighting, a bi-chromatic light with both wavelengths in the human field but at the margin of murine vision, may be a suitable alternative[369]. Light emitted from sodium lamps is orange to yellow, and humans perceive it as brighter than it actually is. 18W low pressure sodium lamps, with an average lumen output of 1650 to 1800, did not disturb the nocturnal activities of a variety of mouse strains within a facility[369].

(xvi) Although mice should not be exposed to high light intensity, staff in animal rooms need enough light to perform tasks. One study concluded that 210 lux at working height is sufficient for health and performance of technicians, but would be the minimum under which they should be expected to work for any length of time[370].

Recommendations

4.2.1.1 Lighting within cages during the light phase should be maintained at a luminance below the threshold of aversion for mice. It is important to keep lighting type intensity and duration constant to avoid experimental variability. For most pigmented strains this is below 60lux and for albino strains it is below 25lux. To enable staff to perform tasks in mouse rooms it may be necessary to increase the lighting to 210lux at working height for the period while workers are in the room.

4.2.1.2 Light intensity can be reduced by using recessed lighting consoles in the ceiling with fluorescent lights of about 25-36 watt and a low spectral intensity (wavelength). This can be achieved by using a low colour number, e.g. colour 33 tubes.

4.2.1.3 Shading should be provided over the top shelves of racks and cages and racks should be positioned in a way that protects mice in the top cages from overhead lights and provides more uniform light levels between cages on different shelves.

4.2.1.4 Lighting should be diffuse and uniform to avoid glare, heat clusters and fluctuating lighting conditions for individual cages.

4.2.1.5 If halogen lighting is used, a silica glass cover must be interposed between the bulb and mice to minimise genotoxic and carcinogenic effects.

4.2.1.6 If mice are observed during the dark phase red or sodium lamps should be used to minimise any disruption to their nocturnal activities.

4.2.2 Light Cycles

Principles

(i) The circadian clock drives 24 hour variations in a range of physiological and behavioural parameters in mammals, including mice[371]. For example, processes that regulate growth, metabolic, endocrine, and immunological parameters in mice are affected by circadian rhythms[372, 373]. Circadian rhythms are predominantly synchronised by the environmental light:dark cycle[374] and the visual perception of light[375].

(ii) Exposure to constant light, as may occur with a faulty light clock or timer, may be stressful for mice. Male BALB/cAnNCr1BR mice exposed to continuous light for a week had increased urine corticosterone:creatinine ratios, and demonstrated a shorter latency to their first agonistic encounter when compared with controls[376]. In addition, these mice had increased weight, despite eating and drinking less than controls. However effects may vary significantly in different strains and even in mice of different gender. Female transgenic growth hormone mice exposed to continuous light over a lifetime grew faster, lived longer and had increased production efficiency than those exposed to a 12:12 light cycle[377]. Exposure to constant light appeared to reduce pro-viral DNA in male BALB/c-H-2k mice inoculated with murine leukaemia virus[378]. Constant light delayed onset of sexual maturity, reduced the rate of weight gain and was associated with irregular activity patterns in female ICR/Alb mice when compared with controls[379].

(iii) The continual process of renewal of retinal photoreceptors (rods and cones) is influenced by the light:dark cycle[470]. This may explain why lack of a dark cycle is a causative factor in retinal degeneration of laboratory rodents including mice.

(iv) Continuous darkness was associated with an increase in severity of arthritis in DBA/1 mice[380], although it was also associated with a decrease in aggression between male sea:ddy mice[381].

(v) Changes in light:dark cycles are stressful for mice. For example, male BALB/cJ, CBA/J and C57BL/10J mice subjected to reversal of the light:dark cycle every four days for 76 days then every two days for an additional 54 days had increased circulating plasma corticosterone and decreased barbiturate sleeping time compared to controls[382]. Lengthening (16:16 L:D)) or shortening (5:5 L:D) the cycle led to increased locomotor activity and corticosterone in male ICR mice[374]. Expanding cycles beyond a 24 hour period may influence food intake and locomotor activity[372]. Advancing the onset of the light cycle by eight hours every second day modified the expression of molecular clock genes and genes involved in carcinogenesis and tumour progression, accelerating tumour growth[383]. In the same study, altering meal times to coincide with the onset of light and darkness helped reduce this effect.

(vi) Mice may require a long period to adapt to changes in light cycles. Male BALB/c, C57BL/6J and CB6 mice subjected to a sudden shift in the light:dark regime (from lights on from 0800 to 2000hrs with half light from 0730-0800 and again at 1930 to 2000hrs (the LD regime) to the reverse, that is, lights on from 2000 to 0800 (the DL regime) demonstrated significant variation in immune parameters between strains, even after five weeks[373]. Daily mean thymic indices and weights, as well as splenic index and weight, were significantly higher in LD mice than their DL counterparts. In addition, the mean daily number of peritoneal leucocytes was significantly lower in LD mice. CB6 mice kept under DL conditions gained more body weight than CB6 and other (BALB/c and C57BL/6J) LD mice.

(vii) While information about the impact of light contamination during the dark cycle on mice is sparse, rat studies suggest that light leaks can have a profound impact on experimental data. For example, minimal light leaks of 0.2lux during an otherwise uninterrupted dark phase inhibited rat melatonin secretion, increasing the rate of tumour growth and lipid uptake[384].

(viii) Flickering light has been shown to be a potent stressor in rats. In one study, exposure to 80 Lux of flickering light for 30 minutes was associated with elevated serum corticosterone and other biochemical markers of stress[385]. Whilst there are no equivalent studies in mice, studies referenced above indicate that mice are sensitive to changes in light and may therefore experience stress when exposed to flickering light.

Recommendations

4.2.2.1 A semi-natural light cycle of 12:12 or 10:14 hours light:dark is suggested. Variations in the light:dark cycle to mimic seasonal change could be considered.

4.2.2.2 The use of dimmers in mouse rooms is suggested to allow the creation of twilight periods between the light and dark cycles.

4.2.2.3 A change in light cycle should be followed by an acclimatization period before commencing a study.

4.2.2.4 Cycles may be disturbed if lighting clocks or timers malfunction. Clocks and timers should be checked regularly. In the event of a disturbance mice should be allowed an additional acclimation/habituation period, as disruption to the light cycle is a source of experimental variability.

4.2.2.5 Care should be taken to prevent light leaks in animal rooms during the dark phase.

4.2.2.6 Lights should be checked for flickering and any flickering rectified. Light intensity should also be monitored

4.3 Temperature

Principles

(i) The thermal biology of the laboratory mouse has been extensively investigated[386-391]. The ambient temperature at which laboratory mice are kept can affect metabolism, cardiovascular function, motor activity, growth and development, body and organ weights, consumption of food and water, haematology and serological parameters, susceptibility to toxins, immunocompetence, reproduction, sleep depth, and behaviour in relation to cohabitants[2, 326, 386, 388, 389, 391, 392].

(ii) In-cage temperature is influenced by factors including, but not limited to, cage design and construction, the position of a cage within a rack and a room, the position of the cage within the flow of air, ventilation rate, presence and type of bedding and/or nesting materials and stocking density. For example, heat dissipates rapidly from cages constructed with a wire mesh floor.

(iii) Mice maintain their core body temperature by a range of mechanisms including varying metabolic rate, shivering, non-shivering thermogenesis[393], increased physical activity[391], grooming (spreading saliva on fur for evaporative heat loss) and thermotropism including huddling with cohabitants[225, 388]. Thermal preferences may vary between single and group-housed mice[390, 393] and may be influenced by sex, current behaviour and time of day[471]. Mice may create habitats with a desirable microclimate by burrowing or nesting[390].

(iv) Huddling allows group-housed mice to reduce cold stress by thermoregulating as one larger animal with a smaller surface area, thus less heat loss, than that of the total number of mice[393]. Even at a housing temperature of 28°C, thermogenic activity of brown adipose tissue was greater in singly housed mice than those housed in pairs or groups of six[393]. Male MA_fSp mice deprived of the opportunity to huddle with cage-

mates consumed significantly more food than their counterparts who were allowed to huddle[225].

(v) Unlike rats, which select an ambient temperature below their thermoneutral zone, mice select an ambient temperature consistent with minimal metabolic expenditure[387, 389]. Decreasing the ambient temperature below the thermoneutral zone is associated with an elevation in metabolic rate, while increasing the ambient temperature above the thermoneutral zone is associated with an increase in evaporative water loss[387].

(vi) A review of studies of laboratory mice found that they have a thermoneutral zone ranging from 26 to 34°C[388]. Strain differences (for example hairless strains that may have a thermoneutral zone at higher ambient temperatures) may account for this large range[390]. Mice kept at lower temperatures may therefore be subjected to varying degrees of cold stress[389].

(vii) One study showed that as the ambient temperature decreased from 30°C to 26°C, heart rate, mean blood pressure, pulse pressure and metabolic rate – as measured by radiotelemetry - increased[391]. Even small (a few degrees Celsius), incremental changes in ambient temperature between this range can lead to significantly higher blood pressure, heart rate and metabolic rate. Because of their higher surface area to bodyweight ratio, mice are approximately twice as sensitive to changes in ambient temperature than rats kept in the same conditions[391]. Mice housed at 23°C consume more calories than those housed between 29 and 33°C and contract their heart approximately 150 times more each minute, or 200,000 extra beats per day, than those housed between 29 and 33°C[326]. For these reasons researchers must pay particular attention to the effects of ambient temperature during studies of cardiovascular function in mice.

Figure 4.3.1 Hairless strains may have a thermoneutral zone at higher ambient temperatures than their furred counterparts.

(viii) Ambient temperature influences sleep under normal conditions, as well as following sleep deprivation and influenza infection. Thus under baseline conditions, adult male C57BL/6J mice spent more time in slow-wave sleep at 30°C compared with those kept at 26°C[2]. Additionally, mice kept at 26°C spent more time in rapid eye movement sleep than those kept at 22°C. Mice infected with influenza displayed hypothermia, reduced locomotor activity and increased slow wave sleep at 22°C. These effects were increased at 26°C but attenuated at 30°C. The findings demonstrate that data collected from mice housed at different temperatures may vary depending on the interaction between the ambient temperature and the condition of the animal.

(ix) In one study the optimal ambient temperature for reproduction, growth and development of JCL-ICR mice housed in acrylic cages with wood shavings for bedding ranged from 20-26°C[386]. Further studies are needed to determine whether behavioural thermal preferences correspond with optimal ambient temperature for reproduction[389, 394].

(x) Variations in environmental temperature outside the compensatory capacity for mice will affect reproductive performance with decreased litter size, increased embryonic deaths and impaired growth, and cause significant variation in food and water intake and haematological and biochemical parameters. JCL-ICR mice housed in acrylic cages with wood shavings for bedding at temperatures above 28°C demonstrated decreased delivery rate, litter size and weaning rate compared with mice housed in similar conditions with an ambient temperature between 12 and 26°C[386].

(xi) Mice exposed to high ambient temperature (34°C and 35.5°C) demonstrated increased water intake, decreased food intake, weight loss[392, 395] and corresponding reduction in the weight of individual organs[392]. Male ddY mice exposed to an ambient temperature of 38.5°C and relative humidity of 85 per cent for 60 minutes each day for a fortnight demonstrated an increased packed cell volume, and increased levels of corticosterone and vasopressin in the blood[395]. The humoral immune response to sendai virus antigen was suppressed, suggesting reduced resistance to infection[392].

(xii) Exposure to a high ambient temperature may lead to frank infertility, as well as subtle effects on fertilisation, embryo growth and embryo development[396]. Male C57BL and CBA mice exposed to a microclimate of 36°C for two twelve-hour periods on successive days were less likely to fertilise females[397]. Of the mated females that did become pregnant, litter size was reduced. Furthermore, in *in vitro* tests a smaller proportion of oocytes were fertilised by spermatozoa from heat exposed males, and fewer spermatozoa penetrated the ova. Exposure of pregnant mice to temperatures of 43°C for one to twenty hours led to high maternal mortality, abortion and/or foetal resorption[398].

(xiii) JCL-ICR mice housed in acrylic cages with wood-shavings for bedding exposed to a low ambient temperature (12°C) demonstrated decreased delivery rates, decreased body weight, reduced water intake, and increased heart, liver, kidney and lung weight than mice housed between 20 and 26°C[386].

(xiv) When exposed to a thermal gradient, the selection of ambient temperature by both single and group housed CD-1 mice demonstrated a circadian rhythm[389, 391] with relatively warm temperatures selected during the middle of the light phase corresponding with minimal motor activity[389].

(xv) When exposed to a thermal gradient, single-housed aged CD-1 mice (11 months old) selected higher ambient temperatures (1.0°C warmer during the light phase and 1.5°C during the dark phase) than group-housed mice[389], possibly due to reduced ability to compensate for lower ambient temperature by huddling with cohabitants or increasing motor activity. It should be noted that these tests were conducted while mice were on a wire-mesh floor. Mice housed on wire mesh floors prefer and may require higher ambient temperatures than mice house in plastic cages with or without bedding[75]. Adult female BALB/cBYJIco, hairless Crl:SKH2(hr/hr)BR and C57BL/6JIco preferred a combination of wire mesh flooring and a high temperature (28°C). However, when the temperature of the wire-mesh floored cage was 24°C, all strains preferred a cage with bedding and a temperature of 21°C.

(xvi) Gestating and lactating dams have reduced thermoregulatory ability, as thermoregulatory responses are compromised post-conception to meet the metabolic and nutritional needs of foetuses/pups[388].

(xvii) Type and volume of bedding material can have a significant impact on thermoregulation. Female CD-1 mice provided with 7-10cm of deep wood shavings was associated with a significantly higher (1°C) core body temperature during the day than mice provided with a thin layer of shavings or chips [390]. Mice housed with bedding and nesting material that does not allow burrowing may therefore exhibit increased thermoregulatory lability.

(xviii) Mice generate heat within a cage. The temperature inside a cage may be several degrees higher than room temperature. For example, cages housing groups of four male C57BL/6J mice were two to three degrees higher that the room[399].

(xix) Under laboratory conditions, the ability of mice to control their environmental temperature has been largely replaced by external systems under human control. Strategies which enable mice to regulate or choose their microclimate, such as the provision of nesting or bedding materials, in-cage shelters and compatible cage companions, should be provided.

Recommendations

4.3.1 A room temperature range for mouse housing between 20 and 26°C is recommended. Consideration of the strain of mice used (for example hairless or obese strains) and procedures that may disrupt thermoregulatory ability (for example anaesthesia, viral inoculation) should be taken into account.

4.3.2 Significant fluctuations in temperature should be avoided. In particular, ambient temperature must be carefully controlled where cardiovascular parameters and sleep are assessed.

4.3.3 Mice should be provided with nesting and bedding materials, an in-cage shelter and compatible cage companions to allow them to select an appropriate microclimate, particularly for sleeping.

4.3.4 Special attention should be given to those circumstances where the mouse's thermoregulatory ability is altered or compromised. Cage temperature for lactating mice and pups up to three weeks of age should be at the higher end of the recommended range (24-26°C).

4.3.5 Ambient temperature should be monitored within the cage and at various points within the room to monitor variation so as to optimally manage the microenvironment.

4.3.6 Adjusting the ambient temperature may be a potential approach to promoting recuperation following sleep deprivation and mitigating the effects of viral infection. For more information see Jhaveri et al[2].

4.4 Humidity

Principles

(i) Relative humidity is important to the health and well-being of laboratory mice as it influences their capacity to thermoregulate as well as playing a role in the transmission of pathogens[400], [401]. Evaporative heat loss is essential for core body temperature homeostasis in mice[329]. Environmental temperature and humidity act together on the ability of mice to thermoregulate[400].

(ii) The type of housing, stocking density and husbandry practices can significantly alter intra-cage humidity. For example, the relative humidity in polycarbonate cages was higher (53.2 +/- 9.6 per cent) than in stainless steel wire mesh cages (50.1 +/-11 per cent)[113].

(iii) Low humidity (15.5+/-3.8 per cent) was associated with alterations in tear secretion, goblet cell density and susceptibility to dry-eye related ocular surface clinical signs including increase in corneal fluorescein staining[402]. The study indicated varying susceptibility among different strains, with C57BL/6 mice showing a 47 per cent reduction in tear secretion compared with a 26 per cent reduction in BALB/c strains. Low relative humidity is associated with ringtail in rats and mice[401] [11]. Low relative humidity (25 per cent) combined with low ambient temperature (16°C) was also associated with the development of ringtail in a colony of Swiss albino mice[403]. Relative humidity of 10 per cent was associated with increased contact hypersensitivity to 2,4,6-trinitrochlorobenzene in C57BL/6 mice than those exposed to a relative humidity of 80 per cent[404]. Housing mice at a relative humidity of below 40 per cent adversely affected survival to weaning and growth rates[401]. Low humidity can lead to dehydration in young mice[401, 405].

(iv) High humidity can enhance the proliferation of bacteria and ammonia production in cages[406, 407], placing mice at greater risk of infection. High relative humidity prevents desiccation of urine and faeces, resulting in proliferation of urease-positive bacteria and subsequent production of ammonia[408]. In one study, ammonia was produced at approximately three times the rate in housing at a relative humidity of 75-80 per cent than in housing at a relative humidity of 30-35 per cent when tested five days after the last bedding change[406].

(v) Survival of young mice to weaning tends to be better at higher levels of humidity. As many as 79.8 per cent of young mice housed at a relative humidity of 70 per cent survived to weaning, as compared to 56.1 per cent at 40 per cent relative humidity[401]. The difference was statistically significant.

(vi) In a previous review of guidelines for the housing of rodents in scientific institutions around the world, a relative humidity of 55 per cent +/- 15 per cent was widely agreed upon[409].

Recommendations

4.4.1 A relative ambient humidity at the level of mouse cages of 55 per cent +/-15 per cent (40-70) is recommended for adult mice.

4.4.2 A relative ambient humidity at the level of mouse cages of 50-70% is recommended for young mice prior to weaning.

4.5 Air quality and ventilation

Principles

(i) A single mouse breathes approximately 35 litres of air per day - considerably more than the amount of food and water they consume put together[400]. Air quality and composition is therefore important for the well-being of the animal and experimental outcomes.

(ii) Air can contain particles and/or volatile substances that can irritate and damage the respiratory system, skin or mucous membranes, or be absorbed and cause systemic effects[329]. The level of exposure to these contaminants in the environment can have a major impact on mouse health[400, 405] and will be influenced by the relative humidity in which this occurs, the turbulence of air within the cage, the presence or absence of drafts, species and strain of animal used, stocking density, and sanitation[405].

(iii) Air quality is largely affected by the concentration of micro-organisms, dust particles and noxious gases, in particular ammonia and carbon dioxide. Ventilation within an animal room is affected by the type of supply air diffuser used, its orientation, air temperature and moisture content; room ventilation rate; location and number of exhausts; room pressurisation; rack configuration and cage density and room dimensions[410].

(iv) Ammonia is formed by urease-producing bacteria or bedding which contains heat-labile urease-activating enzymes which convert the urea present in faeces and urine into ammonia[329]. High intra-cage humidity increases ammonia levels[411]. Ammonia is a potent irritant to the mucous membranes of the eyes, skin and respiratory tract[408]. It can cause changes including a reduction in the number of cilia on respiratory epithelial cells, hyperplasia of respiratory epithelial cells, as well as formation of glandular crypts in respiratory and olfactory epithelium[329]. These changes reduce the efficacy of respiratory tract defence mechanisms, rendering mice more vulnerable to pathogens. One study suggested that elevated intra-cage ammonia levels may impair embryo production in superovulated mice[412].

(v) There is no agreement in the literature on exposure limits of mice to ammonia. Many investigators assume that concentrations of over 25ppm are harmful in mice because lung pathology has been reported in rats exposed to ammonia levels above 25ppm[413, 414], however the rats were inoculated with respiratory tract pathogen mycoplasma prior to exposure to environmental ammonia. Published data showing adverse outcomes may have been confounded with underlying infectious agents such as

mycoplasma and sendai virus[134]. The threshold limit value for human exposure is 25ppm over a 40 hour week[415], however there is significant species variation in tolerance to ammonia. While humans cannot safely tolerate ammonia concentrations of 100ppm for over one hour[416], mice did not indicate aversion nor show signs of respiratory tract irritation at 500ppm[416, 417]. In another study, female BALB/c/Bkl mice exhibited no clear aversion to a chamber containing 4, 30, 56 or 110ppm of atmospheric ammonia for a period of two days[418].

(vi) Studies of chronic ammonia exposure in mice are difficult to compare due to variation in mouse strain and nature of exposure to ammonia (static level versus progressive build-up)[407]. It is possible that mice can be exposed to ammonia levels above 25ppm[418], and may be exposed to such levels within days of cage cleaning[419]. C57BL/6J mice exposed to levels exceeding 25ppm of ammonia had histologically normal nasal passages and eyeballs[71].

(vii) Some cleaning products, particularly disinfectants, contain ammonia.

(viii) Carbon dioxide is a respiratory and cardiovascular stimulant with the potential to act as an asphyxiant by displacing oxygen[408].

(ix) Other volatile chemicals such as those associated with bedding material can also result in physiological alterations in mice (see Section 2.7 Bedding).

(x) Air quality, air flow, temperature and humidity can differ significantly between the macroenvironment and the microenvironment depending on room and cage ventilation systems[406, 420], depending on bedding type, cage cleaning frequency, stocking density and ventilation rate[418]. Adequate ventilation of the macroenvironment (the mouse room) does not equate to adequate ventilation of the microenvironment (the cage)[329, 399], particularly where filter-tops are used on cages. In one study, increasing room ventilation above 5 air changes per hour did not improve ventilation of the cage microenvironment and had the negative consequence of lowering room relative humidity to 22 per cent[399].

(xi) Mice generate a considerable heat load which creates a thermal updraft in the room. In filter-topped cages, the heat load of the animals is the principal factor driving intra-cage ventilation. In one study, occupied mouse cages generated at least 10 air changes per hour in an unventilated room[472].

(xii) The position of a cage in a rack, and the position of the rack in respect of an air source, affects cage ventilation. In one study, the middle cage on the top row of a rack directly below an air diffuser had significantly higher ventilation than all other cages on that rack[399]. Cages on the bottom of the rack had the lowest ventilation rates. In this study, increasing the room ventilation rate increased intra-cage ventilation for cages on the top row of

a rack, however ventilation of cages on middle and bottom shelves did not change as ventilation in the room changed from 0-20 air changes per hour.

(xiii) Exhausts located closer to ground level ventilate cages slightly better than ceiling or high-level exhausts when cages are placed parallel to the walls[410]. Low level exhausts reduce relative humidity and ammonia generation rate because air temperature is higher.

Recommendations

4.5.1 The number of room air changes per hour needs to be adjusted to keep air quality and humidity at acceptable levels within cages. Room ventilation rates of 15-20 ACH may be needed depending on stocking densities.

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4.5.2 Racks should be positioned in a room so as to optimise air exchange and avoid animals being exposed to draughts.

4.5.3 Air quality, air flow, temperature and humidity should be measured both in the room and within cages.

4.5.4 Exhausts should be installed close to ground level when cages are placed parallel to walls.

4.5.5 Intra-cage ammonia levels should be kept at 25ppm or below.

4.5.1 Static isolator cages and filter tops

Principles

(i) Static isolator or filter-topped cages have been used to maintain specific pathogen free mice[329, 421]. They result in containment at cage level and may protect the cage environment and mice from microbial contamination, reducing airborne infections and diseases like neonatal diarrhoea. Unlike traditional open cages with wire lids, static isolator or filter-topped cages decrease spillage of contaminated food and bedding (potential fomites) into the cage from surrounding cages[422].

(ii) In one study, there was an increase in growth rate over a seven day period in female Tac:(SW)fBR mice housed in static isolator cages compared to those housed in standard cages despite no significant differences in food or water consumption[423]. This may be due to a variation in activity levels, which were not measured in this study. When compared with mice housed in cages on ventilated racks, mice housed in static isolator cages had lower body weight gain and lower water consumption[420].

Figure 4.5.2.1 Example of a static isolator cage.

(iii) Studies have shown that static isolator or filter-topped cages reduce intracage ventilation, leading to accumulation of gaseous pollutants, in particular ammonia (NH_3) and carbon dioxide (CO_2)[329, 408, 420]. Carbon dioxide concentrations of up to 4000ppm higher than those found in the macroenvironment have been reported[424]. This effect increased with an increase in cage population. As the population of female RFMfICR/UnWg mice in three different filter-topped cages doubled, intracage carbon dioxide levels rose by 1.5-2 times, while the concentration of ammonia increased exponentially[422].

(iv) Relative humidity is higher in static isolator cages when compared with cages without microisolator or filter tops[411, 423], or with ventilated cages[420, 421]. This is due to decreased water vapour transfer, which occurs primarily through diffusion through the filter[421]. In one study, relative humidity was 20 per cent higher in cages with microisolator tops compared to those without[411]. Ammonia production rates increase significantly in a higher humidity environment [410] (see section 4.4 Humidity).

(v) Humidity, and therefore ammonia concentrations, in both cages and rooms can be reduced by increasing the temperature of supply air. Raising supply air temperature from 18.8°C to 22°C at 15ACH raises the room temperature approximately 3°C and intra-cage temperature by 2.7°C, reducing ammonia concentrations by up to 50 per cent[410].

(vi) When compared with individually ventilated cages, static cages had higher relative humidity, higher levels of ammonia and higher levels of carbon

dioxide[420]. With increasing mouse population density these cages can become unlivable[3].

Recommendations

4.5.2.1 Static isolator cages must be cleaned once a week to avoid excessive ammonia and carbon dioxide levels.

4.5.2.2 Supply air temperature should be maintained at 22 degrees, and room ventilation at 15ACH, to minimise ammonia concentration.

4.5.2.3 The population density of mice in static isolator cages should be kept to a minimum.

4.5.2 Individually ventilated cages

Principles

(i) Individually ventilated cage systems increase ventilation rates and improve intracage air quality by supplying conditioned air directly into the cage[408], whilst maintaining mice in a separate microbiological unit[425]. A number of systems provide high-efficiency-particulate-air (HEPA) filtration. Other advantages of individually ventilated cages include that intracage ventilation is independent of cage location within the macroenvironment; the ability to maintain low intracage ammonia and carbon dioxide concentrations; the ability to maintain a dry environment; the ability to increase cage density and reduce allergens and odours in the animal room[426]. Forced ventilation reduced intracage humidity, as well as reducing in-cage build up of ammonia and carbon dioxide[407, 408, 421]. In a comparison of static isolator cages with ventilated systems, all ventilated systems had significantly lower ammonia accumulation compared with static isolators[408].

Figure 4.5.3.1 Example of individually ventilated cages.

Figure 4.5.3.2 Example of individually ventilated cages.

Figure 4.5.3.3 Example of individually ventilated cages.

Figure 4.5.3.4 Example of individually ventilated cages.

(ii) There are a variety of cage ventilation systems available. The method of air supply and exhaust from the cage and rack will influence air velocity and airflow patterns within the cage[407]. Ventilation rates vary from 25 to 120 air changes per hour and can be maintained with either positive or negative intracage pressure[427]. When individually ventilated cages are under positive pressure, particulate matter may spill into the macroenvironment, exposing personnel. Taking into account heat load, odour and macro-environment particulate concentration, the ideal set-up involves individually ventilated caging under negative pressure with all air exhausted out of the room via a heating, ventilation and air-conditioning (HVAC) system[427].

(iii) Relative humidity levels in ventilated cages tend to be lower than those in static microisolator cages, however they may not be as low as relative humidity in open air cages or the macroenvironment[421]. The amount of moisture that can be absorbed by air passing through the cage is dependent on the volume and temperature of the air. Sources of moisture within the cage, including animals, soiled bedding and water bottles exceed the capacity of air to remove the moisture as it passes through the cage[421]. In contrast, traditional open-air cages have minimal impediment to vapour transfer from the cage microenvironment to the macroenvironment. Intracage humidity in ventilated cages is thus affected by air exchange or ventilation rate[420].

(iv) Temperature in cages of pair-mated C57BL/6J mice decreased with increasing ventilation, although temperature in cages of trio-mated animals had no clear trends[407].

(v) Ventilation can be controlled by setting the rate for number of air changes per hour (ACH). In a study of the microenvironment of different populations of C57BL/6J mice with different ventilation rates and varied frequency of bedding changes, ammonia concentration remained <25ppm for 30, 60, 80 and 100 ACH in cages housing adult males when bedding was changed weekly[426]. When frequency of bedding changes was reduced to fortnightly, 60ACH was sufficient to maintain ammonia concentration below 25ppm in cages housing adult males. However, in cages housing breeding groups consisting of 2 adult females, 1 adult male and up to 9 pups 100ACH was required to keep the ammonia concentration below 25ppm. In some of these cages, additional moisture thought to have come from leaky water bottles increased the RH to more than 61% and the ammonia concentration rose to 150ppm[426]. When frequency of bedding changes was reduced to fortnightly, 60 ACH was sufficient for cages housing adult males. 100 ACH was necessary for cages housing breeding trios (two female and one male adult) and pups. In cages with a relative humidity exceeding 61 per cent, and a biomass of at least 200g (3 adult mice and 9 pups), ammonia concentrations exceeded 150ppm. When the frequency of bedding changes for breeding animals was reduced to fortnightly, mean ammonia concentrations were 25ppm for 100ACH and 50ppm for 30 and 60 ACH. Carbon dioxide concentrations increased as bedding grew soiled. The authors speculate that this effect may be due to the release of carbon dioxide as bacteria broke down faecal material in soiled cages. In a further study, the authors found that a regime of 30 ACH and weekly cage cleaning led to ammonia concentrations higher than those of mice living in microisolator cages[407]. Additionally, pre-weaning mortality was higher at 30 ACH than 60 or 100 ACH. Weanling weight was lower at 100 ACH when compared with 30-60 ACH, and pup mortality was increased when cages were changed weekly. The authors concluded that 60 ACH is optimum for mouse health and reproduction. Another study confirmed that longer periods between cage changing required an increase in ACH to maintain a lower ammonia level[428]. This study demonstrated that 20 ACH led to increased ammonia levels regardless of bedding type, when compared with 60-80 ACH. The author concluded that weekly cage changes would be required at 20 ACH.

(vi) High intra-cage ventilation could induce chronic stress or heat loss. The housing of male C57BL/6 mice in forced-air ventilated cages lead to reduced serum corticosterone levels and a suppressed delayed-type hypersensitivity reaction when compared to counterparts housed in static cages[429]. They also gained less weight than their counterparts, suggesting a chronic stress response. Depending on the location of air inlet, cage size and presence of nesting material BALB/c mice avoided the high ventilated cage compared to a low ventilated cage[473].

(vii) The provision of nesting material (see Section 2.8 Nesting Material) and/or an in-cage shelter[473] (see Section 2.9 In-cage shelters) may offset some of the effects outlined in vi. Female outbred Crl:CD1(ICR) mice housed in individually ventilated cages with 70 ACH under positive pressure preferred nest boxes located on the floor of the cage, where the ventilation

rate was lower[430]. Interestingly, use of a cage-lid mounted nest box closer to the ventilation source increased with time.

(viii) Individually ventilated cage systems or racks generate significantly more noise than background room levels. In a 1996 study, noise level at lower Hz values was significantly higher in ventilated cage systems, while at higher Hz values noise levels were significantly lower within cages for most systems[408]. While investigators concluded that noise levels produced at the Hz levels measured probably had little impact on the female Tac:(SW)FBR mice in the study, the measuring device did not allow for quantification of noise levels over 16kHz. It is thus possible that some ventilated systems studied produced noise at higher frequencies that could adversely affect mice.

(ix) External acoustic stimuli are attenuated with mice housed in individually ventilated cage systems. Male C3H mice housed in individually ventilated cages had reduced startle thresholds when compared with controls[102]. The authors speculate that these mice may react more sensitively to acoustic stimuli because they have been raised in an acoustically attenuated environment, and that the response may indicate anxiety. This effect was strain specific, and did not occur in male B6J mice housed in the same conditions.

(x) Housing of male C3H and B6J mice in individually ventilated cages was associated with a reduction in activity and an increase in anxiety-related behaviours[102]. In addition, B6J mice had reduced latency to grooming.

(xi) Ventilation systems can impact on reproductive parameters. When the effect of individually ventilated versus static isolator and open racks on the breeding performance of DBA/2 mice was investigated, fewer mouse pups were born in individually ventilated cages[431]. Individually ventilated cage-housed mice had their first litters later, and had a higher abortion rate, than those housed in static isolator or open cages.

(xii) Other disadvantages of individually ventilated caging systems include substantial cost for purchase, operation and maintenance[408]. These systems require an uninterrupted power source to ensure constant air supply. Some systems may be difficult to manipulate and clean[408].

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Recommendations

4.5.3.1 A minimum of 5 ACH may be sufficient to maintain room air quality but should be determined on engineering advice and in accord with expected workflows in the room.

4.5.3.2 The choice between positive and negative pressure in ventilated cages should depend on study requirements and the protection of animals and personnel - Ideally ventilated systems should be set up so that individual cages are under negative pressure with all air exhaust entering out of the room via a heating, ventilation and air-conditioning system.

4.5.3.3 For individually ventilated cages housing non-gravid adult mice, a ventilation rate of 60 ACH is recommended if cages are changed fortnightly - rigorous testing may show good air quality results for some systems at lower flow rates.

4.5.3.4 For individually ventilated cages housing breeding trios and/or pups, a ventilation rate of 60-100 ACH is recommended - rigorous testing may show good air quality results for some systems at lower flow rates.. Cages should be changed fortnightly.

4.5.3.5 It is imperative that nesting material and an in-cage shelter are provided in ventilated cages.

4.5.3.6 In individually ventilated cages cleaning regimes should be managed to maintain ammonia levels within a cage below 25ppm.

4.5.3.7 Investigators should be aware of the potential impact of individually ventilated cages on the emotionality and behaviour of particular mouse strains. For example, different systems may produce different levels of noise and draught, some of which may be aversive or harmful to mice.

4.5.3.8 As air supply can be interrupted by power failure, installation of an air-flow controller in the supply air duct (positive pressure) or exhaust duct (negative pressure), which is connected to an alarm system, is essential.

4.6 Sound and vibration

Principles

(i) There are sounds in animal rooms which may have negative effects on mice, including sounds which cannot be detected by the human ear.

(ii) The range of frequencies audible to mice at a standard sound intensity of 60 decibels (dB) is from 2300Hz to 85,500Hz[38]. The range of good hearing (frequencies audible at 10dB) is affected by the size and position of the external ear or pinna[38], which may vary between strains.

(iii) Mice produce ultrasonic (above 20,000Hz) vocalisations[39, 339] and are therefore probably sensitive to high sound frequencies which cannot be detected by humans (ultrasound).

(iv) Sound can have a negative impact on behavioural patterns and physiologic responses in mice and is used as a stressor in animal studies[432-434]. Environmental sounds in laboratory facilities can alter endocrine, reproductive and cardiovascular function, alter sleep/wake cycles and mask inter-species communication[433]. High levels of sound may predispose some strains of mice to audiogenic seizures[435, 436] or may induce hearing loss and/or damage to the auditory apparatus[436]. Loud sounds may mask or hinder communication between mice[4, 329]. They may also trigger cannibalism in female rabbits[437]. Other examples of the effects of audiogenic stress in mice include:

- Significantly decreased pregnancy rate in CF-1 mice exposed to loud sounds in both pre and post implantation period[438];
- Embryocytotoxicity in CF1S mice[439] and embryolethality in CF-1 mice[438];
- Decreased fertilisation, increased embryonic mortality and reduced embryo size in SWt/Dt mice[440, 441];
- Foetal intrauterine growth retardation in Jcl:ICR mice[442];
- Abortion in CBA/J mice[260];
- Teratogenic effects including cranial haematoma, dwarfed hind limbs tail defects[441] in Swiss Webster mice and cleft palate, polydactylia and encephalocele in ddN mice[443];
- Reduced weight gain in CF#1 weanlings[435];
- A two-fold increase in water-intake and failure to eat in old (20-24 months) C57BL/6J mice[444];
- Reduction in eosinophils in ddN mice[443];
- An increase in activity and aggression during sound or noise stress in C57BL/6J mice[444];
- A reduction in exploratory activity during sound or noise stress in male and female Jax A mice, with an increase in general activity immediately following sound or noise stress[445];
- Increased face and genital washing and body grooming[445];
- Increased weight of adrenal glands with increased width of the fasciculate zone[445];
- Alteration of free oxygen radical production by alveolar macrophages in old C57BL/6J mice (20-24 months) and peritoneal macrophages in young C57BL/6J mice (9-11 weeks)[444];
- A reduction in the splenic lymphocyte population and increased plasma cortisone levels in adult female C57BL/6 mice after acute exposure[446].

Sound and vibrations within animal facilities may therefore adversely impact the welfare of mice, and may also be a source of variance in data[436, 474].

(v) Ultrasound can be produced by equipment commonly found in laboratories, including temperature regulating devices, electronic equipment including computer monitors, video recording equipment and telephones, cage cleaning equipment and vacuum hoses as well as running water and squeaky door hinges, chairs or trolley wheels[447]. If unaccounted for, this can have a detrimental effect on experimental outcomes.

(vi) Acute and chronic exposure to loud sounds may impact differently on mouse physiological parameters. For example, female C57BL/6 mice exposed to acute, unpredictable sound periods over one week decreased the splenic lymphocyte population and increased plasma corticosterone concentration relative to controls, while after four weeks no effects were found[446].

(vii) Human activity within the facility can generate irregular, loud sounds, for example the banging of a metal cage top onto a bench. These sounds generate a large amount of energy spread across a range of frequencies[448], and may also lead to audiogenic stress and associated adverse effects. For example, seizure susceptibility of weanlings was prolonged when they were exposed to extraneous sounds including hammering metal or barking dogs[435]. Sounds produced by using an electric drill and banging a garbage tin lid resulted in 90 per cent incidence of seizures in sensitised mice, compared with the 7 per cent expected incidence. The proximity of animal housing to construction sites may have significant, negative effects[435, 449].

(viii) Events susceptible to disruption by external auditory stressors such as construction or fireworks should be rescheduled as data may be invalidated by the use of highly stressed mice[449].

(ix) Routine monitoring of environmental sound and vibrations in laboratories may provide important information about changes that may affect animal welfare and experimental outcomes. Ultrasound can be easily monitored using bat detectors.

(x) Certain strains of mice are genetically predisposed to auditory dysfunction. For example, C57BL/6 and CD-1 mice suffer early-onset hearing loss[450] which can be exacerbated by environmental sounds[436]. Gender-associated hearing loss has been observed in B6 mice, with female mice older than six months exhibiting measurably more extensive hearing loss than males of the same age and strain[436]. Other inbred strains may exhibit degrees of hearing loss. An extensive list of known affected strains is available on the Jackson Laboratory's "Hereditary Hearing Impairment In Mice" website at www.jax.org/hmr/index.html These strains may be more sensitive to environmental sounds and therefore more likely to suffer from audiogenic stress. Furthermore, if auditory problems are not taken into account, behavioural assessment (for example response to an auditory stimulus) of these animals may be flawed[436].

(xi) Artificial background sound, for example a radio, piped music, a white-noise generator or white noise incidentally arising from an air-conditioning system, may be of some benefit in reducing the impact of sudden sounds, however there is no scientific evidence for this[449].

(xii) The use of music is controversial. It has been suggested that constant background sound, in the form of radio music at a volume of 85dB, may blunt the startle effect and reduce overall excitability in mice. However, mice froze or fled when exposed to a loud sound, irrespective of background music[451]. In another study, music reduced the suppressive effects of stress on immune parameters in male BALB/c mice[434]. In this study, mice exposed to audiogenic stress (100dB daily for 5 seconds/minute during a one or three hour period, over eight days) had reduced thymus, spleen and peripheral blood lymphocytes as well as a reduction in total thymus weight, compared with controls. These adverse effects were partially reversed in mice exposed to <40dB classical music

for five hours the following day. Exposure to music decreased the stress-related increase of plasma ACTH concentrations. In addition, music stimulated T-cell proliferation in unstressed mice. Exposure of Std:ddY mice to classical music at 65-75dB during the perinatal period (gestation day 14 to 60 days old) improved performance and reduced the incidence of errors in a maze task[452]. Music-exposed mice had reduced levels of brain-derived neurotrophic factor, and increased levels of tyrosine kinase receptor B and its target protein kinase 1 (PDK1). However, it may be difficult to assess adverse effects as music is a diffuse medium that is difficult for animals to avoid[449]. Care should be taken to avoid excessive volume. Furthermore, the above studies did not control for the effect of music on animal handlers. It has been suggested that mice may indirectly benefit from a radio if it has enrichment value for humans[4, 449]. No differences were found in mice between responses to a loud sound during exposure to classical music, pop music, no music and new age music, although more resting was observed in the new age group [451]

Recommendations

4.6.1 Investigators should familiarise themselves with the hearing range and any potential auditory dysfunction of the strain of mice being used.

4.6.2 Sources of sound including ultrasound should be considered when assessing sound levels to which mice are exposed. Environmental noise may be a source of variance which may confound results, necessitating the use of additional experimental animals. Computers, or any other equipment likely to emit high-frequency ultrasound, should not be used in rooms where mice are housed. If the use of such equipment is unavoidable then measures, such as packing the equipment in polystyrene foam plating, should be taken to dampen ultrasonic noises.

4.6.3 Sound measuring equipment including sound-level meters, condenser microphones, attenuators, amplifiers, weighting and filter networks must be capable of detecting sounds in the range of frequencies appropriate to the species/strain being used.

4.6.4 Because of the potential for adverse effects, unnecessary sounds or noise should be eliminated from facilities in which mice are kept. In particular, avoid sudden, loud sounds.

4.6.5 Individually ventilated cages and racks should be checked for vibration and vibration in animal rooms, especially of cages, should be eliminated.

4.6.6 Due to the vibrations created, placing motorised equipment on bench tops with cages should be avoided.

4.7 Cleaning

Principles

(i) Cleaning involves two components: handling (see section 3.4 Effects of Handling and Human Activity), and cleaning of cages. Cage cleaning and cage changing can be stressful for mice[336]. Cleaning may disrupt odour cues, expose mice to foreign odours and precipitate aggression. Cleaning may be complete or partial (only substrate/bedding is refreshed).

(ii) When given a preference between soiled or clean cages, group-housed 4 week old out-bred female NMRI mice preferred clean cages while individually housed mice did not demonstrate any preference[453].

(iii) Frequent cage cleaning may adversely affect mice. Daily cage changing depressed weight gain by an average of 20 per cent in individually housed male and female NMRI:Cpb mice, compared to control mice handled but replaced into an unchanged cage[454]. The growth-inhibiting effect disappeared in males after one week, whilst it persisted for at least a week in females. In another study, pup mortality was higher in C57BL/6J mice in individually ventilated cages changed every seven days, compared with cages changed at 14 or 21 days[407].

(iv) Aggression among male mice peaks after disturbances such as cage cleaning[28, 238]. In one study, partial cage cleaning (replacing sawdust) stimulated increased aggression frequency and duration in male CFLP mice[19], as compared with mice transferred to completely clean cages. Disturbing, but not refreshing, the substrate did not have a significant effect on the relative aggressive response. However, when nesting material was transferred to the clean cage, male BALB/cAnNCrIBR exhibited lower levels of aggression than controls who received new nesting material[28]. Interestingly, transfer of bedding material (sawdust) containing urine and faeces intensified aggression, leading to fighting. The different results may reflect strain differences, or the fact that mice in the first study were not provided with nesting material of any kind. The provision of nesting material itself reduces aggression (see Section 2.8 Nesting Material).

(v) Exposure to soiled bedding of mice of a different strain during cage cleaning may increase aggression. Male C57BL/6 mice exposed to urine scent of a different strain exhibited increased competitive aggression towards their cagemates[455].

(vi) The provision of an environmental enrichment object (a glass bottle) significantly reduced post cage cleaning aggression in BALB/c mice[311]. However, this reduction was reversed when the soiled object was transferred into a clean cage.

(vii) Individually ventilated cages require less frequent cleaning. The frequency of cage cleaning was reduced to once every 14 or 21 days without adversely affecting weanling weight, growth, plasma corticosterone concentration, immune function, breeder mortality and breeder

productivity in breeding pairs and trios of C57BL/6J mice[407]. See Section 4.5.3 Air quality and ventilation – individually ventilated cages).

(viii) Effective cage washing can be done using various methods either by hand or with machinery such as a tunnel cage washer. Disinfection may be by use of chemical disinfectants or high temperature water. The process has multiple steps commencing with removal of soiled bedding. Residual organic and inorganic matter such as urine salts and faeces can then be removed with hot water alone but the process may be more effective if a detergent solution is used. The final step is disinfection to destroy vegetative microorganisms. Effective disinfection can be achieved with wash and rinse water at a temperature in the range of 62 ºC - 82ºC or by autoclaving after thorough cleaning where cage level and higher barrier practices are in place[475].

Recommendations

4.7.1 The need for changing bedding depends on the type and amount of bedding used and air quality. Frequency of bedding changes will also be influenced by stocking rates, ventilation system, strains of mice used and particular disease conditions (for example, diabetes). As a guide, bedding is commonly replaced weekly or fortnightly.

4.7.2 Nesting material should be transferred from the old to the new cage during cleaning to minimise aggression. Note, bedding material soiled with urine and faeces should not be transferred to clean cages as this may exacerbate aggression.

4.7.3 Care should be taken to avoid contamination of cages with scents from different mouse strains. Cages should be cleaned thoroughly and steps taken to ensure soiled bedding or nesting material cannot fall into other cages. In addition, steps should be taken to ensure that male mice are not exposed to the urine of other male or female mice when temporarily removed from their social groups.

4.7.4 Plastic cages and bottles should be washed in hot (62-82°C) soft water with a manufacturer-recommended detergent solution. All residue must be removed prior to autoclaving as this may be baked onto the cage.

4.8 Monitoring of environmental variables

Principles

(i) Animal rooms in scientific institutions are technologically dependent and therefore vulnerable in the event of power failure or equipment breakdown[1]. Environmental variables including lighting, temperature, humidity, air quality and ventilation, sound and vibrations should be maintained within limits compatible with the well-being and good health of mice. To ensure this occurs, environmental variables in mouse rooms require regular monitoring.

(ii) Temperature and humidity of mouse rooms should be checked daily.

(iii) At the cage level, temperature, humidity and air quality are affected by the system controlling the air supply to each room, or in the case of individually ventilated cages, the air supply to each cage.

Recommendations

4.8.1 Mouse rooms should have temperature and humidity readings displayed in a position where staff can easily see them.

4.8.2 Regardless of centralised computer systems regulating the general environmental conditions, it is still essential to check these variables regularly in the room to indicate conditions at the cage level.

4.8.3 Sensors should be fitted to monitor and report malfunctions in ventilation, temperature and humidity control on a 24 hour basis, with automatic alarm activation and alerting of appropriate staff so that any unexpected variations can be identified and corrected.

4.8.4 On a larger scale, facilities must be equipped to detect hazards such as fire or entry of unauthorised persons.

4.8.5 Care should be taken that the operation of an alarm causes minimal disturbance to mice[1] (see Section 4.6 Sound and Vibration*)*

5.0 Identification and Records

5.1 Identification

Principles

(i) Clause 4.7.1 of the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* states in part:

> Animals must be identifiable, whether individually or in groups. Where possible, animals should be identified by the attachment of a label to the cage, container, pen, yard or paddock where they are kept. Otherwise, identification of individual animals may require a physical mark such as a tattoo, neckband, individual tag, or electronic numbering device such as a microchip…The method chosen should be the most appropriate for the species and project and result in the least pain and distress to the animal.

(ii) Ideally methods of identification should not be painful, not cause adverse reactions, not be uncomfortable and not likely to catch, tear out or be damaged by conspecifics. For example, ear-punch identification may be obliterated within weeks due to wound healing and/or trauma from inter-mouse aggression[3].

(iii) Tail tattooing resulted in transient increase in fluctuating asymmetry (a measure of developmental instability) in pups[456]. Furthermore, mice subjected to tail tattooing had traces of ink in their faeces for several days following the procedure. Whilst a toxic effect of the ink on fluctuating asymmetry was not ruled out by the study, it is possible that the mice

groomed the tail excessively following the procedure in response to pain. Ear tattooing may not be practical in mice, particularly juveniles or smaller strains where there is a risk of incomplete marking[457].

(iv) Amputation of a 2.5cm section of the tail resulted in long term (at least five weeks) thermal and mechanical hyperalgesia in the remaining part of t he tail as well as the hind paw in four to six-week-old male C57BL/6j and C57BL/6NHsd mice[458]. When a smaller (2.5mm) section of the tail was amputated, mice exhibited a transient reduction in climbing behaviour[456].

(vi) Swabbing tails with 70 per cent isopropyl alcohol to remove excess surface oil prior to marking was found to extend the life of surgical skin marker identification to ten days[459].

Recommendations

5.1.1 Where it is necessary to individually identify mice, the least invasive method that is compatible with the use of mice should be used.

5.1.2 Non-toxic dyes and permanent markers may be used on the fur and tail. These methods of identification usually need to be replaced every two to ten days. Swabbing the tail with 70 per cent isopropyl alcohol prior to marking is recommended to extend the life of marker identification.

5.1.3 Fur clipping may be used but needs to be carried out frequently.

5.1.4 Subcutaneous microchipping, tattooing and ear notching may be used where permanent identification is necessary. Note there is some transitory pain associated with applying these forms of identification. Anaesthesia or sedation and analgesia should be used in applying tattoos and ear notches. The method used for identification must be approved by the AEC.

5.1.5 Toe tip amputation is a painful procedure and should not be used – tail tip amputation is similarly painful and should not be used without the express permission of the AEC and with specific justification in each case.

5.2 Cage labels

Principle

(i) The Australian Code of Practice specifies that animals must be identifiable either individually or in groups (clause 4.7.1).

Recommendations

5.2.1 All cages should have labels attached to them that provide the following information, or cross reference to a central record in the same room containing this information:

** Mouse identification (strain, sex, number of mice);*

** Age (date of birth) of litters or of individual mice;*

** Approval number of project in which mice are being used;*

** Name, location and contact numbers of the chief investigator/teacher and, if applicable, other investigators/teachers using mice;*

** Name, location and contact details of staff associated with the housing and care of the mice;*
** Treatments / procedures;*
** Date arrived.*

5.3 Breeding Records

Principles

(i) Thorough record keeping is an essential adjunct to good observation. Accurate production data may indicate the presence of deleterious genes, or other early changes in the health status of a mouse colony or colonies that might otherwise go undetected.

(ii) ARRP Guideline 16: *Supervision of Animal Supply by Animal Ethics Committees* details the types of records required, and information that must be provided to the AEC on animal breeding activities.

Requirements

5.3.1 To assist in the monitoring and management of mouse breeding colonies, regular reports must be made to the Animal Ethics Committee, for review, on the fertility, fecundity, morbidity and mortality of all mouse breeding colonies. Reports should be submitted every six months, but may be required more frequently if deemed necessary by the Animal Ethics Committee. For further information refer to ARRP Guideline 16: Supervision of Animal Supply by Animal Ethics Committees.

5.3.2 Section 4.5.8 Australian Code of Practice for the Care and Use of Animals For Scientific Purposes states that the person in charge must maintain adequate records to allow effective management of the breeding stock including the detection of the origin and spread of disease. Records should include:

(i) the source, care, allocation, movement between locations, use and fate of all animals;
(ii) details of any diseases;
(iii) the fertility, fecundity, morbidity and mortality in breeding colonies; and
(iv) the health status, genetic constitution and physical environment of the animals.

6.0 Additional resources

Bradley Bays T; Lightfoot T and Mayer J (2006) *Exotic Pet Behaviour: Birds, Reptiles and Small Mammals*. Elsevier, St Louis MO – provides information about normal and abnormal behaviour of mice and other species kept in captivity. Designed for a pet-owners but contains useful information for laboratory staff.

Brown PA and Hoostraten-Miller (2004) Principles of aseptic rodent survival surgery: general training in rodent survival surgery – Part I. In *Laboratory Animal Medicine and Management*, JD Reuter and MA Suckow (eds). International Veterinary Information Service, Ithica NY (www.ivis.org) – online document incorporating images and Quicktime videos illustrating techniques, as well as providing guidelines and instruction for those performing aseptic rodent survival surgeries.

Brown PA and Hoostraten-Miller (2004) Principles of aseptic rodent survival surgery: general training in rodent survival surgery – Part II. In *Laboratory Animal Medicine and Management*, JD Reuter and MA Suckow (eds). International Veterinary Information Service, Ithica NY (www.ivis.org) – as above.

Fox JG; Anderson LC; Loew FM and Quimby FW (eds.) (2002) *Laboratory Animal Medicine, second edition*. San Diego, Academic Press (Elsevier Science) – provides an extensive chapter on Biology and Diseases of Mice, as well as excellent chapters on Transgenic and Knockout Mice and Selected Zoonoses.

Jennings M; Batchelor GR; Brain PF; Dick A; Elliot H; Francis RJ; Hubrecht RC; Hurst JL; Morton DB; Peters AG; Raymond R; Sales GD; Sherwin CM and West C (1998) Refining rodent husbandry: the mouse. Report of the Rodent Refinement Working Party. *Laboratory Animals* 32:233-259.

Hedrich H (ed.)(2004) *The Laboratory Mouse*. London: Elsevier – a comprehensive textbook covering the history, development, genetics, biology, pathophysiology and husbandry of laboratory mice.

Poole, TB (ed.) (1999) *The UFAW Handbook on the Care and Management of Laboratory Animals, 7th edition.* London: Blackwell Publishing – contains species-specific information on requirements of laboratory animals including mice.

Reinhardt V and A (eds.) (2002) *Comfortable Quarters for Laboratory Animals*, 9th edition. Animal Welfare Institute Washington DC. Available online at http://www.awionline.org/pubs/cq02/cqindex.html - contains a chapter by Chris Sherwin on mice in research institutions, as well as chapters on other species.

Richardson VCG (2003) *Diseases of Small Domestic Rodents*, 2nd edition. Blackwell Science Ltd, Oxford UK – provides a general overview of pet rodent husbandry.

Scott DW, Miller WH, Grifften CE (2001). *Muller & Kirk's Small Animal Dermatology*, Philadelphia: W.B.Saunders – contains an extensive, illustrated chapter on dermatoses of pet rodents, rabbits and ferrets.

Silverman J, Suckow MA, Murthy S (eds)(2000) *The IACUC Handbook*, Boca Raton, CRC Press – provides a question and answer format for common problems faced by institutional animal care and use committees. Whilst written for North American based investigators some of the information is useful to Australian investigators.

Van Zutphen LFM., Baumans V, Beynen AC (eds.)(2001). *Principles of Laboratory Animal Science.* Elsevier, Amsterdam.

Additional information as well as **guidelines on the housing of dogs, rabbits, rats and guinea pigs** in scientific institutions can be viewed at www.animalethics.org.au

Newcastle University in the UK hosts a website which provides multimedia tutorials on assessing the health and welfare of laboratory animals at www.ahwla.org.uk

The American Association of Laboratory Animal Science provides a **free, online course on mouse biomethodology**, "Working with the Laboratory Mouse." Visit www.aalaslearninglibrary.org. Note that the course contains some material that does not apply to investigators based in Australia.

The **Mouse Genome Database** (MGD), as well as additional information on the genetics, genomics and biology of the laboratory mouse, can be found at www.informatics.jax.org

The **Royal Society for the Prevention of Cruelty to Animals, Research Animals Department provides free, downloadable resources** on the housing and care of each individual species of laboratory animal (including mice). Originally designed for use by lay members of ethical review processes, they provide brief details on the requirements and welfare problems surrounding each species. Visit www.rspca.org.uk/laymembers and click on 'Housing and Care' in the right-hand column.

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