Canadian Council on Animal Care
Conseil canadien de protection des animaux



# CCAC guidelines: Mice

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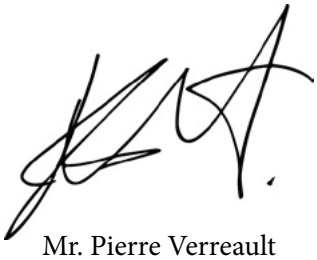
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# Mice

## PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer-review organization responsible for setting and maintaining standards for the ethics and care of animals in science throughout Canada.

The *CCAC guidelines: Mice* provides information for investigators, animal care committees, facility managers, veterinarians, and animal care staff to help facilitate improvement in both the care given to mice and the manner in which experimental procedures are carried out.

CCAC guidelines are intended to provide assistance in the implementation of Russell and Burch's Three Rs (Replacement, Reduction and Refinement) principles for animals in science (Russell and Burch, 1959). The guidelines are based on expert peer advice and current interpretation of scientific evidence. They are intended to provide a framework for the implementation of evidence-based practices, which are constantly evolving. Implementation of evidence-based practices should result in continual improvement in animal welfare, and those involved with mice should keep abreast of the current literature.

For studies outside of Canada, Canadian investigators are subject to these guidelines as well as to the relevant legislation and regulations pertaining to animal care in the country where the study is conducted.

# SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT

## 2. FACILITIES

### Guideline 1

The selection and purchase of caging that is suitable for the needs of the study and the welfare of the animals should be a collaborative decision involving investigators, animal care personnel, and other institutional parties.

*Section 2.1.1 Types of Cages, p. 11*

### Guideline 2

Cages should be of a sufficient size and complexity to allow mice to be housed in appropriately sized groups and to perform behaviours important to their welfare.

*Section 2.1.1.1 Cage Size, p. 12*

### Guideline 3

Cages should provide at least 330 $cm^2$ of floor space, and occupancy should be based on the minimum floor space required per animal.

*Section 2.1.1.1.1 Cage Floor Area, p. 13*

### Guideline 4

Part of the cage should be at least 13 cm in height from the floor to the lip.

*Section 2.1.1.1.2 Cage Height, p. 14*

### Guideline 5

Cages must allow for proper monitoring of the animals, preferably without disturbance, with consideration of research requirements.

*Section 2.1.1.2 Cage Materials, p. 15*

### Guideline 6

Mice should be housed in cages with solid floors.

*Section 2.1.1.3 Cage Floors, p. 15*

### Guideline 7

Mice must only be housed in metabolic cages when necessary for the particular study, and for the shortest time possible.

*Section 2.1.2 Metabolic Cages, p. 17*

## 3. FACILITY MANAGEMENT AND PERSONNEL

### Guideline 8

Laboratory management practices must aim to ensure the macro-environment (room) and micro-environment (cage) maintain the health and welfare of both the animals and personnel, and provide consistency for research outcomes.

*Section 3.1 Managing the Environment, p. 19*

### Guideline 9

Equipment and animals that generate noise and vibration should be located away from areas housing mice, and measures should be taken to mitigate excessive noise and vibration within the animal room.

*Section 3.1.4 Sound and Vibration, p. 22*

### Guideline 10

Mice must be observed daily by trained personnel, with minimal disruption to the animals.

*Section 3.2 Personnel, p. 23*

## 4. PROCUREMENT

### Guideline 11

The health status of the incoming mice should be reviewed before the animals are shipped.

*Section 4.5 Reception of Mice at an Institution, p. 28*

## 5. BREEDING

### Guideline 12

Breeding colonies must be efficiently managed according to approved protocols, anticipated need, and the principles of the Three Rs.

*p. 30*

## 6. HUSBANDRY

### Guideline 13

Mice should be group housed.

*Section 6.2.1 Social Housing, p. 38*

### Guideline 14

Bedding and nesting material must be provided to allow mice the opportunity to build nests and thermoregulate, as well as to dig, burrow, and forage.

*Section 6.3.3 Bedding and Nesting Materials, p. 40*

### Guideline 15

Cages should be changed at a frequency that maintains the intra-cage air quality and bedding within acceptable parameters, while recognizing the stress associated with cage cleaning and the potential impact on study data.

*Section 6.7 Cage Changing and Sanitation, p. 42*

## 7. HANDLING AND RESTRAINT

### Guideline 16

Mice must be handled gently to avoid injury and distress.

*p. 43*

## 8. WELFARE ASSESSMENT

### Guideline 17

All mice maintained in an animal facility should be subject to routine welfare assessments.

*p. 45*

## 9. HEALTH AND DISEASE CONTROL

### Guideline 18

All mice should be included in an animal health program, irrespective of where they are housed.

*p. 48*

### Guideline 19

Strategic measures for disease prevention should include a program for disease control and a system of regular monitoring and reporting for health assessment purposes.

*Section 9.1 Disease Prevention, p. 48*

### Guideline 20

Standard operating procedures should be developed for assessing animal health, providing health care, and treatment of common health problems for the animals; these should be reassessed every three years to ensure relevance.

*Section 9.2 Health Monitoring and Disease Detection, p. 49*

### Guideline 21

A management plan must be in place to deal with unanticipated disease outbreaks.

*Section 9.3 Disease Management in the Event of an Infectious Outbreak, p. 50*

## 10. EXPERIMENTAL PROCEDURES

### Guideline 22

The least invasive method suited to the goals of the study must be used, with consideration of the potential impacts of the procedures on mice, including other mice in the room, and consideration of measures to reduce those impacts.

*p. 52*

### Guideline 23

Endpoints must be developed and approved by the animal care committee prior to the commencement of the study, to minimize any negative impacts of the procedures on the animal.

*p. 52*

### Guideline 24

Mice should be provided with analgesia for invasive procedures that are likely to be painful.

*Section 10.10.2 Analgesia, p. 63*

### Guideline 25

Post-procedural care and monitoring must be planned based on the particular procedure and the individual needs of the animal, and adapted as necessary when unforeseen situations arise.

*Section 10.12.1 Monitoring, p. 66*

## 11. EUTHANASIA

### Guideline 26

Euthanasia of mice must be carried out by competent personnel only, using the method best suited to the particular animals, their housing situation, and the impact on the study data.

*p. 68*

# 1 INTRODUCTION

Throughout this document, the term 'should' is used to indicate an obligation, for which any exceptions must be justified to, and approved by, an animal care committee. The term 'must' is used for mandatory requirements.

Mice are the most common mammal in science in Canada. The most recent CCAC animal data at publication reported that mice represent 31.2% of all animals involved in science (see the CCAC website for the most up-to-date information).

Mice are selected for many different types of studies. Characteristics that make them popular in research include: 1) the wide variety of mouse strains and models available (see the Mouse Genome Informatics website); 2) their small size; 3) their relatively short lifespan; and 4) their short generation time.

There are a number of challenges that may be associated with mouse-based studies, which include:

- recognition, evaluation, and alleviation of pain, discomfort, and distress;
- technical difficulties for studies involving surgical procedures, due to small animal size;
- maintenance of aseptic technique for recovery surgery where there is limited technical support; and
- potential negative effects on animal welfare of genetically modified mouse models and other specialized disease models being generated.

As with any animal-based science, the scientific validity of any protocol involving mice must be established carefully, and the Three Rs of replacement, reduction and refinement (Russell and Burch, 1959) must guide decisions concerning experimental design and the care of the mice.

Replacement is an important consideration in planning any animal-based study. Consideration must also be given to reduction, to determine the fewest number of animals appropriate to provide valid information and statistical power, while still minimizing the welfare impact for each animal. Sample size calculations should be carried out and a biostatistician should be consulted when necessary.

The present guidelines focus primarily on refinement, both in terms of the care of mice in a facility, and of procedures carried out on mice as part of an animal-based protocol that has been approved by an animal care committee. Animals living in an environment where facilities and practices are oriented toward the promotion of good animal welfare are less likely to be stressed and more likely to exhibit normal behaviours and physiology (Poole, 1997; Garner et al., 2017), whereas unmanaged pain and distress can influence the reliability of experimental data (Jirkof, 2017).

The following sections provide a brief overview of the behavioural biology of importance to mouse welfare (Section 1.1, "Behavioural Biology"), the sensory abilities of mice (Section 1.2, "Senses"), the particular anatomical and physiological characteristics of mice (Section 1.3, "Anatomy and Physiology"), and potential

inter-animal variations (Section 1.4, "Sources of Variation"), which form the basis of this guidelines document and have an impact on welfare considerations. It is important to consider the characteristics of the species, strain and sex of the mice, as well as the specific characteristics of individuals, when considering the impact of a procedure or condition on the welfare of mice and on the research results.

## 1.1 BEHAVIOURAL BIOLOGY

Addressing the welfare of mice in the laboratory environment requires consideration of their natural behaviours (which will vary with the strain) and providing opportunity for those behaviours to be expressed where appropriate.

In their natural environment, mice are social animals that live in groups (Crowcroft and Rowe, 1963; Berry and Bronson, 1992). The population density depends on the environment (e.g., availability of resources and shelter), with complex environments having the potential to support higher densities of mice than open areas (Gray et al., 2000). A group may consist of one dominant male, subordinate males, and breeding females (see Latham and Mason (2004) for a review), although social organization can be influenced by population density. Territories are marked through urine (Hurst, 2005) and fecal deposits (Goodrich et al., 1986), with the dominant mouse marking more frequently than subordinates (Hurst, 2005).

Mice are prey species and generally avoid open spaces (Baumans, 2005); they have a tendency to remain close to walls (thigmotaxis). They are nocturnal (Baumans, 2010; Baumgardner et al., 1980) or active during twilight hours (Olsson et al., 2003), and tend to avoid bright light.

Mice are active, highly agile and exploratory animals (Baumans, 2010) and engage in the following activities: burrowing, foraging, climbing, nest building, gnawing, playing (e.g., leaping and chasing), grooming, sexual activities, and mounting behaviours (both males and females) (see Baumans, 2005, 2010; Olsson and Dahlborn, 2002). The ability to gnaw is important for their physical welfare, as their incisors grow continuously at a rate of 1-2 mm/week (Baumans, 2010).

Social contact with compatible animals is the most important factor in promoting species-typical activities and reducing abnormal behaviour, including stereotypies (Curley et al., 2009a; Hunt and Hambly 2006; Kercmar et al., 2014). Additionally, the structure of an animal's environment plays a role in its ability to perform natural behaviours. In a cage where subordinate mice are unable to flee from a dominant mouse or move out of its territory (as occurs in the natural environment), the dominant mouse may respond with increased aggression (Van Loo et al., 2003). Fighting, leading to wounding and death, is one of the most common causes of morbidity and mortality in laboratory mice (Marx et al., 2013).

An inability to control social structure, temperature, conspecifics, etc., may lead to abnormal behaviours, including stereotypies (Gross et al., 2012; Mason and Latham, 2004). Stereotypic behaviour and other repetitive behaviours may suggest motivational frustration or brain dysfunction and must be considered as a possible sign of poor animal welfare, though other factors should also be considered (Mason et al., 2007).

Animals with true stereotypies have altered brain function (Garner, 2005; Garner et al., 2006), although there is not a simple link between levels of stereotypic behaviour and brain function (Gross et al., 2012). In laboratory mice, stereotypies include behaviours such as bar-gnawing, jumping (Garner and Mason, 2002; Würbel and Stauffacher, 1997), circling, looping and twirling. Other abnormal behaviours include food grinding, barbering and repetitive mounting (especially in BALB/c mice) (Clipperton-Allen et al., 2015). Abnormal behaviours including stereotypies are most frequently seen in the dark phase, when the mice are

active. These behaviours are more common in some strains than others, and can have negative health effects and impacts on research outcomes.

For additional information on mouse behaviour, see the resources in Appendix 1, " Resources for Additional Information on the Characteristics of Mice".

## 1.2 SENSES

Olfaction and odour production play an important role in:

- social interaction (e.g., marking territories and recognizing groups or individuals (Hurst, 2005; Goodrich et al., 1986; Arakawa et al., 2008));
- reproductive cycles and sexual maturation (Hurst, 2005);
- detecting the presence of other animals, food, and potential threats (Bind et al., 2013);
- sexual attraction, courtship, and dam-pup interactions (Bind et al., 2013); and
- transmission of stress (Sterley et al., 2018).

In the laboratory, mice also use odours to divide cages into specialized areas, such as for defecation (Sherwin, 2002).

Disruption of chemical signals (e.g., during cage cleaning) may result in aggression (Gray and Hurst, 1995; Hurst, 2005; Van Loo et al., 2000) or other signs of stress (Lerch et al., 2016). Unfamiliar odours, such as those associated with humans, may cause adverse stress responses (Sorge et al., 2014).

Mice have acute hearing and respond to ultrasonic frequencies (Baumans, 2010). Ultrasonic vocalizations occur during non-aggressive interactions, particularly among socially housed animals, and within enriched cages (Portfors, 2007).

Noise and vibration can be significant causes of distress in mice (Jensen et al., 2010; Naff et al., 2007). Adverse effects include audiogenic seizures in young mice (Willott, 2007), reduced fertility (Fathollahia et al., 2013), and autoimmune effects (Hillhouse et al., 2013). High noise levels can also contribute to hearing loss, depending on the age and strain of mouse (Ohlemiller et al., 2000). Impaired auditory capabilities in mice can affect research that requires mice with normal auditory function, and other stress-related impacts of noise and vibration may contribute additional variables to particular studies (Willott, 2007).

Mice have poor visual acuity; however, light levels affect both their physiology and behaviour (Peirson et al., 2018). The high light intensity common in the laboratory environment can cause retinal damage, particularly for albino mice (De Vera Mudry et al., 2013). Light intensity can also influence activity levels, maternal behaviour, and various aspects of reproductive physiology. Additionally, the light-dark cycle can impact behaviour and reproductive physiology (Small and Deitrich, 2007); for example, reproductive disorders have been identified in mice housed under constant light (Miller et al., 2004).

## 1.3 ANATOMY AND PHYSIOLOGY

Some anatomical and physiological characteristics unique to mice may have important implications for their care and use in science, such as continuously growing incisors, inability to vomit, and large surface area to body mass ratio, which causes them to lose heat rapidly (a list of resources providing information about

the detailed anatomical and physiological characteristics of mice is provided in Appendix 1, " Resources for Additional Information on the Characteristics of Mice"). Consultation between the investigators and the veterinarian can be valuable in identifying the particular anatomical and physiological features of the mice of interest that could have implications for the housing and care of the mice, and for the results of the study.

## 1.4 SOURCES OF VARIATION

### 1.4.1 Outbred Stocks and Inbred Strains

Laboratory mice are often divided into two distinct groups: outbred stocks and inbred strains. Outbred stocks are intentionally not bred with siblings or close relatives, as the purpose of an outbred stock is to maintain maximum heterozygosity. Outbred mice have a relatively long lifespan, are resistant to disease, and have high fecundity (MGI, 2013). An inbred mouse strain originates from a single ancestral pair and has been mated brother to sister for 20 or more consecutive generations. The mice are then considered genetically identical and homozygous at almost all loci (MGI, 2013; Peters et al., 2007). However, genetic drift and factors such as poor colony management and genetic contamination (see Taft et al., 2006) may result in notable diversity between the same strains obtained from different sources; these are considered substrains (Zurita et al., 2011; Fahey et al., 2013; Sellers, 2017). Different strains of mice will behave differently (Olsson et al., 2003; Sluyter and Van Oortmerssen, 2000) and vary in their physiology and anatomy. Substrains of a given strain will also be genetically and phenotypically different.

The genetic and phenotypic variations between the different sources of mice can have implications both for their welfare and for the research data. Differences found between derivatives of inbred strains such as recombinant inbred strains, congenic strains, advanced intercross lines and others can be useful in the elucidation of basic biological processes and mechanisms of disease (Peters et al., 2007).

Examples of differences among strains and substrains include:

- litter size and gestation length (Murray et al., 2010);
- altered responsiveness to anesthesia and analgesia (Tanaka et al., 1993; Sonner et al., 1999; Neilan et al., 2003);
- agonistic behaviour – some inbred strains (e.g., SJL, BALB/c and FVB) are genetically predisposed to high levels of agonistic behaviour (Hurst, 2005; Canastar and Maxson, 2003; Clipperton-Allen et al., 2015), while others have a reduced ability to distinguish mice of the same strain via olfactory cues, which can lessen agonistic behaviour (Nevison et al., 2000; Hurst, 2005);
- performance of natural behaviours – some inbred strains (e.g., BALB/c and C57BL/6) exhibit behaviours reflecting adaptations to natural environments, such as tunnelling and nest building, while others (e.g., CBA) appear to lack the ability to perform these behaviours effectively (Sluyter and Van Oortmerssen, 2000);
- vocalization and hearing – different strains show differences in ultrasound vocalization rate and acoustic structures; some strains are genetically predisposed to auditory dysfunction and hearing loss (Willott, 2007);
- immunology – for a review of immunological variation between inbred strains and substrains of laboratory mice, see Sellers (2017); and
- phenotype – spatial learning, alcohol preference and other features commonly measured in behavioural neurosciences vary between inbred mouse strains and between different substrains of a popular strain such as the C57BL/6 (Kiselycznyk and Holmes, 2011).

Mice of different strains and mice that have undergone genetic modification can have very different preferences and requirements for the environment in which they live. It is important to ensure the stock, strain or substrain is appropriate for the study, and that the housing and care are aligned with the animal's needs. For example, C57BL/6 mice are resistant to many diseases and are more cold adaptive than other strains. For information on various mouse strains and their attributes, see Appendix 2, "Databases for Genetically Modified Mouse Lines".

### 1.4.2 Individual Differences – Sex, Health Status, Microbiome

The importance of recognizing and understanding the implications of sex differences in research has been well established (McCarthy et al., 2012). McCarthy et al. (2012) and Becker et al. (2005) discuss considerations for identifying the nature and implications of sex differences in studies, in order to improve study design and interpretation of results. While the estrous cycle can impact variability in some studies involving females, and sex differences may be more prominent during a particular stage of the estrous cycle (McCarthy et al., 2012), there are some studies where the estrous cycle is not a contributing variable that needs to be monitored (Prendergast et al., 2014). Moreover, variability among males, which is influenced by factors such as dominance hierarchies, may also need to be considered (McCarthy et al., 2012).

The health status of mice has implications for their use in research and how they are housed within a facility. Quarantine and sentinel programs, and other means of monitoring the colony for pathogens, are important in maintaining animals of a particular health status (see Section 9, "Health and Disease Control").

The gut microbiota of an animal affects its physiology and the onset of numerous diseases (Clavel et al., 2016; Laukens et al., 2016). The composition of the gut microbiota is influenced by factors such as genetics, age, gender, diet, housing, environment, antibiotic treatment and stressful procedures, and has been linked to the variation in mice models among animal facilities and among suppliers (Turner, 2018; Laukens et al., 2016).

### 1.4.3 Effects of the Environment and Previous Experience

The animal husbandry environment can be a source of variation. Mice exhibit developmental plasticity, with features of the early environment affecting aspects of the adult phenotype (see Lonetti et al., 2010). Fewer abnormal behaviours, including stereotypies, occur when mice are provided with environmental resources (Clipperton-Allen et al., 2015), and this has been shown to be particularly relevant for male mice (Lehmann and Herkenham, 2011).

Standardization is generally employed in research to minimize variability and maximize reproducibility, but even with apparent standardization of environmental conditions, animal behaviour can show considerable variation across experiments due to minor differences in treatment. Reproducibility may be improved by increased heterogeneity in environmental conditions (Richter et al., 2009; Mogil, 2017; Gaskill and Garner, 2017). These potential effects should be carefully considered as part of the experimental design process.

# 2 FACILITIES

For general guidance on facilities, see the *CCAC guidelines on: laboratory animal facilities – characteristics, design, and development* (CCAC, 2003). Additional guidelines and information of particular concern for mice is presented in this section.

## 2.1 HOUSING

Housing must confine the animals securely and safely, and ensure their welfare by permitting normal postures and behaviours (see Section 1, "Introduction"). The various components of housing (e.g., floor area, vertical space, configuration of the living area, nesting materials, cage enrichment, and complexity) must be considered together, rather than in isolation, with an awareness of how the needs of the mice may differ according to strain, genotype, age and sex (note the Canadian Institutes of Health Research (CIHR)'s focus on involving both male and females in pre-clinical research (CIHR, 2015)). Housing can influence biological variables and should be taken into account in experimental design. However, changes which are beneficial to mice welfare and do not impact the physiology of the animal are not likely to impact research results (André et al., 2018).

### 2.1.1 Types of Cages

**Guideline 1**

The selection and purchase of caging that is suitable for the needs of the study and the welfare of the animals should be a collaborative decision involving investigators, animal care personnel, and other institutional parties.

Involvement of investigators, animal care personnel and other institutional parties is critical to ensure that caging meets the needs of the research, meets the welfare standards of the institution, and can be properly used and maintained by the animal care personnel.

There are a variety of caging systems available (see Voipio et al. (2011) and Smith and Baran (2013) for a brief overview). Before any new infrastructure is acquired (both cages and cage racks), there should be extensive consultation among investigators or study directors carrying out mouse-based studies and facility managers; input should also be sought from facility managers or directors from other institutions.

The design of the cage should allow for daily observation of the animals with minimal disturbance. While mice do not appear to show preference for cage shape, their fear of open spaces should be considered in cage configuration, with an awareness that the importance of this fear may vary among strains (McGlone et al., 2001) and across studies. Cages should have rounded edges that facilitate sanitation and are less likely to injure the animals.

In general, rodents prefer non-ventilated cages, and have been shown to suffer from cold stress in ventilated cages (David et al., 2013; York et al., 2012), which can have an impact on the reproducibility of experimental data (see Burman et al., 2014). On the other hand, the use of ventilated cages provides a very controlled environment for the animals, with HEPA-filtered air being delivered with the same pressure, speed and temperature into each cage on a ventilated cage rack (Spangenberg et al., 2014); ventilated cages also minimize the spread of diseases between cages. The use of ventilated cages maintains acceptable air quality when cage changing intervals are extended beyond the standard one week for static cages (Rosenbaum et al., 2009). Where cages are ventilated, mice have been shown to prefer cages with the lowest numbers of air changes per hour. They have been shown to avoid cages with high air movement (Krohn and Hansen, 2010), will attempt to block drafts from air intakes at floor level, and will choose to nest away from air inlets (Baumans, 2010). As thermoregulation and preferred temperatures in mice are based on a number of factors (age, reproductive state, sex, genotype, etc.), the easiest way to accommodate the needs of all mice housed in a ventilated cage rack is to provide sufficient nesting material for the mice to be able to establish their own microclimate (Gordon, 1993, 2004, 2012; Gordon et al., 1998; Gaskill et al., 2012, 2013a; Gaskill et al., 2013b).

For standard ventilated cages, the factors to be considered in acquiring cages include:

- air exchange – the rate, position and velocity of the air supply and the type of cage top can impact air quality and movement of air within the cage; in turn, these features can affect the welfare of mice. Additionally, the way in which air is provided to individually ventilated cages (forced air versus motor-free) has been shown to influence body weight, food and water consumption, and the preferred intra-cage location of growing male mice (Kostomitsopoulos et al., 2012);
- vibration – vibration from blowers, etc., is an important consideration, especially for breeding colonies (Norton et al., 2011), although mice are able to adapt to continuous vibrations (Reynolds et al., 2010);
- noise – design features, such as the location of the blower (on top or on the side), and whether the noise produced by those features is audible to mice, should be considered;
- ability to access animals – the configuration of cage components can affect the ability to handle the animals and, consequently, affect the welfare of those animals;
- ability to monitor animals;
- risk of ergonomic injury for personnel;
- ability to sanitize the cages and racks; and
- biosecurity – examples include HEPA-filtered versus regular-filtered cages, and the ability to switch between negative versus positive rack pressure.

Installation designs should consider using room or building supply air and exhaust, eliminating the need for mounting blowers on racks (Norton et al., 2011).

#### 2.1.1.1 Cage Size

**Guideline 2**

Cages should be of a sufficient size and complexity to allow mice to be housed in appropriately sized groups and to perform behaviours important to their welfare.

Positive welfare encompasses environmental comfort, freedom from pain and distress, freedom to express natural behaviours, and appropriate social interactions (CALAM, 2007; CCAC, 2017).

The choice of cage should support social housing of mice (see Section 6.2, "Housing Management"). Cage size will affect the number of mice that can be housed and their ability to perform locomotory and exploratory behaviours (Jennings et al., 1998), as well as the capacity to add elements that can positively affect their welfare. Cage size and complexity should be considered together, as increasing the amount of empty space in a cage may not improve the welfare of the animals and, in some cases, may have negative welfare implications (Jennings et al., 1998; McGlone et al., 2001; Baumans, 2010).

Space requirements for mice must take into account the strain, age, reproductive status (Jennings et al., 1998; Baumans, 2010), and the potential for animals' needs to change during the time they are held. The suitability of the living area should be evaluated based on factors such as the animals' tendency to perform normal behaviours, the occurrence of abnormal behaviours including stereotypies, agonistic encounters, weight changes, incidence of illness, or reproductive performance (Nicholson et al., 2009).

Young animals may require proportionally more space than adults to exhibit developmentally appropriate behaviours (Jennings et al., 1998). Weight gain, fecal corticosterone metabolite levels, and barbering have been shown to differ significantly with housing density among young mice (Nicholson et al., 2009).

As institutions acquire new cages, these cages must, at least, meet the minimum standards for floor area and height indicated below, to address the behavioural needs of the animals, including the ability for social housing in appropriately sized groups. Larger cages that allow additional enrichment, to accommodate the behavioural needs of the animals, are encouraged.

Cage size must account for the weaning size and weight of all pups, in addition to adult animals in the same cage, if animals are breeding.

#### 2.1.1.1.1 Cage Floor Area

**Guideline 3**

Cages should provide at least 330 $cm^2$ of floor space, and occupancy should be based on the minimum floor space required per animal.

Potential variables in determining floor space requirements for mice include group size, strain, sex, age, stage in breeding cycle, type of space (open versus structured), lighting, and resources provided (Fullwood et al., 1998). The complexity of the space can influence how it is used by the animals; larger cages should be appropriately structured and furnished to promote animal comfort and use.

At a minimum, the floor area should accommodate the following physical and behavioural requirements:

- room for shelter and rest;
- choice for determining thermal comfort (Gaskill et al., 2012);
- opportunities for physically avoiding or being removed from the visual sightline of other mice in the same cage;
- ability to nest on dry bedding; and

- opportunity to perform important behaviours without necessarily touching other animals (e.g., rearing, burrowing, grooming, as noted in Section 1.1, "Behavioural Biology").

As a minimum, cage floor area should be at least 330 $cm^2$, even if fewer than three mice occupy the cage. Each mouse should be provided at least 100 $cm^2$ of space. As a standard group size is 4-5 mice per cage, the cage floor area should be a minimum of 400-500 $cm^2$. Whittaker et al. (2012) reviewed available evidence on the impact of space and housing density on measures of well-being of mice; however, empirical data concerning the impact of group size and space allocation is not currently available. Bailoo et al. (2018) did not find any negative impact on the welfare of mice within the bounds of the space allowances listed below. However, they caution that increasing group size may lead to increased levels of aggression between male mice of some strains.

**Table 1 Space Allowances**

| REQUIREMENT | MINIMUM SPACE ALLOWANCE | NOTES |
|---|---|---|
| Minimum cage size | 330 $cm^2$ | 1-3 adult mice can be kept in the minimum cage size |
| Minimum space per mouse | 100 $cm^2$ | 5 weanling mice or stock mice require a minimum cage size of 500 $cm^2$ |
| Minimum space for trio breeders | 330 $cm^2$ | Male and 2 females, no litters |
| Minimum space for pair or trio with litter[1] | 500 $cm^2$ | At weaning age, pups require their own cage |
| For additional adult mouse in breeding cage | add 100 $cm^2$ for each adult | |

#### 2.1.1.1.2 Cage Height

**Guideline 4**

Part of the cage should be at least 13 cm in height from the floor to the lip.

At least part of the cage should allow mice to stand on their hind legs and stretch up fully, hop, jump, and climb on the bars of the lid (Jennings et al., 1998; Pietropaolo et al., 2007).

Although greater cage volume is often desirable, cages must not be of a height that prevents mice from accessing food or the bars on the cage top, unless modifications are made to allow such access (Jennings et al., 1998). For cages fitted with shelters, the height of the cage should allow mice to climb on top of the shelter to maximize the available space.

1 These are general guidelines and consideration must be given to proper breeding procedures and conditions. Some situations may require greater space for separation of litters (see Section 5, "Breeding").

### 2.1.1.2 Cage Materials

**Guideline 5**

Cages must allow for proper monitoring of the animals, preferably without disturbance, with consideration of research requirements.

There is no clear evidence that mice have a preference for opaque or transparent cages. Among the characteristics to consider are that opaque cages filter out harmful glare and allow mice to hide, but impede observation from outside the cage. Conversely, transparent cages allow observation from outside the cage, but may subject the mice to high light levels (Jennings et al., 1998).

Cages must allow for proper monitoring of the animals. As noted in Section 6.6, "Animal Observation", mice must be monitored daily. While electronic monitoring systems are available for a variety of parameters, these systems have limitations and visual monitoring of the animals is still necessary.

Non-disposable cages must be made of materials that are easy to clean, non-toxic, non-absorbent, durable, and resistant to heat and chemicals; they must also be escape and predator proof (Koehler et al., 2003). The advantages and disadvantages of different cage materials are described by Jennings et al. (1998). Some synthetic materials release bioactive substances that may affect mice; for example, polycarbonate or polysulphone cages may expose mice to bisphenol A (BPA) via leaching, particularly with older cages (Howdeshell et al., 2003). This should be considered when replacing cages made of these materials. Cage floors and walls should preferably be plastic, unless special-purpose cages are required.

Cage materials can affect the microclimate (i.e., by modifying light levels and heat exchange); plastic cages are perceived as being warmer than stainless steel cages (Jennings et al., 1998).

### 2.1.1.3 Cage Floors

**Guideline 6**

Mice should be housed in cages with solid floors.

Solid-bottom cages are strongly recommended (Jennings et al., 1998), particularly for long-term studies (Kalliokoski et al., 2013), and must be used for breeding females and their litters up to weaning, to minimize pup mortality.

Where studies require animals to be separated from their excreta, perforated or slatted floors should be used. In the past, cages with wire-mesh floors were used for this purpose; however, prolonged use of wire-mesh floors can be detrimental to the welfare of the animals (e.g., wire-mesh floors have been associated with mouse urological syndrome (MUS) in some strains (Everitt et al., 1988)). See Section 2.1.2, "Metabolic Cages", for additional considerations for studies involving metabolic cages.

#### 2.1.1.4 Cage Components

Bedding and nesting materials are the most important components of the cage environment for mice (see Section 6.3.3, "Bedding and Nesting Materials"). Mice have been shown to perceive these materials as important, as demonstrated by their willingness to work to access them (Sherwin, 1996a).

Cage enrichment through provision of such items as shelters (see Section 2.1.1.4.1, "Shelters and Nest Boxes"), exercise wheels and certain types of dividers (see section 2.1.1.4.2, "Cage Dividers") can increase the range of behaviours that mice perform, increase their use of different areas within the cage (Jennings et al., 1998; Leach et al., 2000), and reduce anxiety (Olsson and Sherwin, 2006). Mice also appear less hyperactive in new situations and display less abnormal behaviour including stereotypies when reared in complex environments (Chamove, 1989; Leach et al., 2000).

Any items added to cages should be monitored and evaluated to ensure they provide benefit to the animals and do not have a negative impact (Jennings et al., 1998). In their review of housing practices and enrichment for male mice, Kappel et al. (2017) concluded that positive or negative welfare outcomes depend on the strain, age, social position, life experiences, and housing and husbandry protocols. Cage dividers and shelters may lead to increased aggression in groups of males of some more territorial strains (Van Loo et al., 2003; Howerton et al., 2008); however, this can sometimes be rectified by ensuring sufficient enrichment items are present for all animals to use (Baumans and Van Loo, 2013).

##### 2.1.1.4.1 Shelters and Nest Boxes

When offered the choice of a cage with a nest box or one without, mice have shown a preference for cages containing nest boxes (Van de Weerd et al., 1998a), although the use and benefit of nest boxes may vary with the strain and sex of the mice, the type and amount of nesting material provided (Sherwin, 1996b), and the type of nest box (Buhot-Averseng, 1981; Van de Weerd et al., 1998a). If nest boxes or shelters are used, they should have more than one entry and provide sufficient space for all animals in the cage; nesting material can act as a shelter for mice if provided in sufficient quantity.

Shelters can reduce convective heat loss in ventilated caging by blocking air movement, and provide opportunity for avoidance of light and escape from other animals. They can also satisfy thigmotactic behaviour by increasing wall space and provide a structure to climb on. Shelters made of animal-safe plastic may provide refuge for animals in the event of accidental flooding from water bottles or automatic watering systems. Animals will chew plastic shelters; these should be discarded when rendered unsafe. Shelters made of chewable, non-toxic paper material can address other behavioural needs, such as chewing and manipulation (Jennings et al., 1998). Shelters may also help facilitate nest building, as some mice will drag nesting material into the shelter (Van Loo et al., 2005).

##### 2.1.1.4.2 Cage Dividers

The use of cage dividers requires careful monitoring to ensure they have a positive impact on the welfare of the mice. Cage dividers have been developed for different purposes: 1) to increase complexity within cages, while still allowing the opportunity for full contact between mice (i.e., partial partitions); and 2) to allow some degree of social contact between mice in situations where full contact may not be suited to the study or the welfare of the mice (i.e., grid partitions).

Partial partitions within cages can increase environmental complexity, increase the cage floor area used by mice (Leach et al., 2000) and facilitate activity (Chamove, 1989). They may also reduce the animal density

perceived by mice (Jennings et al., 1998) and provide an escape route. Cage dividers that resemble burrows and allow for a common area for all mice in the cage may contribute to reducing stress in mice of some strains (Chamove, 1989) and decreasing aggressive behaviours (Tallent et al., 2018). However, cage dividers should not be used in housing aggressive strains, particularly males of those strains (Barnard et al., 1996).

Grid partitions that allow some social contact for mice that cannot be housed together do not appear to provide the intended welfare benefits, and have been found to be more stressful for mice post-surgery than individual housing (Van Loo et al., 2007).

The use of cage dividers is recognized to be an area that requires more research.

#### 2.1.1.5 Feeders and Water Supply

Mice should have access to clean food and clean, fresh drinking water at all times. Food and water should be supplied in such a manner that they are easily accessible for all animals and contamination is minimized.

In general, food should be provided in feeders. These should be designed to ensure the safety of the animals (e.g., no rough edges). For some mice that have restricted mobility (such as some genetically modified mice), it may be necessary to provide food at the cage floor level.

Water may be provided via a bottle, bag or automated watering system, or in the form of an edible gel. It is important to ensure that the animals are able to use the water source provided (Gordon and Wyatt, 2011). The best option should be chosen to ensure that animals always have access to water and the risk of cage flooding is minimized. Some mice (e.g., debilitated animals or young mice immediately post-weaning) may require additional water sources (see Section 5.3.3, "Weaning").

### 2.1.2 Metabolic Cages

**Guideline 7**

Mice must only be housed in metabolic cages when necessary for the particular study, and for the shortest time possible.

There are several aspects of metabolic cages which can have a negative impact on the welfare of mice. The key stressors for mice in metabolic cages are:

- single housing (see Section 6.2.2, "Single Housing");
- barren environment (see Section 6.4, "Environmental Enrichment"); and
- perforated or wire flooring (see Section 2.1.1.3, "Cage Floors").

All of these elements are associated with welfare concerns, which are described in the sections indicated. Where possible, alternatives to metabolic caging should be used, for example, sample collection in the home cage environment.

Kalliokoski et al. (2013) found that mice did not acclimate to barren metabolic cages, and suggested that their physiological condition in these cages cannot be considered normal. Holding mice in isolation in metabolic cages can lead to changes in their physiology that are indicative of a stress response (Stechman et al., 2010), as well as changes in their cardiovascular function (Hoppe et al., 2009), core body temperature, and

fur condition (Kalliokoski et al., 2013). In some mice, isolation can result in increased fecal corticosterone concentration for up to 14 days (Hunt and Hambly, 2006). In addition, mice in isolation show behavioural changes indicative of impaired welfare (Yamamoto et al., 2018).

Where studies dictate that mice need to be held in metabolic cages for longer than 24 hours, animal comfort should be a consideration and the cage should include a resting platform and the means for the animals to establish a microclimate.

## 2.2 SURGICAL FACILITIES

The *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) requires a dedicated surgical suite or a dedicated area in a procedure room, separate from other activities. For mice, a dedicated surgical suite is preferable. Any recovery surgery should be carried out in a dedicated room. The dedicated room or area should be easily cleaned, with impervious surfaces that facilitate the control of contamination and maintenance of aseptic conditions. There should also be a separate surgical preparation area and a recovery area.

Waste scavenging equipment must be available where anesthetic is used. Biological safety cabinets should only be used when necessary. Measures should be taken to address the susceptibility of mice to hypothermia (see Section 10.11, "Surgery"). Any period of recovery from surgery should be conducted in a dedicated room or area.

## 2.3 CORE FACILITIES FOR GENERATION OF GENETICALLY MODIFIED MICE

With the current ability to access high quality, competitively priced transgenic fee-for-services in most of the major academic and research centres in North America, decisions to establish a core transgenic facility are best made in a long-term planning process that involves all stakeholders at the institution. In that manner, the institution can make a broad policy decision about whether to establish a new facility or to outsource the generation of transgenic animals, based on factors such as: the availability of infrastructural resources, the availability of specialized human resources, geographical location and local community need.

Ensuring the appropriate quality of building infrastructure and human resources is costly, time-consuming and labour intensive. For institutions on the cusp of renovation, new construction and programmatic expansion, the building and housing infrastructure, as well as instrumentation and laboratory facilities, should be in place or planned to provide specific pathogen-free derivation facilities and barrier-housing space. Appropriately trained and skilled personnel are critical to the high-quality performance of any transgenic core facility and may be more difficult to attract and retain in geographically isolated areas of the country. Many successful transgenic core facilities are based on core operational funding and are overseen by a range of expertise including senior-level research scientists and institutional veterinarians.

Whether researchers use dedicated core facilities or outsource their requirement for particular animals to reliable providers, a careful decision can help ensure that the most efficient and effective methods of producing animals are used; this has the additional potential to reduce the number of animals involved in providing the necessary supply.

Core facilities usually include facilities for cryopreservation. The freezing units should have backup sources of power and liquid nitrogen, and an alarm system.

# 3 FACILITY MANAGEMENT AND PERSONNEL

## 3.1 MANAGING THE ENVIRONMENT

The *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) should be consulted for general guidelines that are applicable to all species. This section provides additional considerations that are particular to mice.

**Guideline 8**

Laboratory facility management practices must aim to ensure the macro-environment (room) and micro-environment (cage) maintain the health and welfare of both the animals and personnel, and provide consistency for research outcomes.

Procedures for managing the environment will depend on the room layout and type of caging. Particular attention should be paid to maintaining an appropriate environment within cages.

### 3.1.1 Lighting

Room lighting, including prolonged light exposure and task lighting, should contribute to good animal welfare, while enabling appropriate observation and handling of all animals. Light levels within cages are important to the animals, and vary with the type of caging, the provision of nesting material, the location in a cage (e.g., front or back), the position of the cage on a rack, provision of shade from above the rack, and the distribution of cages in a room. Additionally, the sensitivity of mice to light and their susceptibility to retinal damage depends on the strain (LaVail et al., 1987; De Vera Mudry et al., 2013), with albino mice being particularly sensitive to light-induced photoreceptor degeneration (LaVail et al., 1987).

Light levels are typically measured in lux; however, this is a measure of perceived brightness to humans and does not directly relate to the brightness perceived by mice with different spectral sensitivity (Peirson et al., 2018). Depending on the housing conditions, the general lighting conditions for laboratory animal rooms recommended in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003), 325 lux at 1 m above the floor, may be too high for mice (De Vera Mudry et al., 2013), and lighting should be maintained below this level whenever it is not needed for husbandry or experimental purposes.

Light:dark transition testing, a common behavioural test of anxiety in mice, indicates an avoidance of brightly lit areas. Intense light can cause eye pathology (Greenman et al., 1982) and influence behaviour, reproduction and physiology (Lipman and Perkins, 2002). Light damage, even if acute, involves long-term retinal remodelling (Rozanowska, 2012; Organisciak and Vaughan, 2010). In addition to welfare concerns, the development of artifacts in mouse strains due to light-induced remodelling has particular implications for the use of genetically modified mice to model visual development, function and disease (Natoli et al., 2016; Song et al., 2016; Zhao et al., 2014; Kuse et al., 2014).

Changes in light:dark cycles are stressful for mice and may require a long period of acclimation (van der Meer et al., 2004; Loh et al., 2010). The impact of light on circadian rhythms can affect physiological parameters such as growth, metabolism, reproduction, endocrine and immunological parameters, as well as behaviour (Campuzano et al., 1999; Kolaczkowska et al., 2001; Jiang et al., 2006), and development of disease states such as tumour growth (Filipski et al., 2005). This can be compounded by variations in response based on the mouse strain (Kolaczkowska et al., 2001). To ensure a stable baseline for research, there should be a sufficiently long period of acclimation to changes in light cycles to permit homeostasis to be re-established.

Mice reproduce optimally with no behavioural problems when housed under a cycle of 14-hours light and 10-hours dark (14:10); however, a 12:12 light:dark cycle is also acceptable. Consistency in the diurnal cycle is often critical to reliable research results; even opening a door briefly during the dark phase can affect pup production, behaviour, and anxiety (Bedrosian et al., 2013). If making observations during the dark phase, lighting in a spectrum that mice are less sensitive to, such as red (Jennings et al., 1998), or using sodium lamps (McLennan and Taylor-Jeffs, 2004) may aid illumination. However, some physiological parameters may be affected by red light (Hofstetter et al., 2005; Pierson et al., 2018).

Evidence that blue light causes behavioural arousal, elevation of corticosterone, and delay in sleep onset indicates that the use of LED lights may have an impact on some research studies (Pilorz et al., 2016).

It is important to measure light levels in the room and cages once the equipment is in place, including at the top, middle and bottom rows of the cages and inside cages at the front and back. This is very important to understand light exposure, and should be carried out periodically (with adjustment to lighting levels if necessary). Management practices to control light levels should be evaluated in the context of each study; for example, rotation of cages may not be practical where such movement will cause stress to the animals or result in mixing of dose groups for testing purposes. Management practices to control light levels within cages may include:

- rotating cage position on the rack;
- avoiding use of the top shelf of racks, or providing shade over the top shelves if the cage does not have a solid top;
- providing animals with the ability to avoid light within the cage using hiding structures and sufficient nesting material;
- using red light or sodium lamps during the dark period for monitoring the active phase; and
- providing eye protection for the mice if it is necessary to use bright lights for particular procedures (e.g., bringing a few cages at a time into a procedure room and covering them with towels to occlude the light or using opaque restraining tubes when handling the animals).

Additionally, investigators should be aware of situations that expose mice to different light levels (e.g., mice can be exposed to large differences in light levels when removed from their cages in transfer stations or when exposed to room lighting).

### 3.1.2 Temperature and Relative Humidity

Temperature and relative humidity should be considered together, as the combined effects of these two parameters influence the welfare of animals. Facilities should have a system in place to monitor temperature and relative humidity within cages. The type of cage, the number of mice, and the quality and quantity of nesting and bedding material influence temperature and relative humidity within a cage. Other factors to be

considered are the position of cages within a rack and within the room, ventilation rate, frequency of cage changes, cage enrichment items (e.g., shelters), and animal density. Even with adequate ventilation, cage temperatures may be several degrees above room temperature (Reeb et al., 1997).

Room temperature should be checked daily or continuously monitored by a building automation system equipped with an alarm or notification system, and maintained in the range of 20-26 °C (Yamauchi et al., 1983). Reproductive performance is enhanced at warmer temperatures in the range of 22-28 °C (Helppi et al., 2015). Gaskill et al. (2012) suggest that mice prefer temperatures in the range of 26-29 °C (depending on strain and sex). These higher temperatures can be achieved in practice by providing an appropriate type and amount of nesting material to allow creation of a microclimate by the animals within each cage. Gaskill et al. (2013a) and Johnson et al. (2017) recommend 8-12g of nesting material, sufficient to be manipulated into a complete dome.

For lactating mice and pups up to 3 weeks of age, room temperature should be at the higher end of the range (i.e., 24-26 °C) (Gordon, 1993). Again, this requirement will be influenced by whether nesting material is provided. Special consideration should also be given to young, old and obese mice, and mice undergoing particular procedures (e.g., anesthesia and surgery), as they may have a reduced ability to thermoregulate.

Sudden changes in temperature should be avoided (Swoap et al., 2004) and appropriate nesting material may help avoid sudden intra-cage temperature changes (Gordon, 2004; Gaskill et al., 2013c).

Ambient temperature can affect metabolism, cardiovascular function (Overton and Williams, 2004; Swoap et al., 2004), motor activity (Overton and Williams, 2004), growth and development, body and organ weight, consumption of food and water, hematology and serum chemistry parameters, susceptibility to toxins, immune competence, reproduction (Yamauchi et al., 1983), sleep (Jhaveri et al., 2007), behaviour toward other mice (Greenberg, 1972) and development of disease states (Hylander and Repasky, 2016).

Ideally, room relative humidity should be 40-60%, as stated in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003); however, a lower limit of 30% may be acceptable for mice to control ammonia levels, with a caution that low humidity can lead to dehydration in younger animals (Hessler and Leary, 2002). The health status of the animals and the impact of humidity levels on animals undergoing experimental procedures are important considerations in determining the appropriate relative humidity level. Supplemental humidification should be provided where low relative humidity is a problem.

Health concerns for mice attributed to low relative humidity include alterations in tear secretion, goblet cell density, susceptibility to dry-eye related ocular surface clinical signs (Barabino et al., 2007), dehydration in young mice (Hessler and Leary, 2002), and increased susceptibility to certain respiratory infections. Although ring tail has been traditionally attributed to low relative humidity, it has been suggested that it is a multifactorial disease (Recordati et al., 2015).

Conversely, high relative humidity affects the thermoregulatory capacity of mice (Clough, 1982; Donnelly, 1989) and enhances proliferation of bacteria, leading to increasing ammonia levels (Memarzadeh, 2005; Reeb-Whitaker et al., 2001).

The health and comfort of personnel should also be taken into consideration.

### 3.1.3 Air Quality and Ventilation

As noted in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003), "the rate of air exchange within a room must be such that clean, fresh air is available to all animals and personnel at all times". See Section 12.3, "Heating, ventilation and air conditioning (HVAC)" of the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003) for factors affecting room air quality. Intra-cage levels of temperature, humidity, and ammonia are generally higher than room levels.

The main components of air quality of concern in laboratory animal facilities are ammonia, carbon dioxide, particulates and volatile organic compounds (CCAC, 2019). Cage-level ammonia concentration should be monitored on a regular basis. It has been suggested that ammonia levels should be maintained within guidelines recommended for humans, i.e., < 25 ppm (Rosenbaum et al., 2010), or that cages should be changed when the intra-cage ammonia concentration reaches 50 ppm (Silverman et al., 2009). Mice exposed to ammonia at 52 ppm for 13 days showed degeneration of nasal epithelium in post-mortem analysis (Vogelweid et al., 2011). Similarly, the most sensitive indicator of damage from chronic ammonia exposure in rats has been reported as the appearance of histological changes in the nasal passages (Broderson et al., 1976).

The relationship between cage-changing frequency, nasal histopathology and intra-cage ammonia levels is dependent on cage size and stocking density. The interaction has been explored by Mexas et al. (2015), and provides support for current practices (a minimum of weekly cage changes) for mice that are group housed in static shoebox-style cages. For individually ventilated cages, studies considering the interaction between bedding volume, air changes per hour and ammonia levels indicate that changing cages every two weeks is likely the best timeframe for the welfare of the animals (Ferrechia et al., 2014; Rosenbaum et al., 2009 and Reeb-Whitaker et al., 2001).

More information is needed on mouse preferences for cage changing intervals (e.g., a study by Green et al. (2008) failed to show any avoidance of high ammonia levels by the mice in a preference test). Similarly, there is little documented evidence on how mouse welfare is affected by soiled cages.

### 3.1.4 Sound and Vibration

**Guideline 9**

Equipment and animals that generate noise and vibration should be located away from areas housing mice, and measures should be taken to mitigate excessive noise and vibration within the animal room.

It is beneficial to measure environmental noise within the animal facility (Turner et al., 2007; Turner et al., 2005). Lauer et al. (2009) monitored noise levels in rodent rooms and identified the source of loud noise and noise variability to be primarily human activity. Similarly, Turner et al. (2005) note that noise in animal facilities is generally greatest when personnel are present, due to both the activity of personnel and the increased activity of animals in the presence of people.

Mice and humans perceive various sounds differently, and the effects of sound on the welfare of the animals and on study results should be considered from the mouse's perspective (Reynolds et al., 2010; Turner et al.,

2005). In general, the audible frequency range for mice at a standard sound intensity of 60 decibels (dB) is 2,300-85,500 Hz, depending on the strain (Heffner and Heffner, 2007); humans have a hearing range of 20-20,000 Hz (Turner et al., 2005). Strains of mice differ in auditory sensitivity, the rate of progressive hearing loss, and susceptibility to noise-induced seizures (Turner et al., 2005).

Mice are very sensitive to ultrasound and use it to communicate. Ultrasonic noise in the environment should be minimized as it can potentially result in adverse health effects for mice and confound research results (Turner et al., 2007). Sources of ultrasonic noise include dripping taps, trolley wheels, computers, light ballasts, movement of furniture, vacuum cleaners and cage washers (Turner et al., 2007).

High ambient levels of sound or intense brief sounds may induce hearing loss or damage to the auditory apparatus, depending on the strain and sex of the mice (Willot, 2007). Loud noise may also result in audiogenic seizures (characterized by wild running, convulsions, and possibly death from respiratory paralysis) in some strains of mice (Willott, 2007). Other possible non-auditory impacts of noise include alteration of reproductive efficiency (Rasmussen et al., 2009; Turner et al., 2007; Turner et al., 2005), endocrine and cardiovascular function, and sleep/wake cycles (Turner et al., 2007; Turner et al., 2005). It is particularly important that mouse breeding colonies be located as far away as possible from noise-generating equipment and noisy animals (e.g., dogs and nonhuman primates).

Chronic exposure to moderate levels of low-frequency noise (< 0.5k Hz) at 70 dB has been shown to impair balance in mice (Tamura et al., 2012); however, this level of noise may not be detrimental to all strains (e.g., young adult female C57BL/6 mice exposed to the noise of a vacuum cleaner did not show increased concentrations of fecal corticosterone metabolites or express anxiety-related behaviour (Jensen et al., 2010). Exposure to even a single period of intensely loud noise has been shown to affect learning in young mice (Tao et al., 2015).

The director of the animal facility should establish a relationship with the institutional general facilities management and planning department to ensure that the animal facility is alerted to any new construction or renovations and can coordinate with the researchers to minimize the impact on the animals and on research activities.

Sources of vibration within laboratory animal facilities include the room ventilation system, ventilated racks, and equipment related to husbandry or research activities, as well as activities occurring outside of the room (Reynolds et al., 2018; Norton et al., 2011). Vibrations can result in various physiological and pathological effects, and the impact of vibration caused by a particular source will depend on the species and age of the animal (Reynolds et al., 2018; Norton et al., 2011). In a study involving construction equipment, mice were found to experience more vibration than humans (Norton et al., 2011), and therefore, measures should be taken to dampen all potential sources of vibration.

## 3.2 PERSONNEL

**Guideline 10**

Mice must be observed daily by trained personnel, with minimal disruption to the animals.

Sufficient personnel are needed to ensure that: 1) cages are cleaned, food and water are provided, and other husbandry requirements are addressed as appropriate; and 2) animals are observed daily.

Mice must be observed once daily, seven days a week, by trained personnel who can: 1) recognize welfare concerns and health problems in mice and resolve them through institutional standard operating procedures; and 2) follow proper record keeping and reporting procedures to ensure the facility manager and veterinary team remain informed, and researchers are alerted to any changes.

A major consideration for personnel working in facilities where mice are held is the large numbers of animals housed. This, coupled with the small size and behaviour of mice as a prey species, can make it difficult to see each animal and detect problems of individual mice. Where welfare concerns are identified, any additional demands on personnel time (e.g., for the implementation of appropriate mitigation strategies) also needs to be considered. In collaboration with the investigator and veterinary team, the animal care committee should define the level of monitoring beyond daily observation that is necessary for the particular animal model or study.

All personnel should use appropriate practices to observe animals that respect the welfare of the mice (e.g., not tapping on the cage, not moving cages in a way that causes disturbance).

The impact of changes in personnel and perfumes or scents on the animals is discussed in the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017). Perfumes and other strong odorants should be avoided when working with mice.

# 4 PROCUREMENT

The *CCAC guidelines on: procurement of animals used in science* (CCAC, 2007) should be consulted for general guidelines that are applicable to all species. This section provides additional considerations that are particular to mice.

## 4.1 SOURCE

Many mouse models, especially genetically modified mice, may develop an adverse phenotype and need special care and management to mitigate potential welfare concerns (Pritt et al., 2006). Investigators must be aware of the likelihood that the mice they are working with will have special needs, and inform all personnel involved in the care of these animals (Pritt et al., 2006).

There are many confounding issues that may prevent the use of pre-existing animal lines, including intellectual property protection, scientific barriers (e.g., assurance of the same site of insertion of the gene of interest), animal quality concerns, incorrect analysis, compromised background strains, health status, availability, and accessibility. However, investigators should take reasonable steps to ensure that they are not generating new genetically modified mouse lines when scientifically adequate lines already exist and are accessible. See Appendix 2, " Databases for Genetically Modified Mouse Lines", for a list of databases of available mouse lines.

If mice have not been subject to major invasive procedures, they may be transferred to another study or protocol. The transfer of animals that have been surgically prepared (e.g., adrenalectomy, thymectomy or cannulation) by breeding institutions for inclusion in an animal-based protocol is also permitted. However, these animals should only be transferred when they are fit to travel.

## 4.2 DOCUMENTATION

An effective documentation system should be in place to provide information on the identity and welfare of mice, including health concerns and any special needs, to ensure their proper care and to inform decisions regarding any animal-based procedures. This information should accompany the mice and be accessible to all involved in their care and in the conduct of procedures.

Correct nomenclature should be used to identify the animals, especially in the case of genetically modified mice. For guidance, see the *Guidelines for Nomenclature of Mouse and Rat Strains*, published jointly by the International Committee on Standardized Nomenclature for Mice and the Rat Genome and the Nomenclature Committee, and updated annually. Consistent use and understanding of all abbreviations is important (e.g., a KO (knock-out) mouse is by definition homozygous for the null allele -/-; KO should not be used for a heterozygote +/- mouse).

Genetically modified mice that are brought into an institution should have accompanying documentation, which should include:

- accurate and correct nomenclature of strain and genetic modification(s) in accordance with the *Guidelines for Nomenclature of Mouse and Rat Strains*;

- information on the phenotype of the animal, including welfare status, mitigation strategies for pain and/or distress, and humane endpoints;
- special conditions for husbandry and breeding (e.g., special requirements to address behavioural needs, nutritional needs, or sensitivity to temperature);
- the procedure used to distinguish genetically modified animals from wild-type animals; and
- archiving strategy (e.g., cryopreservation) or archive location if the line is archived off-site.

## 4.3 SHIPPING AND RECEIVING

Standard operating procedures and documentation are needed for moving animals within an institution, as well as for procuring animals from other institutions or suppliers. These standard operating procedures should be applicable to all mice in science, including those that are procured as live feed for other animals, such as for captive wildlife species. Prior to accepting new genetically modified mice for breeding purposes, the animal care committee should ensure that the investigator has a plan in place to appropriately manage breeding, including how animals carrying the genotype of interest will be identified.

Transportation plans must be in place to manage any unanticipated delays. Facilities should develop a policy or standard operating procedure for performing a risk assessment and determining necessary precautions for transportation (see Section 5, "Transportation", in the *CCAC guidelines on: procurement of animals in science* (CCAC, 2007)). The policy or standard operating procedure should ensure the transport route is well planned in advance, with consideration to the ambient conditions. The assessment of risk should be based on the type, duration, and route of transportation, the weather, and the likelihood of delays. Short-duration movement of animals (< 1 hour) may not require a risk assessment if the animals can be returned to the original location should problems arise; however, even short transportation within an institution needs to be planned in advance (e.g., avoiding people and public elevators).

## 4.4 TRANSPORTATION

### 4.4.1 Shipping Conditions

As noted in Section 5, "Transportation", of the *CCAC guidelines on: procurement of animals in science* (CCAC, 2007), "[i]nstitutions, in consultation with animal users (particularly for protocols using nontraditional or field species), are responsible for selecting the method and timing of transportation of animals from the suppliers, and monitoring the transportation process."

Temperature limits should be specified and measures taken to protect mice from exposure to temperatures outside these limits (e.g., transporting mice at night during hot weather and providing sufficient bedding and nesting material for thermoregulation (Syversen et al., 2008). Mice are more susceptible to extreme temperatures and sudden changes in temperature than larger animals, and it is important that the shipper can ensure the appropriate environmental conditions that will safeguard the welfare of the animals (see Syversen et al. (2008) for a description of the prevalence of inappropriate temperatures and temperature variations experienced by mice during shipping).

Mice must be transported in a safe, non-compressible container from which they cannot escape. The container should be a specifically designed transport box to contain all wastes, protect mice against potential contamination, prevent exposure of personnel and the public to allergens, zoonotic diseases or any hazards

(e.g., biological, chemical, or radiation), and keep the animals out of view. Containers must permit adequate ventilation for the mice, even when stacked. Specifications for containers are covered in the *CCAC guidelines on: procurement of animals in science* (CCAC, 2007) and further details concerning the design and construction are given in ILAR (2006) and Swallow et al. (2005).

When a divider is used, individual mice in a container must be identifiable in case the divider is breached. The divider should be constructed so as to prevent the mice from crossing to the other side. Whenever possible, sexually mature male and female mice or those of different ages or health status should be shipped in separate containers rather than separated by a divider within the same container.

Mice must have food and water prior to and during transport. Mice lose more heat and calories and become dehydrated more quickly than larger animals; for this reason and the necessity of anticipating potential delays, the amount of food and water available during transport should be at least twice that required by the animals for the expected length of travel (CCAC, 2007). Gels are the preferred method for providing hydration during transport, and they should be provided to the mice the day before shipping to allow for acclimation. Water must not be placed in the animal container as it is likely to spill. If mice become wet, hypothermia may develop rapidly, depending on the external temperature.

The transport of cryopreserved embryos or sperm is a useful alternative when the well-being of live mice may be compromised by long periods of transport or when their health status is not compatible with transportation. This is particularly important for genetically modified mice. However, the receiving institutions must have facilities available to handle embryos and sperm appropriately. Those involved in the transport of mice should be aware of further refinements to transport methods, as they become available (see Takeo et al., 2014).

Institutions acquiring gametes should be aware that this material can be a source of contamination and introduce pathogens into a facility (Janus and Bleich, 2012).

### 4.4.2 Moving Mice Between Institutions

Care should be taken not to ship mice that are already welfare compromised to the extent that they will experience distress as a result of transportation practices.

An American Association for Laboratory Animal Science (AALAS) and Federation of European Laboratory Animal Science Associations (FELASA) working group has developed a health monitoring report form that can be used to convey standard health information between institutions (Pritchett-Corning et al., 2014).

### 4.4.3 Moving Mice Within an Institution

Short-duration transport of mice (e.g., within an institution) should be in accordance with policies or standard operating procedures that emphasize good procedures for protection of both animals and people.

Mice should be transported in a covered, closed, and secured cage or container. Home cages may be used, provided they are properly secured and the water bottle removed. Care should be taken to maintain the cage in a horizontal plane during transport to optimize ventilation and to minimize potential accidents, including escape. Personnel transporting mice should be aware that mice are sensitive to vibration and noise, and choose a transport method that reduces those elements. For example, placing a folded towel or drape under the cage will significantly diminish the vibration transferred between the cart and the cage (Hurst and Litwak, 2012).

If mice are to be moved outside between buildings, consideration must be given to the weather. Suitable precautions must be taken to protect the welfare of the animals if they are to be moved during extreme weather conditions (i.e., both winter and summer). Mice must not be left unattended in a non-secure area during transport and should be placed in a secure location and attended to as soon as possible upon arrival at their destination.

## 4.5 RECEPTION OF MICE AT AN INSTITUTION

**Guideline 11**

The health status of the incoming mice should be reviewed before the animals are shipped.

Mice are obtained from a variety of sources, including breeding companies and individual laboratories sharing animals. However, as pointed out by Shek (2008), animals are imported from a wide variety of research institutions with variable biosecurity, husbandry and health monitoring practices, resulting in the potential for parasite infestations and microbial infections that are largely eliminated by commercial specific pathogen-free rodent suppliers.

In addition to reviewing the health status of the animals in advance of their arrival, it is also important to obtain as much information as possible on the details of the husbandry and other mouse-related practices of the source institution shipping the mice. This will assist in establishing quarantine conditions for the animals upon arrival.

Reception conditions should be described in a standard operating procedure and include procedures to be followed upon opening containers, such as:

- verifying that the animals received correspond to the order;
- decontaminating the exterior surfaces of non-disposable containers;
- opening the container in such a way as to prevent escape;
- preferentially opening the container and transferring mice to their cage under a laminar flow hood;
- handling the mice in such a way as to prevent contamination (e.g., not touching the mice with the hand that touched the exterior of the container);
- verifying that all animals have been removed from the transport container; and
- dealing with animals that are sick or dead on arrival.

Assessment of the condition of the animals upon receipt should be carried out according to the institution's standard operating procedure. A visual examination of the mice upon arrival is valuable to assess any need for immediate treatment (e.g., for dehydration, trauma). Observation of animals received from a shipper is also important to ensure that the new groupings of mice are compatible.

Animals brought into the facility must undergo a period of quarantine when required by the health status of the incoming animals.

A period of acclimatization, which can run concurrently with the period of quarantine, is important to ensure any stress associated with transportation has been alleviated and the physiology of the animal has

returned to a normal state. The length of time required will depend on the conditions of the transport, age of the animals and the particular animals involved (e.g., animals with stress-sensitive genotypes may require a prolonged period of acclimation (Obernier and Baldwin, 2006)). During this period, the animals should be habituated to the method of food and water delivery and to the new environment. Animals should also be acclimated to study conditions and procedures that will be conducted while they are conscious.

## 4.6 PROCUREMENT OF MICE FOR FEED

If mice are to be procured as live feed for maintaining other animals (e.g., wildlife or reptiles), they should be acquired from a reputable supplier (as defined in the *CCAC guidelines on: procurement of animals in science* (CCAC, 2007)) to avoid bringing diseased or contaminated animals into the facility. The type of transport container and the procedures for transportation and reception should follow the requirements for other mice. An animal protocol is required for any mice to be procured or bred in-house for live feed. The mice should be killed prior to feeding.

# 5 BREEDING

**Guideline 12**

Breeding colonies must be efficiently managed according to approved protocols, anticipated need, and the principles of the Three Rs.

Prior to undertaking in-house breeding, careful consideration must be given to factors such as the necessity of the breeding program, the availability of suppliers, and the availability of appropriate space and personnel (see the *CCAC guidelines on: procurement of animals used in science* (CCAC, 2007), Section 4.2, "In-House Breeding Colonies"). Large numbers of animals may be required in the maintenance of breeding colonies of genetically modified mice. Every animal from a breeding colony should be on a protocol and requires assessment of the phenotype for identifying genetic drift and animal welfare concerns (see Section 8, "Welfare Assessment").

Breeding protocols must be approved by the animal care committee. Any projected or expected effects of genetic modification on mouse health must be considered, along with strategies to mitigate those effects. The breeding protocol should also include screening protocols so that the appropriate animals can be identified based on their genotype or phenotype. The facility manager or institutional veterinarian can be a resource for developing breeding protocols.

As soon as the genetically modified animal with the desired genetic modification or phenotype has been generated or acquired, a breeding strategy is required if there is a need to further propagate the particular line of animals. Well-established institutional guidelines for colony management and animal monitoring are necessary to ensure animal welfare and the quality of experimental data.

## 5.1 RECORD KEEPING AND OVERSIGHT

Proper record keeping is extremely important to ensure effective and efficient management of breeding colonies, including detection and spread of disease, and the reproducibility of research experiments (Casellas, 2011). Records must be kept, and should include the following:

- the particular animals involved in the breeding program (e.g., source, date of birth, unique identifier (e.g., ear notch, tattoo), health status, genetic information, breeding history and productivity, previous care, and involvement in any procedures or studies);
- housing and husbandry requirements;
- details of expected and demonstrated phenotypes (including behaviours);
- details of diseases and treatments;
- breeding parameters, such as fertility, fecundity, morbidity, and mortality;
- intended assignment to studies; and
- criteria for retirement from breeding.

Cage cards are important as a quick visual reference, but additional records are necessary for managing breeding programs. Breeding colony cage cards should include date of birth for litters and projected weaning dates. Where there are numerous inbred strains in a facility or room, record keeping can be complex and computerized systems are strongly recommended. Colony-management software can provide automatic notification of when animals require weaning and details of life histories. Tyeus (2006) describes the benefits of relational databases in managing breeding systems involving more than 100 animals over three generations, and a free database has been provided by Jax.

Current systems for managing digital data and records are becoming more capable of interfacing between protocol review processes, grant officers, financial administrators, and human resource data management (i.e., personnel certification and training records). Efforts that incorporate management of digital records for mouse colony management facilitate the integration of animal data into such systems. Digitally based colony management and the need for added infrastructure or computer applications might be considered in the planning and building of new animal care resources.

Breeding logs must be available to the veterinary team, animal health personnel, and the animal care committee to ensure appropriate procedures are followed. Institutional measures for post-approval monitoring should be developed and applied to ensure approved protocols and standard operating procedures are followed and to assess their efficacy. This can be facilitated through shared access to digitized records.

Where investigators have approval to undertake breeding, they must demonstrate competence in managing breeding colonies (see *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015)) and demonstrate that they (and their personnel) can keep complete records.

All animals in a breeding colony (including both the number born and the number weaned) must be documented within the institution and reported at least annually to the animal care committee. The number of animals born should be determined as soon as they can be counted without disturbance. These numbers should be recorded per line. Annual reports to the animal care committee as part of protocol renewal should also include information on the breeding system, reproductive performance (including any sudden change that could indicate genetic contamination), morbidity/mortality (based on number born versus number weaned), criteria used for culling animals, and other factors related to the way the breeding program is managed to meet the requirements of the research while avoiding overproduction of animals.

## 5.2 IDENTIFICATION OF BREEDING COLONY ANIMALS

Procedures for the timely identification of genetically modified founders and offspring should be developed and submitted to the animal care committee as part of the breeding protocol and approved prior to commencement of breeding. See Section 6.1, "Identification of Animals", and Appendix 3, "Methods of Identification for Mice". For further details on particular methods, see Dahlborn et al. (2013).

When mice will be involved in long-term breeding, they must be permanently identified (see Section 6.1, "Identification of Animals").

## 5.3 CONSIDERATIONS FOR BREEDING MANAGEMENT

As with all breeding of animals, careful attention must be paid to limiting the production of surplus animals; in many cases, overproduction can be avoided through more precise planning (Finlay et al., 2015).

Communication with investigators or study directors and facility managers is essential to ensure planning of animal numbers is well considered far in advance. Specific training for people involved in breeding colony management is encouraged.

Careful tracking of all animals (including the number born, the number weaned, and the number transferred to experiments) provides data for investigators and animal care committees to use in applying the Three Rs. It also avoids shortfalls in the numbers of animals required. Inefficient breeding results in more animals being needed to produce a given number of pups. Purpose-designed colony management software may be useful (Hetherington et al., 2000).

Strategies should be in place to use any surplus animals that are produced (e.g., for training exercises, tissue or blood collection that can be stored for subsequent use, or live feed). A registry can be developed to notify researchers of any surplus animals that may be available. See CCAC (2014) for best practices in facilitating the sharing of animals and animal tissues.

Breeding animals should be carefully selected based on genetic characteristics and factors related to their history and health that may have an impact on breeding success and the requirements of the scientific studies. Strain differences in reproductive performance should be taken into account (Byers et al., 2006; Jax). Breeding management should aim to minimize genetic contamination, and a program should be implemented to monitor and limit genetic drift.

Deleterious mutations are frequently expressed as impaired reproductive performance and if the mutation is not the subject of the approved research, these animals should not be bred further. However, mutations may be very subtle and contamination of the genome may have occurred several generations prior to noticing a change in phenotype; in some cases, control of the offspring according to phenotype may be insufficient and a genome scan is necessary to detect contamination (Casellas, 2011; Fahey et al., 2013).

For outbred animals, founding populations should be large enough to ensure long-term genetic heterogeneity of breeding colonies (Chia et al., 2005). Nomura and Yonezawa (1996) provide a comparison of four systems of group mating that are utilized to avoid inbreeding.

Investigators are responsible for describing the phenotype for the specific strains they are working with, and sharing that information with the animal care committee and veterinarian. The investigator, animal care committee, and veterinarian should collaborate on the development of a phenotyping plan, which should be described in a protocol approved by the animal care committee.

Monitoring of animals for which the phenotype is lethal or anticipated to have severe negative effects on animal welfare must be undertaken, and should be a collaborative effort involving the investigator, animal care personnel, and the veterinarian. Collaboration is important to ensure that welfare indicators are identified early and efficiently, and that animals with valuable phenotypes are not euthanized before they are identified.

Appropriate biosecurity measures must be in place to prevent genetic or pathogenic contamination of mouse lines.

### 5.3.1 Breeding Systems

Breeding systems can vary widely from strain to strain. Considerations for breeding include the strain or stock of mouse (inbred or outbred), space availability, the requirement for post-partum breeding, and the need to maintain a particular health status (Harkness et al., 2010; Hampshire and Davis, 2005; Murray and

Parker, 2005; Pritchett-Corning et al., 2011). The type of breeding system has also been shown to influence maternal behaviour as well as weanling weight and behaviour, with these influences varying with different strains (Braden et al., 2017). Investigators should work with animal health personnel and the veterinarian to devise the best breeding system strategy for the experiment, while minimizing surplus mice.

Trio breeding, which involves one male and two females and the removal of pups at weaning, makes efficient use of cage space. However, records for trio breeding can be difficult to maintain (Harkness et al., 2010) and this breeding system may lead to overcrowded cages (see Section 2.1.1.1, "Cage Size") or reproductive suppression (Garner et al., 2016).

Monogamous pairing simplifies record keeping and allows for post-partum breeding, which maximizes the number of litters per female; however, this system requires a large number of males (Harkness et al., 2010).

Polygamous or harem mating requires moving pregnant females to separate cages and results in greater milk production, larger young, and more young weaned per litter; however, this system does not use post-partum breeding and results in fewer litters per female (Harkness et al., 2010). For further details on common mating systems, see Baumans (2010), Harkness et al. (2010) and Berry and Linder (2007).

For all breeding systems, steps must be taken to limit the production of surplus animals or the loss of newborns due to the presence of unweaned older pups. This includes obtaining animal care committee approval for the breeding scheme, close monitoring of the reproductive success of the colony, and reporting the numbers of mice born and the numbers of mice weaned to the animal care committee. A standard operating procedure should be developed describing breeding schemes approved by the animal care committee and the care to be given to the breeding mice and their pups.

Where short-term breeding is carried out (e.g., mice are bred in order to study the products of reproduction or reproductive behaviour) and the mouse strain is not intended to be maintained, the use of breeding schemes may not be necessary. However, good breeding practices and record keeping should still be in place.

### 5.3.2 Breeding Age

A number of factors will influence ideal breeding age. Mice are generally first bred after reaching at least 50 days of age, at which time they weigh 20-30 g (Harkness et al., 2010). Initiating breeding before this time or after 70 days of age results in reduced fertility (Harkness et al., 2010).

It is important to keep good records of the reproductive performance of the animals, as breeders should be replaced according to reproductive history. Indicators used to determine when to replace breeders include litter size, pup weight, and numbers of pups born and pups weaned, which are all strain specific (Harkness et al., 2010; Murray and Parker, 2005), as well as the interval between litters and the general health status of the breeder. Inefficient breeding ultimately increases the number of animals that are needed to produce a given number of pups.

The reproductive lifespan of female mice is approximately 6 to 8 months (Berry and Linder, 2007), depending on the strain. Breeding beyond this age results in decreased litter size and parturition difficulties (Baumans, 2010). Male mice can reproduce for a longer period (Berry and Linder, 2007); however, they should not be used for breeding beyond one year of age due to the increased likelihood of age-induced susceptibility to disease and decreased efficiency of breeding.

### 5.3.3 Weaning

Mice should be weaned at approximately 21-28 days or when 10-12 g in weight (Baumans, 2010; Harkness et al., 2010), depending on the strain. Smaller mice of slow-growing strains should be weaned at 28 days of age (Harkness et al., 2010). It is recognized that weaning age influences behaviour and sexual dimorphism in the brain (Curley et al., 2009b). Weaning earlier than 21 days may result in higher anxiety levels for the pups, lower levels of maternal behaviour when they mature, and reduced ability to adapt to social groups (Kikusui et al., 2005). Weaning procedures should be included in standard operating procedures approved by the animal care committee to describe the care to be given to the breeding mice and their pups.

For some genetically modified strains with developmental impairment, it may be best to leave the pups with the dam for at least 28 days, provided the cage is not overcrowded and the dam was not mated in the post-partum period. When planning for late weaning, post-partum mating is discouraged.

Newly weaned mice should be closely monitored to ensure they are able to access food, water, and nesting material. Initially, they may be provided with food on the cage floor, more than one source of water (i.e., water substitute (e.g., gel) or water bottle, in addition to the automatic drinking valves), and pre-shredded nesting material, until they are noted to be thriving on their own.

### 5.3.4 Post-Partum Breeding

Post-partum estrus occurs 14-28 hours after giving birth (Foster et al., 1983). Pairing a male and female mouse together takes advantage of this estrus and results in the maximum number of litters in the shortest time possible. However, this practice can result in the delivery of a second litter before the previous litter is weaned and pups may be lost as older pups out-compete the younger ones (Hickman and Swan, 2011). This system can also lead to health and welfare issues for the female breeder and potential overcrowding in cages.

### 5.3.5 Cryopreservation

Archiving technologies can lead to a reduction in numbers of animals. Archiving can be used to:

- provide backup in case of catastrophic loss of a breeding colony;
- overcome problems associated with loss of fertility due to aging;
- preserve a line not currently being studied;
- limit genetic drift (in particular, the loss of the transgene expression in subsequent breeding generations);
- repopulate a breeding colony;
- enable a line of genetically modified mice to be shared and thereby reduce the need for the line to be generated elsewhere; and
- facilitate sharing of a line where transportation would result in considerable stress for the animals.

Cryopreservation of gametes and embryos should be used to minimize genetic drift and reduce the risk of losing a line due to disease, contamination, human error in screening and managing breeding colonies, or catastrophic events (fire, flood, etc.; Liu and Takahashi, 2010). It can also be more cost-effective to cryo-preserve lines that are not currently being used for research, rather than maintain live animals (Liu and Takahashi, 2010). See Prins (2011), Mochida et al. (2011) and Takeo and Nakagata (2011) for descriptions of methods of cryopreservation and recovery.

Services for cryopreservation are available. Where cryopreserved samples are maintained in-house, they should preferably be saved in at least two geographically separate locations to avoid loss of cryopreserved gametes or embryos. See Section 2.3, "Core Facilities for Generation of Genetically Modified Mice".

## 5.4 FACTORS AFFECTING REPRODUCTION

### 5.4.1 Environmental Factors

Light, temperature, relative humidity, noise and vibration are discussed in Section 3, "Facility Management and Personnel". As noted in that section, a light cycle of 14 hours light and 10 hours dark is optimal for reproduction; however, a 12:12 cycle is also acceptable. Maintaining a consistent dark phase is important, as even opening a door briefly during the rodent dark phase can affect reproductive performance (Bedrosian et al., 2013). In addition, it is important to house breeding mice away from sources of noise and vibration or to take measures to minimize the effects.

Pups up to 3 weeks of age may require higher temperatures than older mice. The room temperature should be 24-26 °C (i.e., the higher end of the normal range) (Gordon, 1993), although this requirement will be influenced by the provision of sufficient nesting material.

As noted in Section 1.2, "Senses", odours can influence various aspects of breeding and pup-dam interactions. The housing situation (i.e., the presence of other mice) and disruption of pheromones through such activities as cage cleaning can affect the reproductive physiology of mice (Hurst, 2005; Bind et al., 2013) and the quality of maternal care provided to pups (Bind et al., 2013).

### 5.4.2 Housing and Husbandry Influences

Cage sizes and requirements for bedding and nesting materials are discussed in Section 2, "Facilities". The type of caging should be consistent throughout a study, as changes may affect breeding (Czarnomska and Wezyk, 1974; Porter et al., 1963). Nesting material is important for all mice, and is of even greater importance during breeding. The provision of nesting material has been shown to increase the numbers of pups born and weaned (Gaskill et al., 2013c). While the effects of thermal stress may be greater for nude mice, all laboratory mice are housed below their lower critical temperature, typically 26-28 °C (Gordon, 1993; Speakman and Keijer, 2013); therefore, providing mice with the opportunity to behaviourally thermoregulate can reduce heat loss and lead to improved breeding performance (Gaskill et al., 2013a; Gaskill et al., 2013c; Gaskill et al., 2013d).

Cages should not be cleaned during the first few days after delivery, to avoid disturbing the pups. If it is necessary to clean or change cages during that period, some of the nesting material should be retained (Harkness et al., 2010).

## 5.5 HEALTH ISSUES

When new lines are being produced, investigators may not know the resulting phenotypes of the mice. In such cases, the veterinarian must be involved in decisions regarding the care and welfare of those animals.

If health concerns occur, the veterinarian must be consulted to ensure they are dealt with in a timely manner. Standard operating procedures should be in place to address common health problems associated with

breeding. Health issues tend to be specific to the particular line of mice, but may include pre-weaning pup mortality, dystocia, and vaginal or rectal prolapse.

Important factors related to litter survival are nest building before parturition and having an uncomplicated parturition. The probability of litter survival has been shown to increase dramatically with nest building behaviour, and decrease with the female being outside the nest (Weber et al., 2016).

Cannibalism is not necessarily the cause of pup mortality. Dead pups are often cannibalized by their parents, but Weber and colleagues have not found any evidence that C57BL/6 females actively kill their pups (Weber et al., 2013a; Weber et al., 2013b). Where cannibalism is a concern, steps must be taken to minimize the occurrence, for example, by reducing light intensity and noise, providing nesting material (Baumans, 2010), and leaving the female undisturbed for two days following the birth of a litter (Harkness et al., 2010); however, these measures complicate monitoring (Weber et al., 2016).

## 5.6 GENOTYPING

Genotyping is important for determining the genetic makeup of mice that have been genetically modified and for monitoring a breeding colony for genetic drift. The genotyping methodology (which may include outsourcing to a specialist company) must be approved by the animal care committee and should be described in a standard operating procedure.

The choice of method for obtaining genetic material should be based on: 1) the aim to minimize pain and distress for the animals; 2) the amount of tissue needed, which will depend on the type of analysis required; and 3) whether a suitable tissue sample can be obtained from the method used for identification of the animal (see Sections 5.2, "Identification of Breeding Colony Animals", and 6.1, "Identification of Animals"). For a review of welfare concerns and potential refinements for methods of genotyping, see Appendix 4, "Recommended Practices for Genotyping".

# 6 HUSBANDRY

The *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017) should be consulted for general guidelines that are applicable to all species. This section provides additional considerations that are important for mice.

## 6.1 IDENTIFICATION OF ANIMALS

If only short-term identification is needed, possible methods include the use of non-toxic dyes, permanent markers, and fur clipping. Where permanent identification is necessary, methods include microchips/transponders, tattoos, ear tags and ear punches (for additional examples, see Appendix 3, "Methods of Identification for Mice").

Genetically modified mice should be identified according to standardized nomenclature, which should be included in documentation that is maintained and transferred with the animals.

## 6.2 HOUSING MANAGEMENT

Addressing the physical and behavioural needs of mice through housing includes providing opportunity for social contact and locomotor and exploratory behaviours, as outlined in Section 1.1, "Behavioural Biology". Space requirements for mice are discussed in Section 2.1.1.1, "Cage Size"; the cage size should support group housing of mice (Clipperton-Allen et al., 2015) and the inclusion of a shelter (Van Loo et al., 2004; Clipperton-Allen et al., 2015).

Abnormal behaviours including stereotypies are often associated with poor environments, and must be considered a potential sign of stress. Efforts must be made to reduce the development of these behaviours by providing opportunities for mice to perform important species-specific behaviour. Some stereotypic behaviours have been found to be reduced when mice were provided with enrichment (Bechard et al., 2011; Gross et al., 2012); however, other factors may also contribute to the occurrence of abnormal behaviours, including stereotypies. For example, litter size has been shown to be positively correlated to stereotypic behaviour in females of some strains of mice (Bechard et al., 2012), as have age and weight at weaning (Würbel and Stauffacher, 1997).

Once abnormal behaviours or stereotypies have started, they are very difficult, if not impossible, to extinguish. For example, barbering is a learned behaviour that can be transmitted to offspring. Barberers are more likely to be female and the incidence of barbering has been linked to reproductive status and genetic background (Garner et al., 2004a), while cage design and location, relatedness of cage mates, and the presence of other barberers also influence the incidence of barbering (Garner et al., 2004b). It does not appear to be an expression of dominance (Garner et al., 2004b), but is affected by a variety of factors such as poor environment (Bechard et al., 2011) and diet (Dufour et al., 2010). Barbering is a welfare concern that should be evaluated and addressed (Tynes, 2013). For other examples of abnormal behaviours including stereotypies, see Section 1.1, "Behavioural Biology".

### 6.2.1 Social Housing

**Guideline 13**

Mice should be group housed.

Both male and female mice show a strong preference for social housing (Sherwin, 1996a; Van Loo et al., 2004; Van Loo et al., 2001a).

Fighting, leading to wounding or death, is especially common in male laboratory mice. Where aggression occurs in group-housed males, it must be addressed immediately.

Aggressive behaviour can sometimes be prevented by improving the housing conditions before males are grouped (Van Loo et al., 2001a; Clipperton et al., 2015; Weber et al., 2017), with an evaluation of the risk of fighting and injuries documented. Approaches to reduce or avoid aggressive behaviour include:

- maintaining stable post-puberty groups – aggression toward new mice may begin at 32-36 days of age, suggesting that any mixing of unrelated animals should occur prior to this age (Gaskill, 2014);
- ensuring that group-housed male mice have sufficient cage resources to limit aggression (Baumans and Van Loo, 2013; Clipperton-Allen et al., 2015) – providing only a single shelter or enrichment item can lead to increased aggression due to competition (Van Loo et al., 2003; Howerton et al., 2008);
- ensuring appropriate cage configurations with multiple choices for escape, hiding or being out of the sightline of other males (Gray et al., 2000), in conjunction with observation to ensure mice do not become more aggressive by defending resources (Van Loo et al., 2003) – for example, Tallent et al. (2018) showed that partial partitions within cages that resembled burrows and were linked to a common area for all mice in the cage may reduce aggression;
- maintaining room temperature at 20-22 ºC (Weber et al., 2017) and providing nesting material (Weber et al., 2017; Van Loo et al., 2003);
- consolidating cage cleaning with other activities to minimize disturbances and transferring nesting material to the new cage to maintain scent markings (note that bedding material should not be transferred as it appears to increase aggression (Van Loo et al., 2003; Weber et al., 2017));
- not re-housing males back together after mating (Annas et al., 2013); and
- limiting group size in a standard cage to three animals (Weber et al., 2017; Van Loo et al., 2001b).

Additional general recommendations to reduce aggression include minimizing stress and pain in handling mice and in the conduct of procedures, providing pain control when necessary, and avoiding exposure to potential endocrine disruptors (Weber et al., 2017).

### 6.2.2 Single Housing

Single housing of mice is only permitted in exceptional cases with strong scientific, welfare or medical justification. Social isolation has been shown to have an impact on various stress-related parameters (Kalliokoski et al., 2014; Nary et al., 2002; Arndt et al., 2009; Koike et al., 2009), which could also impact research data. However, some males that show aggression and fight may need to be single housed (Kappel et al., 2017) if the measures noted in Section 6.2.1, "Social Housing", do not sufficiently address this behaviour. Providing

sufficient environmental enrichment within the cage is particularly important in addressing the welfare of any singly housed animals (Clipperton-Allen et al., 2015).

## 6.3 FOOD, WATER, AND BEDDING

### 6.3.1 Food

Mice eat up to 20% of their body weight daily; however, the amount and composition of the diet should be adapted to the age and nutritional needs of the animals. For example, breeding animals require a higher fat content and mice subjected to stress (such as drug testing or surgery) or mice maintained in a germ-free environment may have different nutritional requirements (NRC, 1995). Bachmanov et al. (2002) provide information on requirements of food intake for 28 different strains of mice.

There should be a transition period to acclimate mice to any significant changes in their food (e.g., if mice will be given a new food post-surgery, they should be introduced to that food in advance) and the animals should be monitored to ensure they are eating.

In general, food should not be sprinkled on bedding in order to minimize contamination. However, there are many situations (e.g., post-surgery, weakened or limited mobility animals, newly weaned animals or enrichment) where food can be provided at the floor level.

Sufficient food should be added to hoppers to ensure that mice have access to clean food at all times. Prior to adding fresh food, the remaining food should be examined and any spoiled or soiled food should be discarded.

Any imported food must follow the feed regulations from the Canadian Food Inspection Agency (CFIA, 1983). Pathogens (e.g., parvovirus and rotavirus) can potentially be introduced through food and bedding (Clifford and Watson, 2008; Watson, 2013). Irradiated food or autoclaved food that is specifically intended for autoclaving should be used in specific pathogen-free facilities, although pathogens can still be introduced through this food.

Where special diets are required, arrangements for supplies should be made in advance, and the criteria for storage and use should be well understood to ensure the stability of any dietary additives.

If food is not a standard commercial food, there should be a standard operating procedure on preparation and maintenance. Where the prepared food is to include an experimental compound, the properties of the compound (e.g., lipophilicity) need to be considered in relation to the actual dose to be delivered to the animal (see Hayes and Kruger (2014) and the United States Food and Drug Administration *Redbook 2000: IV.B.1. General Guidelines for Designing and Conducting Toxicity Studies* (FDA, 2003)).

### 6.3.2 Water

Mice may require specially treated water (e.g., acidified, chlorinated, reverse osmosis, autoclaved, or ultraviolet (UV) irradiated) to prevent introduction of pathogens. Facility managers and investigators should be knowledgeable about the available options for providing water to mice and select the method best suited to the particular animals and study.

Bachmanov et al. (2002) indicate that water consumption ranges from 3.9 to 8.2 ml/mouse/day, depending on the strain.

### 6.3.3 Bedding and Nesting Materials

**Guideline 14**

Bedding and nesting material must be provided to allow mice the opportunity to build nests and thermoregulate, as well as to dig, burrow, and forage.

Both bedding and nesting material are important to enable mice to create a comfortable microenvironment for resting, breeding, and the performance of some natural behaviours (Jennings et al., 1998; Sherwin, 2002; Gaskill et al., 2012). Bedding is also important for absorbing urine and feces and controlling ammonia levels (Rosenbaum et al., 2009; Freymann et al., 2017), and may be incorporated into nest structures.

Provision of nesting material is a standard cage requirement (Gaskill et al., 2013c), as many strains of mice are highly motivated to build nests (Van de Weerd et al., 1998b). Nests allow mice to regulate exposure to light, temperature and perceived threats. The amount of nesting material required (typically 8-12 g per cage) should be of sufficient quantity in at least one area of the cage to cover all animals within the cage (Gaskill et al., 2012). Hess et al. (2008) provide an example of a scoring system for nest quality, and suggest that the quality of the nest is greatly influenced by the type of material provided. It is recommended that mice be given a choice of two types of material (e.g., paper strips and tissue), as this can result in complex nest construction and improved reproduction (Gaskill et al., 2013a). Mice that are unable to shred material for a nest (e.g., sick mice, disease models or otherwise debilitated mice) should be given pre-shredded or prepared nesting material (Aubert et al., 1997; Gaskill and Pritchett-Corning, 2016).

Nesting materials and bedding must be non-toxic, not harmful if ingested, absorbent but not dehydrating for neonates, consist of particles that are suited to the needs of the mouse (e.g., ease of manipulation), and produce a minimal amount of dust (Baumans, 2010). In addition, when the mice are involved in an experiment or study, the materials should not have an impact on the experiment or study. Mice show a preference for large fibrous particles that they can manipulate, such as shredded paper, over wood chips, sawdust or floors with no bedding (Blom et al., 1996). Preference for certain materials may be based more on the structure of the material (i.e., the ability to manipulate the material into a nest) than on the composition of the material (Van de Weerd et al., 1997). Mice are able to build better nests with shredded paper strips than other materials (Hess et al., 2008). While paper may be provided as nesting material, some paper can quickly become wet from urine, and should be avoided as bedding material.

Selection of materials should also be based on the strain of mice and the purpose of the study. For nude mice that lack eyelashes, some types of paper bedding with small fibres can cause swollen eyelids and abscesses (White et al., 2008), and cotton bedding can lead to conjunctivitis (Bazille et al., 2001). Additionally, some types of bedding derived from wood or corncobs can affect the physiology of mice and impact research results (White et al., 2008; Ambery et al., 2014; Leblanc et al., 2014). Nesting material with fibres of inappropriate length may lead to gangrenous pododermatitis in pre-weaned animals (Barthold, 2016).

It should be noted that pathogens (e.g., parvovirus and rotavirus) can potentially be introduced through bedding (Lindstrom et al., 2018).

See Section 6.7, “Cage Changing and Sanitation”, for guidance on changing bedding and nesting material.

## 6.4 ENVIRONMENTAL ENRICHMENT

Important basic requirements that address the physical and behavioural needs of mice are discussed in other sections of this document:

- cages should be of a sufficient size to allow mice to perform behaviours important to their welfare, and promote the conduct of additional behaviours that will improve their quality of life (see Section 2.1.1.1, "Cage Size");
- nesting material and shelters are standard features in mouse housing, as they address behavioural needs and provide comfort for the animals (see Section 2.1.1.4, "Cage Components"); and
- mice should be group-housed (see Section 6.2, "Housing Management").

Additional elements that may enrich the animals' environment are listed below, but must be considered in the context of the individual animal and the research requirements:

- features to promote activity (e.g., structures to climb on or around, exercise wheels);
- substrates for burrowing;
- structures to facilitate hiding or avoidance of people or other animals (e.g., tubes, dividers);
- objects to chew (e.g., nylon bones) to help prevent overgrowth of teeth and stereotypic bar chewing;
- variety in food (e.g., a piece of vegetable, cereal, or mouse treats), provided that these do not cause any biosecurity issues; and
- encouragement of foraging by providing selected food items in the bedding (Hutchinson et al., 2005).

## 6.5 HUMAN CONTACT AND HANDLING

Mice are nocturnal animals and therefore, routine observations and cage changing should be conducted quietly and efficiently to minimize disturbance of animals, especially when these activities are performed during the mouse resting phase (i.e., in the light part of the diurnal cycle). Breeding colonies and experimental animals should be separated, whenever possible, to minimize the disturbance of other animals when particular mice are removed for procedures.

For further details on handling, as well as restraint, see Section 7, "Handling and Restraint".

## 6.6 ANIMAL OBSERVATION

Daily observation of animals should include evaluation of both health and behaviour, although it is recognized that the full range of behaviours is best observed in the dark phase of the light cycle. Each facility should have a standard operating procedure describing how daily observation is to be done.

Observing mice may prove more difficult than other species due to their size, overall number, and use of nesting material. The housing and rack configuration can also impact the ease and efficiency of observation. This, however, does not suggest that observation of mice should be done with any less rigour than for other species.

Some aspects of Appendix 5, "Indicators of Disease", and Appendix 6, "Indicators That May Be Used to Assess the Welfare of Mice", may be useful to incorporate into daily observations of mice.

## 6.7 CAGE CHANGING AND SANITATION

**Guideline 15**

Cages should be changed at a frequency that maintains the intra-cage air quality and bedding within acceptable parameters, while recognizing the stress associated with cage cleaning and the potential impact on study data.

Specifications for bedding and nesting material are provided in Section 6.3.3, "Bedding and Nesting Materials".

Cage changes, although an essential element for appropriate laboratory mouse husbandry, can be disruptive to mice (Duke et al., 2001; Sharp et al., 2002; Rosenbaum et al., 2009). Cages need to be changed often enough to allow mice to have separate areas for living, sleeping, and soiling. The frequency of changes is dependent on a number of variables, including cage size, type and number of occupants, type and amount of bedding, number of air changes per hour, etc. (Reeb-Whittaker et al., 2001). Ideally, both air quality monitoring and visual inspection should be used to assess the need to change cages. There should be clean bedding in the living and sleeping area of the cage, away from the soiled areas. Standard operating procedures should be developed for cage changing, with consideration of the factors listed above (see Section 3.1.3, "Air Quality and Ventilation" for details on air quality in relation to cage changing). Irrespective of the standard operating procedure, cages should be changed if nesting material appears wet or soiled, as this material will no longer help with thermoregulation.

Adequate sanitation should ensure the removal of infectious agents to prevent their spread within the facility (Compton and Macy, 2015), as well as the removal of urine and fecal waste to prevent excessive contact with mice and to minimize ammonia levels.

## 6.8 RECORD KEEPING

It is important that all records identified in the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017), Section 12, "Record Keeping", are maintained. In many cases, group records are kept for mice; however, individual records are necessary for animals that undergo treatment, procedures, or breeding. Requirements for breeding records are detailed in Section 5.1, "Record Keeping and Oversight", in this document.

# 7 HANDLING AND RESTRAINT

**Guideline 16**

Mice must be handled gently to avoid injury and distress.

Handling can have a significant impact on both the welfare of the mice and on experimental results. Handling of mice can cause a number of changes indicative of stress: elevation in heart rate, body temperature (Kramer et al., 2004) and blood pressure; hyperthermia; altered immune system responses (Moynihan et al., 1989); and increased corticosterone levels (Irwin et al., 1986).

Attention must be given to ensuring appropriate methods are used and the personnel involved are competent in using the chosen methods. Mice should be habituated to handling whenever possible.

## 7.1 HANDLING

Hurst and West (2010) compare handling techniques that have been developed to reduce stress for mice, and provide links to online training resources. Alternatives to handing mice by the tail, such as the use of a tunnel placed in the home cage, should be used to minimize handling stress (Gouveia and Hurst, 2013; Hurst and West, 2010). Mice that are handled by the tail have been found to be less responsive to reward, compared to those handled using a tunnel, which has implications for both animal welfare and research results (Clarkson et al., 2018). If mice are habituated to handling, scooping into a cupped hand can also be used to move them.

Picking mice up by the tail can cause aversion and anxiety. If mice are to be handled by the tail, proper technique must be applied, with their weight supported right away. Mice must not be handled by the last third of the tail to avoid 'tail slip' (also known as "de-gloving" injuries), and should not be held by the tail for long periods of time.

The negative impact of handling mice can be reduced through habituation (see Kramer et al., 2004). The response of mice during habituation should be monitored, and a balance achieved between handling the mice enough for habituation, and handling them as little as possible. All mice in an experimental group should undergo habituation to the procedures, where possible, and the habituation program should be applied consistently within the group.

## 7.2 RESTRAINT

Mice should only be restrained if less stressful procedures cannot achieve suitable results. Any restraint should be for as short a period as possible, and a restraining device that is appropriately sized to the animal should be used. Mice should be monitored constantly while being restrained.

Several types of mechanical restraining devices are available; the devices are designed for purposes such as injection and withdrawal of blood and short-term cannula collection of bodily fluids. Habituation to

restraint devices and positive reinforcement techniques should be used as much as possible, and the period of restraint should be the minimum necessary. Alternative procedures that can reduce the need for restraint (e.g., catheters) are encouraged.

To minimize the duration of restraint, it is important that it be conducted by personnel competent to perform the particular method. There is evidence that mice respond differently to male and female personnel (Sorge et al., 2014), and that they do not generalize handling across handlers; therefore, maintaining the same handlers during a study should be a consideration (Gaskill and Pritchett-Corning, 2016).

# 8 WELFARE ASSESSMENT

**Guideline 17**

All mice maintained in an animal facility should be subject to routine welfare assessments.

Where mice are involved in protocols, the assessment should be tailored to the particular strain of mice and the specifics of the study.

Welfare assessment is a necessary component of animal-based studies, both for ensuring a good quality of life for the animals within the constraints of the study, and for the quality of the scientific data. While all personnel involved with the animals have a role in collecting information for welfare assessments, primary responsibility for planning and documenting welfare assessment rests with the investigator in consultation with the veterinarian, as approved by the animal care committee.

When possible, investigators should refer to basic standard operating procedures approved by the animal care committee and adapt them to suit the specific mouse strain. If a new genetically modified mouse model is to be generated, or a line that is not well-characterized is to be brought into the facility, the investigator should provide plans for assessing and monitoring the welfare of the strain during the animals' lifetime (i.e., during experiments and the maintenance of the breeding colony), keeping in mind that the level of monitoring required for that strain may change over time.

There should be a plan to integrate information from various sources to provide a measure of mouse welfare at the individual and group level. The overall focus of any welfare measurement should be to identify the need for mitigation strategies or endpoints, with the aim of improving the welfare of the animals (i.e., monitoring should be conducted to benefit the animals, not for the sake of monitoring).

Investigators should ensure proper documentation of the results of welfare assessment procedures and the identification (if appropriate) of any mitigation strategies to address welfare concerns; assessments and concerns should be reported to the animal care committee. Assessments of the welfare of the animals can be done in parallel with research on newly derived or received genetically modified strains; any additional information provided by the results of welfare assessment should be communicated to those involved in the assessment and care of the animals.

The large numbers and variety of genetically modified mice available for research means some facilities will house mice with a wide range of phenotypes (e.g., disease or susceptibility to develop a condition), which makes the assessment of animal welfare particularly important.

The development of a welfare assessment plan should be based on the characteristics of the mice to be assessed, with consideration of potential sources of variation such as strain or stock, sex, age, health status, and previous experience (see Section 1.4, "Sources of Variation"). Consideration should also be given to the entire lifespan of the animals in terms of the number, frequency, and invasiveness of manipulations they will experience.

Factors to be taken into consideration when developing welfare assessment plans for mice include:

- their small size, group housing, and the large numbers held in facilities;
- the numerous different strains and disease models, which can exhibit very different behaviours and needs (see Section 1.4, "Sources of Variation");
- their susceptibility to small changes in the environment as a result of their small size (e.g., fall of temperature, cage flooding); and
- their short lifespan, with changes in life stage occurring quickly.

As welfare assessment is an integral part of the daily care of the animals, various aspects of welfare assessment are included in other sections of this guidelines document:

- daily observation of the animals and their environment (i.e., visual observation of the animals, the cage, food, water, and other aspects of their housing) is covered in Section 6, "Husbandry", and in the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017);
- the suitability of the physical and social environment of mice is addressed in Sections 2, "Facilities", and 6.2, "Housing Management", in this document;
- details of health monitoring for mice are provided in Section 9, "Health and Disease Control"; and
- additional monitoring requirements for mice that have undergone surgery or other procedures are outlined in Section 10, "Experimental Procedures", and in the *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017), Section 10.3, "Animal Care Monitoring in Relation to Research, Surgery and Anesthesia".

Welfare assessment should involve integration of information collected during the daily care of the animals and the conduct of any procedures, rather than a duplication of effort, with additional information from other sources incorporated where necessary. Welfare assessment is more comprehensive than routine checks of the animals, and assesses their quality of life. Some indicators of welfare can only be observed once negative states are quite advanced (e.g., poor coat condition), while others are much more sensitive to gradual changes in the welfare of the animals (e.g., attenuated growth rate). Welfare assessment also includes indicators of positive states, for example, an assessment of the quality of nest building (Rock et al., 2014; Gaskill et al., 2013c). Hawkins et al. (2011) have developed a guide to defining and implementing procedures for the welfare assessment of laboratory animals.

When developing welfare assessment criteria, it is essential that investigators critically consider the strain and disease model and how manipulations will affect the welfare of the animals. The current literature should be reviewed as a starting point for developing plans to assess a particular situation. For example, Spangenberg and Keeling (2015) provide methods of assessing mice in their home cage when they are not undergoing procedures; Leach et al. (2008) report on the results of a Delphi consultation aimed at identifying valid and feasible measures of mouse welfare; and Flecknell et al. (2011) and Urban et al. (2011) focus on pain research involving animal models and the importance of being able to assess the affective or emotional component of pain as well as the sensory component.

A number of assessment tools have been developed, such as body condition scoring (Foltz and Ullman-Culleré, 1999), facial grimace scale (Langford et al., 2010), nest building assessment (Rock et al., 2014; Gaskill et al., 2013c), and burrowing behaviour (Jirkof et al., 2013; Jirkoff, 2014; Deacon, 2012). It is important that any assessment tool be applicable and validated (i.e., to ensure the assessment tool is reporting the situation reliably); each tool will have limitations that should be investigated (see Miller and Leach, 2015).

Validation is best carried out by the research team in collaboration with animal care technicians and veterinary personnel. Assessment tools can evolve and should be reassessed over time. Standardizing the terms used to describe the condition of the animals will assist in the clear understanding of welfare issues (see Mouse Welfare Terms, 2017; Fentener van Vlissingen et al., 2015).

Appendix 6, "Indicators That May Be Used to Assess the Welfare of Mice", provides a list of indicators that may be used to assess the welfare of mice.

# 9 HEALTH AND DISEASE CONTROL

Maintaining healthy animals is important to the welfare of those animals and to the quality of the scientific data. Genetically modified mice and mice with compromised immune systems may have increased susceptibility to disease from other animals in a facility, which can have an impact on research (Franklin, 2006).

**Guideline 18**

All mice should be included in an animal health program, irrespective of where they are housed.

Veterinary professionals must be engaged in the development of the health program, which should be approved by the animal care committee and overseen by people competent in evaluating the health of mice. The animal health program should include:

- prevention of conditions conducive to ill health, with prevention strategies suited to the health status of the animals and the intended studies;
- health monitoring and detection of latent disease by systematic evaluation of individual animals and the health status of each colony; and
- an emergency plan for the management of disease in the event of a suspected outbreak.

## 9.1 DISEASE PREVENTION

**Guideline 19**

Strategic measures for disease prevention should include a program for disease control and a system of regular monitoring and reporting for health assessment purposes.

Animals should be free of unwanted pathogens and clinical diseases. A veterinarian should be integral in developing standard operating procedures to limit the risk of introduction of disease into the facility, and should be available for consultation on all matters relating to the health of the animals (Mähler et al., 2014).

The disease prevention and control plan should address the following:

- procurement – mice coming from a supplier should have a recent satisfactory health report provided by the supplier (commercial or non-commercial), and undergo a thorough health assessment upon arrival (see Section 4, "Procurement");
- quarantine – newly arrived animals should be kept separate from other animals in the facility (see Section 4.5, "Reception of Mice at an Institution");

- facilities and their management – facilities, equipment, and management practices should be in place to prevent airborne, direct contact, or fomite transmission of microorganisms (e.g., ventilated cages), water contamination, pest infestations, and contaminants from external sources;
- husbandry – mice should be fed a high-quality diet (irradiated or autoclaved if specifically intended for autoclaving) and practices should be in place for effective sanitation, prevention of overcrowding, and regular bedding changes;
- biosecurity – standard operating procedures should limit access and detail the use of personal protective equipment and handling of animals under a biosafety cabinet; and
- temporary holding – plans should be in place for holding contaminated animals separate from other animals in the facility in the event of a disease outbreak, and should include a disease prevention strategy.

Biosecurity is an important component of a preventative health program. Quality assurance programs that focus on biosecurity procedures and health and environmental monitoring are important to maintaining animals of a particular health status (Shek et al., 2015; Rehg and Toth, 1988). Infectious agents may affect experimental data without causing disease. A routine and comprehensive health monitoring program is essential; however, due to limitations associated with testing, it will not be sufficient to ensure the animals are free of pathogens or infectious agents that may affect research (i.e., testing is always retrospective (Franklin, 2006)).

## 9.2 HEALTH MONITORING AND DISEASE DETECTION

**Guideline 20**

Standard operating procedures should be developed for assessing animal health, providing health care, and treatment of common health problems for the animals; these should be reassessed every three years to ensure relevance.

Standard operating procedures should be developed for routine health checks and welfare assessment for individual animals and for each colony, based on the strain and health status of the animals, the type of research, and the potential effects on other animals in the facility. Animal monitoring requirements for health and disease control will also depend on the length of time the mice are housed and the type of facility in which they are housed. Health monitoring programs may include the use of environmental monitoring (e.g., through exhaust plenum testing) or the use of sentinel or colony animals, depending on factors such as the strain, sex, age of the mice, and caging system. Evaluation procedures need to be determined (e.g., test intervals, selection of agents, and verification (Mähler et al., 2014; Brielmeier et al., 2006; Fox et al., 2007)). It is important that testing methods and samples are specific to the disease of interest, and where possible, adhere to the Three Rs principle of reduction.

Quality assurance programs should be updated over time in response to the regional prevalence of diseases in the area. The literature should be reviewed for information on diseases affecting mice and procedures for their detection. The use of molecular assays to test directly for pathogens may be used to replace sentinel animals, including polymerase chain reaction (PCR) testing of the environment through the exhaust air duct (Henderson et al., 2013; Jensen et al., 2013a; Zorn et al., 2017). This approach is strongly encouraged as it can result in a significant reduction in the numbers of animals maintained in the facility.

There are numerous murine viruses and many have been identified in breeding colonies and research facilities. Although most of these viruses cause no overt disease, they may increase susceptibility to other microbial infections, alter the phenotype of experimental animals, or erupt into overt clinical disease when the animals are under stress (e.g., due to an experimental procedure or treatment). Breeding colonies and research facilities, in particular those that hold long-term, valuable colonies, should consider regular screening for these viruses as a preventive diagnostic procedure (Henderson et al., 2013). The most prevalent murine viruses include, but are not limited to, mouse parvovirus, mouse hepatitis virus, and minute virus of mice (Carty, 2008; Clifford and Watson, 2008). Other less prevalent viruses can also cause health problems.

Bacterial agents prevalent in colonies of mice include, but are not limited to, *Helicobacter spp*. (Carty, 2008; Lofgren et al., 2012), *Pasteurella pneumotropica* (Carty, 2008; Hayashimoto et al., 2007) and *Staphylococcus aureus* (Pritchett-Corning et al., 2009). Other less prevalent bacterial agents can also cause health problems.

Parasites, such as fur mites and pinworms, are usually associated with asymptomatic infection in immunocompetent mice. However, these parasites are a persistent problem in laboratory mouse colonies and can affect both the health of the animals and experimental results (Ricart et al., 2010; Leblanc et al., 2014; Pritchett, 2007). Karlsson et al. (2014) and Ricart Arbona et al. (2010) provide an evaluation of techniques for the detection of fur mites.

See Mähler et al. (2014) for more information on potential viruses, bacterial agents and parasites, as well as considerations for monitoring.

There should be procedures in place to ensure any animal health concerns or other potential animal welfare issues are documented and communicated to the veterinarian in a timely manner.

## 9.3 DISEASE MANAGEMENT IN THE EVENT OF AN INFECTIOUS OUTBREAK

**Guideline 21**

A management plan must be in place to deal with unanticipated disease outbreaks.

A management plan must be developed to deal with serious disease outbreaks within the facility and from outside sources, and to prevent pathogen transmission and infection recurrence. Plans should include a communication strategy involving veterinarians, veterinary and animal care personnel, investigators, the facility manager, and the animal care committee. Access to quarantine facilities or a means of isolating the animals must be available.

For infectious disease outbreaks, the veterinarian must be consulted to ensure that the techniques employed will eradicate the pathogens. Typical procedures may include quarantining the room in which the disease is discovered and tracking and testing any animals that were recently moved from that "source" room (Mähler et al., 2014). Follow-up actions, such as treatment, depopulation, re-derivation etc., will depend on the nature and extent of the outbreak, the health status of the animals, and the type of research (Mähler et al., 2014). If infected animals are to be euthanized, proper containment measures must be in place for handling and disposal of the animals and bedding, as well as decontamination of cages and rooms to prevent the spread of disease (Mähler et al., 2014).

Re-derivation of mouse strains to address contamination may involve invasive procedures such as harvesting embryos or sperm, and their transfer to a recipient (clean) female, or birth by hysterectomy under sterile conditions and cross-fostering to clean foster mothers. These measures may be necessary to avoid having to re-generate genetically modified animals, which would involve significantly greater numbers of animals. Re-derivation procedures can be outsourced to take advantage of external expertise. Non-invasive procedures such as cross-fostering may be employed when appropriate, to eliminate some agents from a colony.

Embryo transfer is more effective than caesarean re-derivation for production of pathogen-free pups. Embryos can be produced that are free of infectious viruses that are transmissible vertically (from mother to pups); appropriate application of this re-derivation approach will lead to specific virus-free seronegative recipients and re-derived pups (Mahabir et al., 2009).

Where surgery is used for embryo transfer, all requirements for surgery, anesthesia, and analgesia must be followed (see Sections 10.10, "Anesthesia and Analgesia", and 10.11, "Surgery"). When possible, alternatives to surgical embryo transfer should be used to reduce the amount of pain and distress experienced by the recipient mice; alternatives include nonsurgical embryo transfer (NSET) (Green et al., 2009).

# 10 EXPERIMENTAL PROCEDURES

**Guideline 22**

The least invasive method suited to the goals of the study must be used, with consideration of the potential impacts of the procedures on mice, including other mice in the room, and consideration of measures to reduce those impacts.

The institutional veterinarian must review all protocols involving experimental procedures (see CALAM, 2007). For routine procedures, standard operating procedures approved by the animal care committee should be available to all personnel involved with the animals to ensure consistency of procedures and animal care. Where new procedures are proposed, standard operating procedures should be developed in consultation with an expert in the subject matter and stakeholder input (from researchers, safety officers, animal care personnel) should be sought before the standard operating procedure is approved and implemented. Standard operating procedures should be reviewed regularly and updated as new information becomes available. All procedures should be documented and records should be kept in close proximity to housing or procedure areas and be accessible to the veterinary team, animal care committee, and the research team.

Institutions should have a policy or standard operating procedure on repeated procedures on animals, including mice. The frequency, the duration of intervals between procedures, and the total number of procedures that may be performed on the same mouse during its lifetime must be considered. The standard operating procedure must take into account the invasiveness and pain and distress associated with those procedures and their impact on the welfare of the mouse, both in the short and longer term (CCAC, 1998).

Procedures that adversely impact animals should be avoided where alternative methods are effective in achieving the study outcomes.

All procedures have the potential to cause pain and distress. Many seemingly routine procedures are more complicated when conducted on mice because of their small size. Procedures may only be performed by competent people that have been properly trained by personnel with appropriate expertise. Where possible, it is preferable to use the expertise of a veterinarian and experienced animal care personnel to carry out these procedures.

As techniques advance, refinements will continue to evolve in many of these areas and investigators, veterinarians, and animal care committees should evaluate new evidence on refinements and consider their implementation (Smith and Hawkins, 2016).

**Guideline 23**

Endpoints must be developed and approved by the animal care committee prior to the commencement of the study, to minimize any negative impacts of the procedures on the animal.

The *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (CCAC, 1998) provides the following definition of an endpoint: "the point at which an experimental animal's pain and/or distress is terminated, minimized or reduced, by taking actions such as killing the animal humanely, terminating a painful procedure, or giving treatment to relieve pain and/or distress."

Investigators, in consultation with the veterinarian, must establish appropriate and study-specific endpoints (e.g., initiation of treatment, termination of a procedure, and euthanasia) and plans for monitoring. Key references relevant to the particular study should be consulted in determining the earliest practical endpoints (e.g., Urban et al., 2011; Trammell et al., 2012; Trammell and Toth, 2011). Where a mouse model may be in development or new to a researcher, pilot studies should be performed to establish endpoints.

Appropriate monitoring frequency must be established based on the level of invasiveness of the protocol and expected clinical or other signs, as well as the progression of the condition of the animal, the animal model, and the individual animal (e.g., previous experience). Monitoring should be documented.

Where appropriate and in accordance with the level of invasiveness of the protocol, monitoring score sheets incorporating several parameters of assessment can be helpful in monitoring for endpoints. Monitoring for endpoints should be a cooperative effort involving investigators, veterinarians, and veterinary and animal care staff.

Animals experiencing pain or distress that cannot be relieved and that is not approved as part of the research protocol must be euthanized promptly.

## 10.1 ANIMAL MODELS

Investigators or study directors should decide whether mice are required for the study and if so, which mouse strain(s) provide the best model of the biological processes involved in their work, taking into account the special needs of the strain, the ethical or welfare considerations of working with the strain for a given experiment or study, and the strain availability.

Strains that have special requirements must not be obtained until measures are in place to care for them appropriately. Particular studies may need to be redesigned if those requirements could pose difficulty with maintaining the health and welfare of the animals or be intensified as a result of the experimental interventions. The measures required in these situations may include special or additional technical expertise and highly trained personnel.

## 10.2 ADMINISTRATION OF SUBSTANCES

Administration of substances requires careful planning to ensure the substance is delivered effectively and negative impacts on the welfare of the mice are minimized. Planning includes selection of an appropriate administration route and use of appropriate equipment; determination of the required volume, frequency of delivery, and properties of the substance and diluent/solvent (i.e., pH, viscosity, sterility, osmolality); and consideration of requirements for restraint (Turner et al., 2011a,b; Morton et al., 2001). Standard operating procedures should be developed and approved by the animal care committee, following consultation with the institutional veterinarian. When the side effects or safety of a substance are unknown, pilot studies should be conducted.

Examples of common methods of administration are described in the following sections. Less common methods should be researched and approved by an animal care committee before use.

### 10.2.1 Injections

Injection solutions must be sterile or at least filtered (0.22 micron filtre) and maintained in a sterile container, as infectious agents could be present in solutions and affect the animal, the facility, or the study.

The procedures must only be conducted by people competent in the technique, using appropriate handling and restraint. Needles should be the smallest size suitable for the situation, keeping in mind that very small needles are easily dulled (for example, while withdrawing from a multi-use vial) and should not be reused. Substances to be administered should not be irritating.

For mice, acceptable routes for injecting substances include subcutaneous, intraperitoneal, and intravenous (see Newcastle University's *Procedures with Care* for descriptions of procedures for each of these routes). Intramuscular injections are discouraged because of the potential for pain and pathology (i.e., subsequent lameness and sloughing of skin and muscle) due to the lack of sufficient muscle mass in mice. If intramuscular injections are performed, the volume injected must be very small (0.05 ml per site on contralateral limbs for sequential doses, or 0.1 ml if only on one occasion (Turner et al., 2011a; Workman et al., 2010). Intradermal injections are also discouraged because they are technically difficult to perform; however, if used, the volume injected must be very small (Turner et al., 2011a), with consideration of the particular mouse involved, the specific protocol, and the particular site of the intradermal injection.

The tail vein is the blood vessel of choice for injection into an unanesthetized mouse; however, intravenous injections require training and competence. Warming the tail with warm water or using a heat lamp can improve visualization of the tail vein; however, both approaches should be performed with caution to avoid injury to the tail. Magnification, such as with a head-mounted magnifier, can also be used to improve visualization of the tail vein. Restraint devices are available that facilitate intravenous injections into the lateral tail vein (e.g., a cylinder of appropriate diameter for the mouse, with an adjustable-length divider to hold the body and a slotted end for exteriorizing the tail).

The injection site should be monitored for perivascular irritation (i.e., redness and swelling at the site; the vein may appear white), and if it occurs, veterinary advice should be sought.

For maximum volumes and more information about injections, Turner et al. (2011a) and Diehl et al. (2001) should be consulted. Where repeated injections are required over an extended period (hours), osmotic mini-pumps or intravenous catheters should be considered. When catheters or mini-pumps are used to administer substances, animals with chronic infusion of fluids should be monitored for signs of fluid overload and pulmonary edema (Turner et al., 2011a). Catheters and the catheter site should be checked and cleaned regularly to ensure proper functioning and to prevent infection (Turner et al., 2011b).

Suggested volumes for bolus and slow injections (single and repeat doses) and infusion for various sites are provided by Turner et al. (2011a) and Diehl et al. (2001), with a caution that the volumes tolerated by the animals will be influenced by the physiochemical properties of the substance that is administered. Whenever possible, the smallest practical volume should be administered.

### 10.2.2 Oral Dosing

Methods of oral dosing include adding the drug to the animal's regular food (including as a compounded medical diet), water (typically not recommended for mice as they may avoid drinking the water) or a food treat, and oral gavage. The method required will largely depend on the specifics of the study and whether the animals will accept food or water that has been treated. Where other methods can be used (e.g., add-

ing compounds to food or a small amount willingly consumed by the animal), they are preferred over oral gavage. In some cases, oral gavage may be necessary because of the specific dosing requirements or where the properties of the substance and its absorption may be affected by the presence of food. Veterinary advice should be sought in determining the most appropriate method for the study. Pilot studies are encouraged when dosing in food or when using other forms of compounding, to ensure the actual dose received by the mouse is equivalent to that of studies using gavage.

Oral gavage can be a technically difficult procedure. It must therefore only be performed by personnel who have demonstrated competency in the proper technique and perform the procedure routinely on mice, to minimize stress, injury, or mortality (Arantes-Rodrigues et al., 2012). Prior to the procedure the animal should be habituated to restraint.

Mice must be properly and firmly restrained during the oral gavage procedure. This involves holding the mouse by the skin on either side of the base of the neck and exerting gentle pressure with fingers of the restraining hand to tilt the head up to align the oral cavity and pharynx with the esophagus; this positioning creates a near-straight line from the mouth to the stomach.

Gavage administration of substances is best performed by inserting a long, bulbous-ended needle (feeding needle) of pre-measured length (the tube should extend from the tip of the nose to the last rib of the mouse (Morton et al., 2001)) over the tongue into the distal esophagus where it enters the stomach. Gavage needles should be the correct length for the size of mouse. There are advantages and disadvantages of using steel versus plastic gavage needles. Steel gavage needles, when not perfectly used, can do more damage to internal tissues and organ systems than plastic gavage needles. Plastic gavage needles are less damaging and small, soft, flexible, disposable needles can work well. However, some plastic gavage needles of a larger diameter can bend and be bitten or swallowed, in whole or part, by the mouse. These complications of using plastic material can necessitate repeating gavages and thus involve more stress for the mouse. Additionally, consideration should be given to the size of the mouse's stomach; even the smallest flexible, plastic needles available may not be small enough for some mice.

For habituation and tolerance of repeated oral dosing or gavage, gavage needles can be dipped in a solution, such as a sugar solution, that is pleasing to the mouse. Such needle treatment also stimulates the swallowing reflex, which facilitates the gavage procedure (Hoggatt et al., 2010). However, consideration should be given to the potential effects of the dipping solution on mouse physiology in the context of the experimental outcome.

Agents to be administered must be in suspension or solution at room to body temperature. The smallest volume possible should be administered, optimally 5ml/kg (Turner et al., 2011a), and consideration should be given to the particular mouse involved (e.g., a pregnant mouse has a reduced stomach size). Larger volumes can result in passive reflux (if the stomach is overfilled) or in aspiration pneumonia (Turner et al., 2011a).

The conduct of gavage procedures might need special consideration when performed in barrier conditions, which often require working within the confines of a biological safety cabinet. Personnel working in barrier systems should adapt techniques to optimize ergonomics for themselves while minimizing injury and stress to the mice.

## 10.3 COLLECTION OF BODY FLUIDS OR TISSUE

Standard operating procedures for the collection of body fluids or tissues should be developed and approved by the animal care committee before use. The institutional veterinarian should be consulted on experimental protocols.

### 10.3.1 Blood Collection

#### 10.3.1.1 Survival Blood Collection

Because mice are small and there is the potential for stress from handling and invasive procedures, only personnel competent in the specific procedure on mice may perform procedures for the collection of blood or tissue. The least invasive method for the volume needed should be used, taking into account the amount of animal handling required and potential problems.

For a healthy mouse, up to 6 ml/kg of blood (i.e., 10% of the blood volume) can generally be taken through a single sample, and 3 to 4 weeks should be allowed for recovery before further samples are taken (Morton et al., 1993). The blood volume of a healthy mouse will vary, but it is typically around 1.5 ml for a 25 g mouse. For obese animals, the percentage of total blood volume that can be taken in a single sample should be reduced to approximately 7% of the estimated circulating blood volume, as the circulating blood volume to body weight ratio is less than for non-obese mice (Morton et al., 1993). It is important that the tissues at the blood sampling site be allowed to recover between sampling.

Blood sampling (except for terminal blood sampling) should not be performed on mice less than 14 days of age due to the risk of hypovolemic shock (Robinson et al., 2003).

Following collection of blood, and before the animal is returned to the cage, there must be assurance that bleeding has stopped. Fluid replacement should be implemented if necessary.

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) provides a decision tree that may be useful in determining the most appropriate technique for the size of sample required. Information on appropriate techniques is also available on their website (Blood Sampling: Mouse), and in Parasuraman et al. (2010).

The effect of the sample site on study results must also be taken into consideration (Diehl et al., 2001; Hoggatt et al., 2016). For example, concentrations of white blood cells have been shown to differ between samples taken from the heart and those taken from peripheral sites, such as the tail vein (Nemzek et al., 2001), the facial vein, (Mella et al., 2014), as well as the caudal vena cava (Schnell et al., 2002). Additionally, stress associated with handling may impact blood parameters (e.g., levels of glucose, corticosteroid), providing further reason for proper habituation and handling. This emphasizes the importance of using the same method throughout a study, and reporting the method used in subsequent publications (Hoggatt et al., 2016).

Examples of acceptable sites for blood collection in mice include the lateral tail vein, facial vein, and the medial and lateral saphenous veins. As mentioned in Section 10.2.1, "Injections", heat may be required to improve visualization of veins; however, it should be applied with caution.

While taking a blood sample from the facial vein is relatively easy technically, the ease of access poses a significant risk of inadvertently sampling too much blood. Additionally, this sampling site has been shown to be stressful for mice and can result in excessive tissue damage (Teilmann et al., 2014), and therefore its application requires that personnel be highly trained. Francisco et al. (2015) compare blood collection from the facial vein using a needle versus a lancet and found there was no significant difference in the welfare of the mice or the quality of sample obtained with the two approaches. However, the technique must only be performed by highly competent staff, and an anesthetic is recommended when developing competency in this technique.

#### 10.3.1.2 Terminal Blood Collection

Cardiac puncture is only acceptable as a terminal procedure after the mouse has been euthanized or where the mouse is under deep anesthesia prior to euthanasia. Blood collection from the abdominal aorta under deep anesthesia is also acceptable. Where euthanasia by decapitation has been approved by an animal care committee, trunk blood can be collected (see the NC3Rs webpage on blood sampling in mice).

Retro-orbital bleeding has been associated with negative animal welfare consequences and therefore should only be performed as a terminal procedure. The sample obtained from retro-orbital bleeding is not representative of venous blood, as it is a mixture of venous blood and tissue fluid (see the NC3Rs webpage on retro-orbital blood sampling in mice).

### 10.3.2 Urine and Feces

The least invasive method of collecting urine or feces that suits the requirements of the study should be used. Kurien et al. (2004) review various methods of urine collection, ranging from voluntary voiding to the use of metabolic cages and surgical methods. It is important to address the requirements of the study, in terms of obtaining samples that are free of contamination and of sufficient volume and/or obtained at a particular time, as well as the need to minimize any pain and distress experienced by the animals, both for the welfare of the animals and for the quality of the samples obtained. Methods of free-flow collection of urine on plastic wrap are described by Kurien and Scofield (1999). It is also possible to collect urine using a form of non-absorbent sand. For guidance on metabolic cages, see Section 2.1.2, "Metabolic Cages".

To maintain consistency, consideration should be given to collection of fresh feces rather than random sampling of fecal pellets from cage bedding, which can lead to variability in the viability of any pathogens or other biological materials being assayed in the feces.

## 10.4 EXPLANTS AND IMPLANTS

Animal care and monitoring are critical to the successful use of explanted and implanted devices. Commonly used equipment to deliver substances to mice includes superficial or surgically implanted catheters, vascular access ports, and infusion-delivery pumps (Turner et al., 2011b). Implantation of telemetry and other recording devices is also common.

Surgical insertion of devices poses a risk of post-operative infection, often due to the difficulty of sterilizing and handling the device. Devices act as a nidus for bacterial biofilms, and the exteriorization of such devices allows microorganisms to track from the skin or device into internal tissues. To minimize these risks, all devices should be sterilized using standard techniques, including autoclaving, gas or chemical sterilization, or irradiation. If sterilization is not possible, the institutional veterinarian should be consulted for other options. Aseptic technique should be used during surgical insertion of any device and during device handling and manipulation.

The size or weight of a device and the site of attachment or insertion should be chosen to minimize any effects on the mouse's ability to perform normal activities. For telemetry, the mass of the device is often calculated relative to the mass of the animal; however, this can be disproportionately large for mice, compared to other species (Morton et al., 2003). In all cases, the details of the device should be recorded. If there is concern the device may affect the animal's ability to reach food or water, provisions to facilitate access should be made.

Where devices are exposed and can be damaged, monitoring must be sufficient to ensure that there is minimal risk to the animal. Devices must be designed and attached or inserted to minimize the risk of dislodgement, and caging should be designed or altered to minimize the risk of catching any part of the exposed device. Mice should only be singly housed when there are significant welfare risks to housing them in groups.

As far as possible, environmental enrichment should continue to be provided to mice with devices to permit the expression of motivated behaviours (Lidster et al., 2016).

## 10.5 PROCEDURES FOR GENETICALLY MODIFIED MICE

The selection of methods to generate new genetically modified strains should be made with consideration of the Three Rs, for example, reducing the number of animals used in creating and maintaining each line and consideration of overall welfare impacts, as some methods are more efficient than others. Procedures for the generation of genetically modified animals should be reviewed by the animal care committee during protocol review, in keeping with the rapidly evolving nature of genetic modification and advances in research on animal welfare. Submission of protocols for renewal by the animal care committee should include a report from the investigator on the efficiency of the methods used to produce new strains.

Archiving technologies contribute to reduction and refinement; expertise in this area should be sought in the development of a new line.

A genetic monitoring program can also be an important tool to safeguard the genetic quality of the mice used in a specific research program (Fahey et al., 2013).

### 10.5.1 Collecting Samples for Genotyping

The sampling method should be the least invasive method that can provide the quantity and quality of tissue required for the particular genotyping method being used. Ideally, it would also serve as a means of identifying the animal, thus minimizing the handling of animals and the number of procedures carried out on each animal (see Section 6.1, "Identification of Animals"), although this is not always possible (Bonaparte et al., 2013).

Low-invasiveness methods are available for collecting samples for genotyping; however, these methods are more prone to cross-contamination (Cinelli et al., 2007; Robinson et al., 2003) and may not be suitable for some studies:

- stool sampling (Cinelli et al., 2007);
- saliva and buccal cell sampling (Robinson et al., 2003); and
- hair sampling (Robinson et al., 2003; Otaño-Rivera et al., 2017).

Where these methods are not appropriate for a particular study, either ear biopsy or blood sampling should be used. An ear biopsy (also referred to as ear punching) involves the use of an ear punch device to remove a small tissue sample (approximately 2 mm in diameter) from the periphery of the pinna where the tissue is thinnest (Morton et al., 2003). Ear biopsy causes less discomfort to the animals than tail biopsy (Norecopa, 2008) and results in minimal bleeding (Bonaparte et al., 2013); however, it is not always suitable for quantitative genotyping and cannot be performed on animals less than 14 days of age (Norecopa, 2008).

A tail biopsy for DNA analysis involves removing a tissue sample of less than 5 mm in length from the distal aspect of the tail (Hankenson et al., 2008). Where tail biopsy is deemed necessary, consideration of anesthet-

ics and analgesics must include assessment of the pain associated with the procedure, the effects of recovery from anesthesia, and the potential long-term effects (Jones et al., 2012). The use of anesthetics may provide less benefit to young mice undergoing tail biopsy than to adult mice, and anesthesia and associated recovery time may increase anxiety and decrease activity levels for mice of all ages (Hankensen et al., 2011).

Toe clipping is discouraged, and is only permitted in particular situations with animal care committee approval where:

- no other method can be used;
- the mouse is very young (< 7 days old); and
- only one toe is to be clipped (Bonaparte et al., 2013; Schaefer et al., 2010; Wever et al., 2017).

For further information on the application of genotyping methods and associated welfare and experimental concerns, see Appendix 4, "Recommended Practices for Genotyping".

### 10.5.2 Superovulation of Females

Superovulation techniques increase the production of oocytes per female, and thus reduce the number of female mice required to produce a required number of offspring (Nagy et al., 2003; Robinson et al., 2003). For optimal results, consideration should be given to the strain and weight of the mice, and the dose, timing and type of hormone that is injected (Luo et al., 2011; Takeo and Nakagata, 2015). In general, younger females are more responsive to superovulation than older females (Hoogenkamp and Lewing, 1982; Luo et al., 2011).

### 10.5.3 Vasectomy

For vasectomy, methods of anesthesia and analgesia regimes, post-operative care, and pain assessment should be carefully chosen in order to reduce discomfort and pain (Leach et al., 2012; Miller et al., 2012). The animals should be given 2 weeks to recover from the surgery prior to mating.

### 10.5.4 Embryo Transfer Re-Derivation

As described in Section 9.3, "Disease Management in the Event of an Infectious Outbreak", alternatives to surgical embryo transfer should be used to reduce the amount of pain and distress experienced by the mice; alternatives include nonsurgical embryo transfer (NSET) (Green et al., 2009). When surgical embryo transfer is performed, anesthesia and analgesia regimes, post-operative care and pain assessment should be carefully chosen in order to reduce discomfort and pain.

### 10.5.5 Phenotyping

Some procedures that are acceptable for animals that have not undergone genetic modification may not be acceptable for genetically modified mice with altered phenotypes; those procedures may need to be modified or avoided when animals have compromised ability to respond to stress. This includes the choice of procedures for phenotyping.

Once the animals are phenotyped, any additional information related to animal welfare should be given to the animal care committee as soon as possible. Stable germ-line transmission does not necessarily mean that there is a stable phenotype or stable animal welfare, since phenotypes can change (e.g., be age dependent,

have background effects, require homozygosity, or require breeding to other mutant lines). Appropriate monitoring is needed for the lifespan of the animal or when the genetic background is changed.

Investigators should take reasonable steps to publicize to the research community all available phenotypic and welfare information, along with strategies for mitigating problems with genetically modified mouse lines (Brown and Murray, 2006).

Genetically modified mice may respond differently to drugs and food, as well as a number of experimental conditions, when compared to mice that have not undergone genetic modification. These changes in response may be the result of differences in the animal's metabolism and are particularly relevant to the use of anesthetics and to the use of the mice for testing new drugs or in toxicity studies.

## 10.6 ANTIBODY PRODUCTION

For procedures involved in the production of antibodies, see the *CCAC guidelines on: antibody production* (CCAC, 2002).

## 10.7 IMAGING

For imaging, it is imperative that a plan be developed in consultation with a veterinarian. Although studies involving repeated imaging can reduce the number of animals required for a research study, the procedures create numerous occasions for animals to be stressed. Some of the other factors to consider include: repeated injections; anesthesia; handling and transportation; experimental conditions (e.g., tumour burden or surgery); hypothermia; and fasting (Hildebrandt et al., 2008). All of these factors should be addressed in relation to both the welfare of the animals and the validity of the imaging results. In particular, given the significant impact of repeated anesthesia on the physiology of an animal, consideration should be given to the number of times and frequency of imaging. See Section 10.10, "Anaesthesia and Analgesia", for particular considerations regarding the use of anesthetics. For serial imagery, it is particularly important that animals are monitored between imaging sessions.

The schedule for imaging should be developed based on the anticipated outcomes of an intervention or timeline of an age-dependent change and the welfare of the animals.

A number of imaging methods are available (see Zhi et al., 2014; Yuan et al., 2010; Villiger et al., 2009; Leitgeb, 2007; Antony, 2014), as well as intravital microscopic live-animal imaging of open-skin sites. The least invasive method suited to the goals of the study must be used.

Equipment should be thoroughly cleaned and disinfected between uses to minimize the possibility of cross-contamination, especially when sharing equipment.

## 10.8 BEHAVIOURAL STUDIES

A healthy animal with a good welfare status is critical to achieving a valid and interpretable outcome of any behavioural testing regime.

Aversive stimulation, deprivation or restriction of resources should only be used when there is no alternative. Where possible, a reward strategy (e.g., highly preferred food) should be used to motivate an animal rather than using aversion. Motivational studies using shock, aversion stimuli such as puffs of air, food and

water restriction, and/or water escape should be justified to the animal care committee and used in the least invasive fashion and for the shortest duration possible.

For consideration for various types of behavioural studies, see the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (NRC, 2003), Chapter 9, "Behavioral Studies".

Equipment should be thoroughly cleaned and disinfected between uses to minimize the possibility of cross-contamination, especially when sharing equipment.

## 10.9 FOOD AND FLUID INTAKE REGULATION

The use of food or fluid regulation in mice requires consultation with the veterinarian to determine the necessary level of regulation, the potential adverse consequences of the technique, and methods for assessing the health and welfare status of the animals (NRC, 2003). The procedure must be scientifically justified to the animal care committee, and endpoints (i.e., indicators for when to alter or terminate the procedure) to maintain the animals' health and welfare must be defined (NRC, 2003; CCAC, 1998) and approved by the animal care committee.

The least reduction in food and water necessary to achieve the research outcome should be used. Where possible, pilot studies should also be carried out to determine whether or not food or water regulation is essential.

Where it is necessary to fast the animals, the shortest possible period should be used, preferably during the photophase (light phase) to align with the behaviour of the species. In mice, food and water intake show a significant daily variation as they are strongly coupled with circadian rhythm. The effect of food deprivation is greatest when it occurs in the active phase, as mice typically consume two thirds of their daily food intake at night. Significant reductions in liver weight and glycogen content, as well as increases in levels of glycerol, free fatty acids and acetoacetate, have been measured after 3 hours of fasting in rodents (Palou et al., 1981). Fasting times for many outcomes, such as a steady-state blood glucose and insulin levels, are not the same as for human fasting (Jensen et al., 2013b).

Characteristics such as strain (including genetic modification), sex, age, housing density, reproductive status, and room temperature markedly affect fluid and food intake patterns. Food and water regulation can cause marked changes in the physiology and biochemistry of the animal, and those changes become more severe with longer durations of food and water withdrawal (Classen, 1994; Jensen et al., 2013b). There should be documented monitoring of the weight of the mice, and this information should be available to the veterinary staff.

## 10.10 ANESTHESIA AND ANALGESIA

### 10.10.1 Anesthesia

Anesthesia should be used as a tool for procedures that are potentially stressful or painful. Decisions regarding when this is warranted should be made in consultation with the veterinarian and approved by the animal care committee.

Anesthetics can have profound effects on the physiology of animals, affecting both the welfare of the animals and experimental results (Flecknell, 2009). The use of anesthesia should be considered a significant procedure, and careful planning of the anesthetic regime, including management of complications, is a critical component of good experimental design (Flecknell, 2009). The need for planning includes the selection of

drugs and dosage schedules that are suited to the specific anatomy and physiology of the mice, careful monitoring, and appropriate care of the mice during and after the procedure (Gargiulo et al., 2012).

Specific training in the administration of anesthetics is critical to success. Only personnel with appropriate training and demonstrated competency must be permitted to perform anesthesia (CCAC, 2015).

Anesthesia management must reflect phenotype and health status. The possibility of the genetic modification, experimental procedures, and disease model affecting the phenotype should be considered when planning anesthesia. Circadian rhythm should also be a consideration, as there is evidence that anesthesia disrupts circadian rhythm in mice and results in cognitive impairment, which lasts longer in older mice (Song et al., 2018). Precautions must be taken to ensure safe anesthesia. Ideally, animals undergoing anesthesia should be in good health. Mice cannot vomit, and therefore it is not necessary to restrict food and water intake before anesthesia. Determining appropriate dosage must take into consideration the strain, body weight, age, sex, and any genetic alterations (Gargiulo et al., 2012). Animals should be weighed and the drugs diluted accordingly to ensure accurate dosage. Because of potential differences in drug metabolism, a pilot study may be helpful to determine the appropriate effective dosage when anesthetizing a new strain. Vital signs and reflexes of animals under anesthesia should be monitored regularly (Jaber et al., 2014), as mice can easily be overdosed with anesthetic. For more information on monitoring, see Section 10.12.1, "Monitoring".

The type of anesthetic and concentration must be carefully considered, as aversive behaviour is typically greater or more frequent with anesthetics administered at higher concentrations (Leach et al., 2002). There is also evidence that mice that have been exposed to certain anesthetics (e.g., isoflurane) will try to avoid repeat exposure (Wong et al., 2012; Moody and Weary, 2014), suggesting that anesthesia is not an innocuous procedure and is a welfare concern. This must be taken into consideration, particularly for longitudinal studies.

Balanced anesthesia is an approach that combines smaller amounts of multiple drugs to target the many components of an anesthetic state: consciousness, analgesia, muscle relaxation, and alteration of autonomic reflexes (Tranquilli et al., 2007). Balanced anesthesia may eliminate adverse effects that could accompany a large dosage of a single drug (Ilkiw, 1999).

Inhalation anesthesia, using an anesthetic chamber for induction and an appropriate breathing system for anesthetic maintenance with a precision vaporizer, provides rapid onset of, and recovery from, anesthesia, and the level of anesthetic can be accurately and rapidly adjusted during the procedure (Flecknell, 2009). Vaporizers should be calibrated regularly to ensure accurate delivery of anesthetic. Procedures such as intravenous injection may be accomplished more efficiently and with less stress for the animals if the animals are briefly placed in a gas anesthesia induction chamber.

Repeat-bolus dosing to extend a surgical plane of anesthesia must be performed with great care (Jaber et al., 2014). This technique should be justified over other techniques that provide for a more stable plane of anesthesia, such as inhalant anesthesia, as there is an increased risk of animals regaining consciousness during procedures or experiencing anesthetic overdose. Continuous intravenous infusion is also an option, but it must be used with caution to avoid fluid overload.

Adequate oxygenation and ventilation, and the elimination of dead space are critical and must be considered even when injectable anesthetic drugs are used. Hypoxia can affect metabolism and possibly the validity of research data; prolonged hypoxia can result in brain injury or sudden, unexpected death. Oxygen can be administered through a face mask, although intubation is also possible in mice (Konno et al., 2014; MacDonald, 2009). Pulse oximeter monitors that are suitable for mice are available.

Mice are susceptible to hypoglycemia, dehydration, and hypothermia during anesthesia and surgical procedures, and veterinary advice should be sought regarding appropriate preventative measures and treatment. These alterations of normal physiology will affect recovery and may affect the quality of research data.

When available, anesthetic reversal agents should be administered at the end of the period requiring anesthesia to accelerate recovery. Adequate pain control should also be provided for painful procedures, independent of the anesthetic (see Izer et al. (2014) for a discussion of the use of antagonists to anesthesia and impact on pain control).

Animals must be monitored regularly through the recovery period and should not be left unattended until they are able to ambulate. An external heat source should be provided during and following anesthesia until animals are able to thermoregulate on their own (since mice behaviourally regulate body temperature, the ability to thermoregulate is generally recognized as the ability to move about the cage rapidly on their own without external stimulation). As with anesthesia of any animal, care must be taken to prevent overheating and burns when heating devices are used. Other forms of supportive care should be provided, such as fluids and eye lubrication.

For neonatal mice, inhalant anesthetics, such as isoflurane, can be used safely for potentially painful procedures. When inhalants are not suitable, for safety or practical reasons, evidence from studies on 4 day old rats indicate that hypothermia may be appropriate (Huss et al., 2016) and have less negative impacts on neonatal mice than pharmacological approaches (Danneman and Mandrell, 1997). To minimize any potential pain associated with cooling, mice pups should be wrapped in a latex blanket (Danneman and Mandrell, 1997).

### 10.10.2 Analgesia

**Guideline 24**

Mice should be provided with analgesia for invasive procedures that are likely to be painful.

Decisions not to provide analgesia when animals are likely to experience pain (e.g., invasive procedures or models of disease such as arthritis) must be approved by the animal care committee. It is important to consider that mice experiencing pain may not show clinical signs of pain or may express behavioural signs not usually associated with pain. Mice must not be denied analgesic treatment for a procedure that is likely to be painful on the sole basis that they do not overtly demonstrate signs of pain.

The need for analgesia and the type of analgesia should be reviewed with the veterinarian prior to the development of pain from procedures or the development of pain from chronic conditions. Pre-emptive analgesia should be used for procedures that are likely to be painful, as the lack of control of acute pain will increase the possibility of chronic pain that is resistant to analgesics emerging later as a result of wind up (central sensitization).

It is important to learn how to recognize signs of pain in mice (they have a tendency to mask clinical signs of pain) and to develop strategies to evaluate pain as accurately as possible. Physiological and behavioural signs should be monitored (see Kohn et al., 2007; Flecknell, 2009, 2018). Acute pain may be detected through changes in facial expression (Langford et al., 2010; Matsumiya et al., 2012), although this may be more easily evaluated using video or photographs, due to the activity level of mice (Miller and Leach, 2015; Faller et

al., 2015). Observation of behaviours, such as burrowing and nest building (Jirkof, 2014), may be useful to evaluate the level or presence of post-operative pain and discomfort, although these behaviours are subject to modification by other stressors too. Specific signs, such as abdominal writhing and hind leg stretching following surgery, are also helpful indicators of pain and discomfort (Wright-Williams et al., 2013).

Many common analgesic agents are too concentrated for accurate dosing of mice and must be appropriately diluted with a sterile diluent. Multimodal analgesia (i.e., the combined application of several different analgesics, each with differing mechanisms of action) should be considered. This approach can provide more effective pain relief, as the onset of action for the various analgesic agents are different (Flecknell, 2009) and they target different pain pathways or receptors (Gaynor, 2009). Categories of analgesic drugs include non-steroidal anti-inflammatories, opioids, and local anesthetics.

The duration and frequency of analgesic treatment will depend on the type of surgery and the state of the animal; longer-term analgesia may be necessary for invasive surgery. Sustained-release drug formulations are available and can help provide longer-lasting analgesia (Healy et al., 2014), without requiring stressful handling for re-injection.

The least invasive route of administering analgesics should be used (see Abelson et al., 2012; Molina-Cimadevila et al., 2014).

## 10.11 SURGERY

Surgery involves major interventions (penetration of the body cavity) and other invasive procedures, such as stereotaxic surgery with implants and orthopaedic surgeries. Consideration of the invasiveness of the procedure, whether it is recovery or non-recovery surgery, and length of time to recovery inform the requirements for the procedures and the measures to be taken to minimize negative effects on the welfare of the animals.

Major survival surgeries should take place in a dedicated surgical suite (or dedicated area of a procedure room), where aseptic conditions are maintained through the use of sterile instruments and suture material. Appropriate attire (sterile gloves, mask, etc.) should be worn by the surgeon to maintain aseptic technique, and gloves should be changed between surgeries on different mice. See Héon et al. (2006) for additional practices to improve aseptic technique.

Surgical instruments should be of an appropriate size; microsurgical instruments are essential for many surgeries on mice. Ideally, a sterile pack of surgical instruments should be used on each animal. When this is not possible, instruments must be wiped clean and disinfected using an appropriate method (e.g., immersion in cold sterilant and then rinsed; hot bead sterilization and then cooled before reuse). The maximum number of procedures that can be carried out for each pack should be determined in advance in consultation with the veterinarian and current standard operating procedures, and a sufficient number of packs should be available to accommodate this.

Consultation with a veterinarian or veterinary technician can assist in choosing sterile suture material of the appropriate size and type for mice. Inappropriate material will delay healing and contribute to post-operative complications, including discomfort and wound chewing. Suture material and wound clips should be removed when healing is sufficient, generally 7-10 days after surgery.

Special consideration must be given to the small size of the animal and any potential monitoring difficulties that may be encountered during surgery due to the presence of surgical drapes, ancillary equipment, etc., which may limit clinical observation. Strategies and equipment must be in place to ensure appropriate monitoring. Areas of concern include hypothermia, hypoglycemia, dehydration, and blood loss. Prior to surgery, the maximum amount of permissible blood loss should be established (see Section 10.3.1, "Blood Collection").

Hair must be removed from the surgical incision site prior to surgery and an area extending beyond the site should be clipped and the skin prepared. However, the large surface area to body mass ratio of mice causes them to lose heat rapidly, making them very susceptible to intraoperative hypothermia (Taylor, 2007). To minimize this risk, the area shaved for surgery should be only as large as necessary for surgical access and to maintain asepsis. Solutions used for skin preparation should be applied to the surgical area with precision to minimize cooling effects (Skorupski et al., 2017).

Surgery in mice is challenging because of their small size. Recovery surgery must only be performed by personnel with the appropriate training and demonstrated competency. Animal tissues should be handled gently and any unnecessary trauma should be minimized. Minimizing tissue manipulation, trauma, and tension on tissues during surgery will reduce the amount of post-operative pain experienced by the animal and improve recovery.

Magnifying devices (e.g., binocular microscopes, head-mounted magnifiers, surgical glasses or loupes) should be used as appropriate, as well as good lighting, to ensure good visualization. However, as noted in Section 3.1.1, "Lighting", intense light can cause eye pathology in mice, and it is important that the mouse's eyes are closed and/or protected by drapes.

Surgeries on severely immunocompromised mice or mice treated with hazardous agents should be carried out in biological safety cabinets.

Mice should be placed on a warm surface during surgery. Where heating devices are used, care must be taken to prevent overheating and burns.

To ensure an appropriate plane of anesthesia for invasive procedures and surgery, the pedal withdrawal reflex should be monitored regularly. Care should be taken to neither over- nor under-extend the leg while checking the pedal withdrawal reflex. The duration of the surgical plane of anesthesia is defined as the time between the loss of the pedal withdrawal reflex and reappearance of that reflex (Jaber et al., 2014). Alternatively, the tail can be gently pinched if the foot is not accessible.

Warm isotonic fluids should be administered to compensate for evaporation through open surgical wounds and blood loss, and to support blood pressure. Although administration of intravenous fluid is possible, it is difficult because of the small veins in mice. Warm fluids can also be administered by the intraperitoneal or subcutaneous routes. The amount of blood loss should be minimized and monitored during surgery; if it exceeds the established maximum, the animal should not be permitted to recover.

Detailed surgery logs must be kept by the investigators and be accessible to the veterinarian, the animal care committee, and others as needed. There should also be an indication of any procedures performed (and when they were performed) on the cage card for cage-side assessment of the mice.

## 10.12 MONITORING AND POST-PROCEDURAL CARE

### 10.12.1 Monitoring

**Guideline 25**

Post-procedural care and monitoring must be planned based on the particular procedure and the individual needs of the animal, and adapted as necessary when unforeseen situations arise.

Each animal must be monitored throughout the entire procedure, from the point when the procedure could impact the welfare of the animal, until complete recovery, and monitoring should be documented. Monitoring mice may prove more difficult than for other species due to their small size. However, monitoring of mice should be done with the same rigour as for other species. For rodents, changes in their welfare status can occur quickly, and monitoring may need to occur more frequently than for larger animals.

Investigators must obtain the approval of the animal care committee regarding acceptable monitoring schemes and documentation practices after consultation with the veterinarian and prior to starting studies. This includes relevant endpoints (i.e., intervention, treatment, and euthanasia). It should also be noted that as studies are refined, monitoring schemes and documentation may need to change.

Monitoring includes specific measures and observations, such as weight loss, pallor of the extremities (an indicator of anemia), and behavioural indicators (e.g., how well the animal builds a nest, and how well the animal moves around its cage). Where appropriate, scoring sheets should be developed based on validated assessment measures and used to assess mouse condition (see Nunamaker et al., 2013; Paster et al., 2009). Body-condition scoring and regular observation of physical and behavioural indicators of health, such as those as described by Folts and Ullman-Culleré (1999), can be useful elements of a monitoring scheme. The stress caused by handling mice for monitoring (e.g., to determine body weight or temperature) should be taken into consideration when developing a monitoring plan.

Personnel responsible for monitoring mice and documenting their condition must be competent in recognizing and interpreting mouse-specific clinical signs and conditions, as well as mouse pain and pain behaviours, and be aware of the documentation and reporting procedures, including emergency contact information. It is very important that peri-procedural monitoring be a collaboration involving animal care personnel and the research team. The timing of monitoring is very important (e.g., animals should always be weighed at the same time of day), and depending on when procedures occur, plans may need to be made to monitor mice outside regular staffing hours and overnight. Clinical signs and conditions may vary depending on the type of procedure and age or sex of the animal. Animal care personnel should have information about the surgery in advance to assist in identification of departures from normal patterns of post-surgery recovery.

### 10.12.2 Post-Procedural Care

The plan for post-procedural care should be developed in consultation with the veterinarian. Analgesics should be a component of care following any procedure that could cause pain (see Section 10.10.2, "Analgesia"). However, additional nonpharmacological approaches should also be considered to address animal comfort.

The recovery cage should be placed in a warm environment, with low levels of noise and light, to promote recovery. Animals may be housed singly until they are ambulatory (Kohn et al., 2007) and then returned to their regular housing (Jirkof, 2015). Prolonged single housing of mice can add to the stress of procedures and interfere with recovery (Jirkof, 2015). Animals moved to a clean cage after surgery should be accompanied by their nest from the previous cage (provided it is not dirty or wet) to reduce stress invoked by the new cage environment (Jirkof, 2015). Dry, soft and/or more absorbent bedding should be provided for additional comfort and to prevent bedding from sticking to surgical wounds.

If an animal's movement is restricted, food and water should be made accessible. Soft, palatable food should be provided to promote rapid recovery and a return to normal digestive function (e.g., post-anesthesia). Any new food or food supplement to be provided should be introduced to the animal prior to the procedure so that the animal will be familiar with it. Post-procedural care must continue until complete recovery.

Where palliative care is needed, hydration of the animals is particularly important. Alternative water sources, such as hydrogels, may need to be used if the animal is unable to reach the water source. The animals must be monitored and replacement fluids given subcutaneously, intraperitoneally, or intravenously if necessary, under veterinary direction. If body weight is to be monitored daily, the animal should be removed from the cage in a small container (Hurst and West, 2010). Additional consideration is needed to care for wounds, and the advice of the veterinarian should be sought.

# 11 EUTHANASIA

**Guideline 26**

Euthanasia of mice must be carried out by competent personnel only, using the method best suited to the particular animals, their housing situation, and the impact on the study data.

The general guiding principles outlined in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) are applicable to euthanasia of all animals in science. Additional considerations are discussed in a report of the second Newcastle meeting on laboratory animal euthanasia (Hawkins et al., 2016). This section provides additional information that is specific to euthanasia of mice.

For all methods of euthanasia performed on mice, the following are important requirements:

- personnel involved in the procedure must be trained and have their competency assessed with regard to performance of the procedure on mice and their ability to confirm the death of mice;
- equipment must be appropriately maintained and cleaned before use or reuse;
- stress caused by handling should be minimized;
- animals must not be mixed with incompatible animals prior to euthanasia;
- when using a gassing method, ideally, mice should be euthanized in their home cage;
- pups that are still nursing should not be removed from the dam until just prior to euthanasia (unless provided with thermal support); and
- any animals undergoing euthanasia must not be left unattended prior to confirmation of death.

Specific issues that should be taken into consideration when selecting the most appropriate method of euthanasia for mice include: their small size and the difficulty of accessing blood vessels; their social structure; their sensitivity to travel or movement; their susceptibility to handling stress; and the large number of animals that may need to be euthanized at one time. These considerations should be viewed in terms of the impact of methods of euthanasia on the welfare of the mice and not implications for cost or convenience.

## 11.1 INJECTION

As noted in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010), an overdose of an injectable anesthetic suitable for mice (generally defined as double the dose calculated to achieve anesthesia) is an acceptable method of euthanasia, providing death is confirmed either by close observation or by a secondary method. However, intraperitoneal administration of irritating substances (e.g., pentobarbital) may provoke pain and has been shown to produce inconsistent outcomes in rats (Chisholm and Pang, 2016); hence, its use should be carefully evaluated and approved by the animal care committee. Combining lidocaine or another local anesthetic with pentobarbital may reduce the negative welfare effects (Khoo et al., 2018).

Secondary methods are applied following application of the primary method without allowing the animal to regain sensibility. Possible secondary methods listed in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) are exsanguination by blood draw or organ removal, cervical dislocation, decapitation, and opening of the chest. For mice, intracardiac injection of potassium chloride (KCl) and intravenous perfusion (e.g., formalin, paraformaldehyde) can also be considered potential secondary methods for unconscious or deeply anesthetized animals.

## 11.2 INHALATION TECHNIQUES

Inhalation techniques for euthanasia of mice have potential welfare concerns and must be carefully considered.

Overdose of an inhalation anesthetic agent (e.g., isoflurane or sevoflurane) is an effective method of euthanasia of mice, resulting in rapid induction and unconsciousness; however, the time to death can be prolonged and a second method to ensure death of the animal is recommended once the animal is deeply anesthetized and unconscious. See Section 11.1, "Injection", for possible secondary methods.

The *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) provide information on the use of carbon dioxide ($CO_2$) during euthanasia, and recommend the use of inhalant anesthetics prior to $CO_2$ where practical. Exposing mice to carbon dioxide for euthanasia is likely to cause pain and distress (Leach et al., 2004; Moody et al., 2014). While exposure to inhalant anesthetics has been found to be aversive to rodents, isoflurane has been shown to be less aversive to mice than $CO_2$ (Makowska et al., 2009). However, aversion to isoflurane can increase with repeated exposure (Wong et al., 2012; Moody and Weary, 2014), and this should be taken into consideration. There is currently a substantial amount of research being conducted in the area of inhalant techniques for euthanasia and it is important to carefully evaluate any new evidence that becomes available (see Thomas et al., 2012; Valentim et al., 2015; Boivin et al., 2016a; Boivin et al., 2016b; Boivin et al., 2017; Creamer-Hente et al., 2018). Additionally, Baker and Hickman (2018) caution that there is the potential for observer bias in rating the experiences of mice exposed to inhalation anesthetics.

Gasses must be adequately scavenged to ensure operator safety.

As stated in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010), the $CO_2$ chamber must be flushed with air between groups of animals being euthanized. This is of particular concern for mice; if the $CO_2$ in the chamber is not dissipated prior to use, the animals may experience concentrations of $CO_2$ that induce breathlessness and distress (see Djoufack-Momo et al. (2014) for information on dissipation of $CO_2$ from the chamber).

## 11.3 PHYSICAL METHODS

Physical methods are often used to avoid confounding variability introduced by euthanasia or sedative agents. For example, cervical dislocation and decapitation, when properly performed, are generally not associated with elevated cortisol or stress hormone levels.

If physical methods are required, they must only be conducted by individuals who are highly competent in the use of those methods on mice, and must be justified in the animal protocol and approved by the animal care committee. As noted in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010), cervical dislocation and decapitation are considered conditionally acceptable methods of euthanasia, due to

the potential for severe pain and distress if the procedures are performed incorrectly. When possible, alternate methods of euthanasia should be selected.

If cervical dislocation is used, consideration should be given to using a sedative or anesthetic agent beforehand (Carbone et al., 2012). Cervical dislocation requires a high level of skill, experience, and decisiveness, and should not be considered a routine method of euthanasia for mice.

Decapitation of mice requires that the animals are appropriately restrained. Standard operating procedures must be followed to ensure operator safety. For this technique, consideration should also be given to the prior use of a sedative or anesthetic. If sedation or anesthesia is contraindicated by the protocol, the use of plastic restraint devices can aid in animal positioning and help to prevent operator error.

## 11.4 OTHER METHODS

Some studies require the use of focused-beam microwave irradiation for euthanasia of mice to preserve metabolites in vivo for subsequent analysis (Zhang and Good, 2011; AVMA, 2013). Providing the equipment is specifically designed for euthanasia of rodents in laboratory situations, and the operator is competent in the procedure, this method results in rapid loss of consciousness and death (AVMA, 2013). However, this method requires firm physical restraint and must be justified to and approved by the animal care committee before being used.

## 11.5 EUTHANASIA OF PRE-WEANING AGE MICE

Once fetuses become conscious, there is the likelihood that pain perception develops rapidly. Therefore, when pregnant dams are euthanized, the method chosen should provide rapid cerebral anoxia to the fetus with minimal disturbance to the uterus, thus limiting arousal of the fetuses.

If fetuses are required for the study, it is preferable to euthanize them by physical methods, such as decapitation with scissors. Anesthesia followed by exsanguination can also be used. Intraplacental injection of pentobarbital can be used if studies require preservation of the fetal anatomy for histology, or if it is important to avoid hypoxia.

Neonates up to 14 days of age are best euthanized by decapitation. Between the age of 14 days and weaning, mice can be euthanized with isoflurane, followed by a secondary method.

Fetuses and neonates may be killed by rapid freezing in liquid nitrogen only if preceded by anesthesia (Artwohl et al., 2006).

# 12 END OF STUDY

## 12.1 TRANSFER OF MICE BETWEEN FACILITIES OR PROTOCOLS

For mice that are to be transferred to another institution at the end of a study, see Section 4, "Procurement", particularly with regard to regulations, documentation, and transportation. As mentioned, this applies to mice that have not been subject to major invasive procedures, and are fit to travel.

If mice are transferred to an institution that is not CCAC-certified, it is the responsibility of the institution sending the mice to ensure the animals will receive appropriate care.

## 12.2 RE-HOMING

Where permitted by regulatory authorities, institutions may release healthy research mice (not genetically modified mice) that are commonly accepted pet or companion species to individuals who have the knowledge and ability to provide adequate care to the animals. No genetically modified mice may be moved from research facilities to private premises. As with any other species, if mice are to be released to the care of an individual as companion animals, the institution should develop an appropriate policy describing the conditions that need to be fulfilled before release of the animal. Institutions should ensure those who will be adopting the mice are aware of the care required.

## 12.3 DISPOSAL OF DEAD MICE

Dead mice must be disposed of according to the relevant federal, provincial (or territorial), and municipal regulations for the disposal of biological materials.

# 13 HUMAN HEALTH AND SAFETY

Institutions have occupational health and safety programs that are specifically tasked with addressing this topic through risk assessments. The responsibility of the animal care committee extends to ensuring there is an institutional occupational health and safety program in place so that any risks to human health and safety are properly assessed.

Those working with animals must follow institutional policies and standard operating procedures outlining appropriate measures of prevention and protection. They should seek professional knowledge on animal allergens and zoonotic diseases, as well as other risks or hazards that may be associated with a particular study (e.g., exposure to radiation, anesthetic gas, chemical hazards, and human cell lines).

Laboratory animal allergy, triggered by the presence of rodent proteins, can have long-term effects on the health of people working with mice (Palmberg et al., 2015). Measures to control exposure to animal allergens should use a risk-based approach (see Westall et al., 2015). Practices to minimize exposure include:

- engineering controls – good facility design, adequate ventilation, appropriate air pressure gradients, working with mice in biological safety cabinets or vented hoods when possible, use of bedding disposal stations, and use of appropriate types of caging and bedding;
- administrative controls – work practices and training that help reduce duration of individual exposures (e.g., job rotation, good housekeeping, and personal hygiene practices, such as hand washing and showering); and
- personal protective equipment (PPE) – facility-specific clothing, gloves, hair bonnets, shoes or shoe covers, eye protection, and adequate respiratory protection (Harrison, 2001).

A comprehensive approach should be taken; for example, the use of biological safety cabinets alone does not provide sufficient protection against allergens when bedding is disturbed during cage-changing (Westall et al., 2015; Feistenauer et al., 2014).

If mice are purchased from sources other than reputable suppliers (as noted in Section 4, "Procurement"), there can be increased risks to human health due to the potential presence of zoonotic microbes. Wild-caught mice may also have had exposure to a variety of human pathogens (e.g., lymphocytic choriomeningitis virus, hantavirus, leptovirus, tularemia, salmonella).

Mice may be deliberately infected with zoonotic agents if they are to be models of infectious disease as part of a research protocol, or infectious agents may inadvertently be introduced through contaminated biologics or cell lines injected into mice (Peterson, 2008). One of the more important zoonotic agents is lymphocytic choriomeningitis virus (see PHAC, 2011). Personnel with known or unknown immunodeficiency or reduced immune competency may be at increased risk of infection with certain murine pathogens.

Where there are potential biosafety concerns (including the use of viral vectors for transmission of transgenes), the protocol should be forwarded to the institutional biosafety committee or officer for review, prior to review by the animal care committee. The biosafety committee or officer will ensure that the organism is

appropriately classified and an adequate risk assessment is undertaken to be able to ascertain the necessary conditions for housing and care of the animals and for their subsequent disposal.

People working with mice should take precautions against bites and scratches, as appropriate. In addition, caution should be taken when using needles or sharp instruments on mice, as their small size may increase the risk of personnel poking or cutting themselves.

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# APPENDIX 1
# RESOURCES FOR ADDITIONAL INFORMATION ON THE CHARACTERISTICS OF MICE

## BEHAVIOURAL BIOLOGY (SECTION 1.1)

### Behaviour in the Wild

Singleton G.R. and Krebs C.J. (2007) The secret world of wild mice. In: *The Mouse in Biomedical Research*, 2nd edition (Fox G.J., Barthold S.W., Davisson M., Newcomer C., Quimby F., Smith A.L., ed.). New York, NY: Elsevier, pp. 25-51.

### Behaviour in the Laboratory

Mouse Ethogram, http://www.mousebehavior.org

## ANATOMY AND PHYSIOLOGY (SECTION 1.3)

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# APPENDIX 2
# DATABASES FOR GENETICALLY MODIFIED MOUSE LINES

The following list of databases provides a starting point for determining if a genetically engineered animal line already exists and is available for use. Investigators should seek advice from experts who are knowledgeable with these databases to assist in their use; the experts may be able to suggest additional databases that should be checked.

Information about the attributes of various mouse strains can be found at http://mcp.bs.jhmi.edu/phenotyping-core.

International Mouse Phenotyping Consortium (IMPC): https://www.mousephenotype.org/about-impc/

Jackson Laboratory Mouse Genome Informatics (MGI): http://www.informatics.jax.org/

International Mouse Strain Resource (IMSR): http://www.findmice.org/index.jsp

Mutant Mouse Regional Resource Centers (MMRRC): http://www.mmrrc.org/

European Mouse Mutant Archive (EMMA): https://www.infrafrontier.eu/infrafrontier-research-infrastructure/organisation/european-mouse-mutant-archive

Sanger portal: http://www.sanger.ac.uk/mouseportal/

Mouse Phenome Database (MPD): https://www.jax.org/phenome

Inbred Strains of Mice and Rats: https://www.informatics.jax.org/external/festing/search_form.cgi

# APPENDIX 3
# METHODS OF IDENTIFICATION FOR MICE

The following table lists methods according to invasiveness, with the least invasive methods appearing first.

| METHOD | BENEFITS | ADVERSE EFFECTS / DISADVANTAGES |
|---|---|---|
| Non-toxic dye or permanent marker | Low invasiveness<br>Easy to apply and reapply, especially when dealing with a small number of animals<br>May not require extensive restraint | Only visible for 14 days without reapplication, although newer permanent markers are longer lasting on light coloured coats<br>Loss of visibility of marks may result in misidentification, or require additional handling for reapplication |
| Fur clipping | Low invasiveness<br>Quick | Noise of clippers may cause fear or be irritating to the animal<br>Shaving can cause skin irritation<br>Must be repeated as often as the hair grows back<br>May require more restraint than permanent marker |
| Microchip Transponder | Quick, effective<br>Some enable remote monitoring of physiological parameters | May require the animal to be anaesthetized prior to implantation<br>Can induce physiological and behavioural changes indicative of stress<br>May predispose mice to skin wounds if not properly done<br>Requires a scanner; equipment specific to the transponder is required to retrieve the information<br>Material needs to be appropriate for the research (e.g., non-metal if MRI or scanning procedures will be performed) |
| Tattoo | More permanent than dyes<br>For neonatal animals, the technique is somewhat permanent, minimally invasive, and can be clearly read throughout the animal's life | May fade or become illegible over time if operator is not well trained<br>Tattoo needles must be maintained sharp and sterile<br>Commercial tattoo kits should be used (not do-it-yourself tattooing needles)<br>Anesthetics/analgesics should be considered, providing the stress of the restraint and anesthetic does not impose a greater welfare concern than the tattooing procedure |

| METHOD | BENEFITS | ADVERSE EFFECTS / DISADVANTAGES |
|---|---|---|
| Ear buttons | Less potential for loss or being ripped off than ear tags | |
| Tags | Moderate invasiveness<br><br>Quick procedure<br><br>Ease for any personnel to identify individual animals (i.e., highly visible, does not require specialized knowledge of coding systems) | Possible inflammation and tissue damage<br><br>Metal ear tags used in long-term studies have been linked to squamous cell carcinomas (Baron et al., 2005) and *Staphylococcus aureus* infection (Cover et al., 1989)<br><br>Can be lost or ripped off<br><br>Size, shape, and placement of the tag is essential to the health and welfare of the animal and to prevent loss of tags<br><br>Tag material must be appropriate for the research (e.g., non-metal if MRI or scanning procedures will be performed)<br><br>Local anesthetics and analgesics should be considered; however, the welfare concerns associated with additional restraint of the animals prior to tagging may outweigh the benefits |
| Ear notch | Moderate invasiveness<br><br>Quick procedure<br><br>Tissue can also be used for genotyping | Requires training to ensure notching system is legible and the procedure is done properly with no bleeding<br><br>May impact auditory communication<br><br>Ears can be damaged by fighting and ear tearing can render the identification code impossible to read<br><br>The corresponding identification system must be known to the user<br><br>Tissue may grow back, closing the notch<br><br>Local anesthetics and analgesics should be considered; however, the welfare concerns associated with additional restraint of the animals prior to ear notching may outweigh the benefits |

| METHOD | BENEFITS | ADVERSE EFFECTS / DISADVANTAGES |
|---|---|---|
| Toe clipping | High invasiveness<br><br>Tissue removed can also be used for genetic testing where required<br><br>Quick procedure | Only permitted when genotyping must be done on very young pups<br><br>Only permitted for neonatal mice up to 7 days of age when no other individual identification method is feasible and the tissue collected is also needed for genotyping or other protocol requirements; only one toe is to be clipped<br><br>Justification for toe clipping as a means of genotyping must be provided to an animal care committee; other methods of genotyping should be used if possible (e.g., buccal swabs)<br><br>Risk of infection<br><br>Permanent impact on mobility/gait and also thought to cause chronic pain |

## REFERENCES

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# APPENDIX 4
# RECOMMENDED PRACTICES FOR GENOTYPING

## PREFACE

Genotyping is the process of detecting a genetic modification present in the genome of a cell or animal by examining the DNA sequence using molecular biological assays (such as polymerase chain reaction (PCR) and Southern blot analysis) and comparing it to another individual animal's DNA that has not undergone genetic engineering, or to a reference sequence. It identifies which alleles an individual animal has inherited from its parents.

Genotyping animals requires identification of the animals and acquisition of appropriate samples for DNA analysis. The methods used for identification, sampling and genotyping depend on the species and type of analysis required. When evaluating which methods to use, potential welfare concerns must be taken into consideration and efforts should be made to minimize pain and distress for the animals. The procedure to genotype an animal should also provide the method of identification (see Appendix 3, "Methods of Identification for Mice") to minimize the number of procedures carried out on the animals, and hence minimize pain and distress.

## 1. METHODS FOR GENOTYPING ANIMALS

Developing an appropriate assay method that is suited to the requirements of the study and ensuring steps are in place for optimal results are important to the welfare of the animals (i.e., reducing the need for resampling), as well as to the scientific results and efficient use of time and resources (Bonaparte et al., 2013). It is important that the assay is valid and functioning prior to the generation of the animals, to minimize the numbers of animals that need to be bred. Critical steps to ensuring a good assay include proper sample collection, prevention of contamination between samples, and proper storage of samples to prevent DNA degradation, and allow proper DNA purification, amplification and sequencing (Bonaparte et al., 2013).

### 1.1 Polymerase Chain Reaction (PCR)

Small tissue samples obtained from stool, saliva, hair, ear notches or blood can provide sufficient DNA for PCR analysis. PCR should be used for the primary screen and is the recommended practice for routine genotyping in breeding colonies (Robinson et al., 2003). While there are a number of newer methods for genotyping, each of these methods is based on a PCR approach. Commercial genotyping services should be considered, in particular where these procedures are not routinely carried out in-house.

#### Procedural Considerations

- Requires time and effort to optimize the PCR and to achieve an assay that is reliable and sensitive for unambiguous identification of transgenic or targeted animals (Cowan, 2009).
- Care should be taken to avoid cross-contamination between samples used for DNA isolation (Robinson et al., 2003).

- Long-range PCR (used to determine if correct homologous recombination has occurred in the genome of genetically modified animals) can be difficult to optimize and often lacks positive controls for the PCR experiment to assist with optimization.

### 1.2 Southern Blot Analysis

Southern blot analysis remains the technique of choice to unambiguously identify targeted insertions and determine whether any rearrangements occurred during transgene insertion or gene targeting (Bonaparte et al., 2013). It can also be used to identify gene structure surrounding gene trap or transposon insertion sites. Because of the large amount of tissue required relative to PCR-based methods, Southern blot analysis should only be used for founder animals.

#### Welfare Concerns

- Requires a substantial amount of tissue (e.g., up to a 0.5 cm sample of a mouse's tail) in order to isolate sufficient quantities of high-quality DNA.

#### Potential Refinements

- The use of Southern blot analysis is often the technique of choice for identification of correct founders; however, the use of PCR, requiring smaller tissue samples, should be used for the primary screen and is the recommended practice for routine genotyping in breeding colonies (Robinson et al., 2003).
- A refinement to the use of Southern blot analysis and PCR is to incorporate a reporter gene in the construct to be inserted in the animal's DNA, where practical. This can include the Luciferase gene which codes for an enzyme that converts a substrate to a fluorescent product. Other reporter gene systems are available and in use as well.

## 2. METHODS OF OBTAINING GENETIC MATERIAL FOR GENOTYPING

Methods of obtaining genetic material for genotyping are listed in the subsections below, according to the potential pain and distress for the animals (least to most). In addition to minimizing pain and distress for the animals, selection of the most appropriate method requires consideration of the amount of tissue needed, which will depend on the type of analysis required, and should minimize the need for re-sampling. Tissue for genotyping may be available as a result of the method of identification, and this is preferred to minimize the number of invasive procedures carried out on the animals.

### 2.1 Stool Sampling

Stool sampling is an easy and non-invasive method of obtaining material for genotyping rodents (Chen et al., 2012) by PCR (see Section 1.1, "Polymerase Chain Reaction (PCR)"). Stools contain sloughed intestinal epithelial cells which provide a reliable source of DNA for genotyping (Chen et al., 2012). This method can be cumbersome for very large groups of animals and does not provide a method of identification. Rectal swabs can also acquire colonic or rectal cells, but this is more invasive (Bonaparte et al., 2013).

### Welfare Concerns

- May cause stress due to handling, but otherwise should not compromise animal welfare (Robinson et al., 2003).
- Stools are usually readily produced during handling and can therefore be collected directly from the animal (Robinson et al., 2003), or they are often produced within 1-2 minutes of placing mice in a new empty cage (Hamman et al., 2010). However, this type of spontaneous defecation only occurs in rodents more than 14 days of age (e.g., Hamman et al., (2010) did not see spontaneous defecation in 10-day-old mice).

### Procedural Considerations

- A major concern is avoiding cross-contamination when collecting stool samples (Cinelli et al., 2007).
- Fecal samples must be fresh (less than 24 hours old), and normally more than one pellet is required for rodents (Bonaparte et al., 2013).
- A nontoxic reagent to extract and remove PCR inhibitors from fecal DNA is available (Chen et al., 2012).
- Fecal DNA extraction has proved reliable for only a few gene constructs (Hamman et al., 2010); investigators are encouraged to test this method and publish their results to provide evidence of the reliability of the method and promote its use.

## 2.2 Saliva and Buccal Cell Sampling

Saliva and buccal cell sampling are easy, minimally invasive methods of obtaining material (via pipette or oral swab) for genotyping by PCR (Meldgaard et al., 2004; Mitrecić et al., 2008; Robinson et al., 2003). Collection of saliva containing oral epithelial cells using cotton buds (Robinson et al., 2003) can be done on very small animals (Zhang et al., 2006) in a minimally invasive fashion.

### Welfare Concerns

- Forcibly opening the animal's mouth when taking saliva samples may be stressful, and competent handling of the animals is required (Robinson et al., 2003).
- There are reports of mice biting their tongues during the process (Cinelli et al., 2007).
- If using pipettes or other instruments to collect saliva or buccal cells, they must not have any sharp edges that could injure the tongue or buccal cavity.

### Procedural Considerations

- Possibility of contamination from maternal epithelium in nursing pups (Robinson et al., 2003).
- If mice bite their tongues or if the tongue or buccal cavity is otherwise injured, samples can be tainted by blood (Cinelli et al., 2007).

### Potential Refinements

- Stool sampling (Section 2.1)

## 2.3 Hair Follicles

Hairs can be plucked from the belly of an animal with anatomical forceps, with minimal handling involved (Cinelli et al., 2007; Norecopa, 2008). Genotyping from hair roots is a preferred alternative to the use of blood or tissue samples because it involves less distress for the animal, is simpler and faster, and uses an inexpensive reagent (Terrell et al., 2007); however, it is not used frequently as the risk of contamination (see "Procedural Considerations") may outweigh the minimal invasive nature of the technique. It may be useful for re-sampling or confirmatory testing, as the throughput is low. It can also be useful for colonies where invasive procedures are risky (e.g., for bleeding disorders and highly immunocompromised animals (Bonaparte et al., 2013)).

### Procedural Considerations

- Hair samples are difficult to handle; they electrostatically stick to instruments and become a source of contamination (Cinelli et al., 2007; Norecopa, 2008).
- Possibility of cross-contamination between littermates is a major concern (Robinson et al., 2003).

### Potential Refinements

- Stool sampling (Section 2.1) and saliva and buccal cell sampling (Section 2.2).

## 2.4 Blood Sampling

Blood sampling is not used frequently, as it requires technical skill and is risky for small animals. It provides minimal tissue for genotyping in mice and is most suitable when groups of animals are to be separated based on blood cell phenotype (Norecopa, 2008).

### Welfare Concerns

- Sampling procedures are difficult when performed on small animals requiring well-trained, competent personnel to minimize the potential for pain and distress (Norecopa, 2008).
- No more than 10% of the total blood volume should be taken at any one time, and no more than 15% in a 28-day period. For repeat bleeds at shorter intervals, a maximum of 1% of an animal's circulating blood volume can be removed every 24 hours: i.e., 0.01 x circulating blood volume (ml/day) (roughly = 0.6 ml/kg/day) (Morton et al., 1993).
- Removal of blood from mice less than 2 weeks old should be avoided due to the risk of hypovolemic shock (Robinson et al., 2003).

### Procedural Considerations

- The NC3Rs blood sampling site provides examples of recommended practices for blood sampling.

### Potential Refinements

- Stool sampling (Section 2.1), saliva and buccal cell sampling (Section 2.2), and hair follicles (Section 2.3).

## 2.5 Tissue Biopsy

### 2.5.1 Ear Biopsy

Ear biopsy is considered to cause less discomfort than tail biopsy because it is performed in a tissue where there is no bone formation (Norecopa, 2008). It is generally easier to obtain good results from PCR from ear biopsy material, as the sample contains more DNA than samples from the tail or other tissues (Ren et al., 2001; Robinson et al., 2003). The removal of tissue from the ear for genotyping can also be used for animal identification.

#### Welfare Concerns

- The method can cause pain and discomfort (Norecopa, 2008).
- Can cause inflammation and bleeding if not performed properly.
- Should not be performed in mice younger than 2 weeks since the ear is too small (Norecopa, 2008).

#### Procedural Considerations

- Clean, sharp instruments should be used and instruments should be cleaned between animals.

### 2.5.2 Tail Biopsy

Tail biopsy should only be performed when another method will not yield the required results. In particular, it is used for quantitative genotyping (determination of copy number and location) in founder animals. In general, a very small sample from the tail is used to carry out a polymerase chain reaction: no more than 3 mm should be needed. However, Southern blot analysis requires a 5 mm sample.

#### Welfare Concerns

- Tail biopsy should only be carried out once on an animal. Any occasion requiring a second tail biopsy requires strong justification to, and approval by the animal care committee before taking place.
- Tail biopsy has the potential to cause pain, suffering and distress, and the use of non-invasive or less invasive methods should be investigated first (Robinson et al., 2003).
- Pain and behavioural responses to tail biopsy are strain- and age-dependent and associated with vertebral maturation (Hankenson et al., 2008).
- The skin and periosteum of the mouse's tail is well supplied with nervous tissue, and the removal of even a small section of tail is likely to be acutely and chronically painful (Robinson et al., 2003).
- In mice, the caudal vertebrae start to ossify between 2 and 3 weeks of age. There is bone mineralization and clear evidence of vertebrae, even within the last 1 mm of tail, before typical weaning age (approximately 21 days) (Hankenson et al., 2008; Robinson et al., 2003).
- Tail biopsy should be performed only on young mice (14 to 17 days of age) to avoid transection of distal mature vertebrae (Hankenson et al., 2008), and the smallest amount required for the particular genotyping method being used should be taken.

### Procedural Considerations

- There is a higher DNA concentration in biopsies obtained from younger animals (Hankenson et al., 2008).
- Clean, sharp instruments should be used and instruments should be cleaned between animals.

### Potential Refinements

- Less invasive methods, such as stool (Section 2.1), saliva and buccal cell (Section 2.2), hair follicle collection (Section 2.3), or blood sampling (Section 2.4), should be used when only qualitative genotyping (usually done through PCR) is required (Norecopa, 2008). Ear biopsies are considered less invasive than tail biopsies and provide tissue that can be processed using the same techniques applied to tail biopsies; they are therefore easily incorporated into programs currently using tail biopsies for genotyping by PCR.
- Isoflurane anesthesia must be used if tail biopsy is performed after 4 weeks of age, although anxiety-like behaviour is increased in mice exposed to isoflurane (Hankenson et al., 2008), and, re-exposure to isoflurane has been shown to be more aversive than initial exposure (Moody and Weary, 2014). Studies are still needed to determine the potential efficacy of topical anesthetics and analgesics in young mice for the alleviation of adverse effects due to biopsy (Hankenson et al., 2011).

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# APPENDIX 5
# INDICATORS OF DISEASE

General indicators of ill health in mice include the following:

- rough, dull coat, piloerection;
- change in skin or mucous membrane colour (pallor, hyperemia, cyanosis, jaundice);
- decreased activity (reduced movement, reduced exploration of surroundings, seeking shelter or cage corner (Hankenson et al., 2013), reduced interaction with co-housed mice);
- hunched posture, lethargy, prostration, unresponsive to stimulation (slow to move or does not move away from stimulus) (Paster et al., 2009);
- hyperactivity, excessive scratching;
- overreaction to stimulation;
- skin lesion (wound, alopecia, dermatitis (Kastenmayer et al., 2006));
- presence of a mass;
- abdominal distension;
- tachypnea, dyspnea (abnormal breathing);
- neuromuscular signs (lameness, ataxia, tremors, convulsion);
- body condition score 2 or lower (2 = thin, 1 = emaciation) (Ullman-Culleré and Foltz, 1999);
- weight loss (however, this may be masked by the presence of a mass or accumulation of body fluids);
- hypothermia or hyperthermia (Nemzek et al., 2004);
- abnormal behaviours including stereotypies; changes in behaviour;
- ocular lesion, eyes half closed or closed; note that orbital tightening is a sign of acute pain (Langford et al., 2010);
- diarrhea or constipation (Nemzek et al., 2004);
- dehydration;
- sign of pain or discomfort (facial grimacing, belly pressing with or without hind leg extension, twitching, writhing, flinching, rear leg lifting, lying flat, staggering, back arching, abnormal walking, hopping, licking, scratching or grooming at a lesion, lack of nesting behaviour, reduced or supressed normal behaviour); and
- poor breeding success.

Note that mice are prey animals that may not exhibit clinical signs or behaviours suggesting disease. Unanticipated death(s) may be the first indication of a study or colony problem.

The above list is not comprehensive and the indicators required to assess animal health must be tailored to the specific animals being assessed and the specific animal model being used (especially in the case of genetically modified animals).

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# APPENDIX 6
# INDICATORS THAT MAY BE USED TO ASSESS THE WELFARE OF MICE

| INDICATOR | COMMENTS | REFERENCE |
|---|---|---|
| Nest building and time to integrate into nest test (TINT test) and nesting consolidation test scoring | Nest building activity can be affected by thermal stress, aggression among animals within the cage, and when mice experience general malaise or pain | Gaskill B.N., Karas A.Z., Garner J.P. and Pritchett-Corning K.R. (2013) Nest building as an indicator of health and welfare in laboratory mice. *Journal of Visualized Experiments* 82:e51012 (accessed on 2019-06-19).<br><br>Oliver V.L., Thurston S.E. and Lofgren J.L. (2018) Using cageside measures to evaluate analgesic efficacy in mice (*Mus musculus*) after surgery. *Journal of the American Association for Laboratory Animal Science* 57(2):186-201. |
| Burrowing behaviour | Spontaneous burrowing behaviour has been shown to change as a result of inflammation in acute DSS-induced colitis | Jirkof P., Leucht K., Cesarovic N., Caj M., Nicholls F., Rogler G., Arras M. and Hausmann M. (2013) Burrowing is a sensitive behavioural assay for monitoring general wellbeing during dextran sulfate sodium colitis in laboratory mice. *Laboratory Animals* 47(4):274-283. |
| Fecal corticosterone | Corticosterone may be a stress marker | Wright-Williams S., Flecknell P.A. and Roughan J.V. (2013) Comparative effects of vasectomy surgery and buprenorphine treatment on faecal corticosterone concentrations and behaviour assessed by manual and automated analysis methods in C57 and C3H mice. *PLoS One* 8(9):e75948, doi: 10.1371/journal.pone.0075948. eCollection 2013. |
| Grooming transfer test scoring | Sensitivity to detect post-operative pain up to 48 hours after the procedure | Oliver V.L., Thurston S.E. and Lofgren J.L. (2018) Using cageside measures to evaluate analgesic efficacy in mice (*Mus musculus*) after surgery. *Journal of the American Association for Laboratory Animal Science* 57(2):186-201. |

| INDICATOR | COMMENTS | REFERENCE |
|---|---|---|
| Pain face | A pain face, as described by Langford et al. (2010), is thought to be associated with noxious stimuli of moderate duration | Langford D.J., Bailey A.L., Chanda M.L., Clarke S.E., Drummond T.E., Echols S., Glick S., Ingrao J., Klassen-Ross T., Lacroix-Fralish M.L., Matsumiya L., Sorge R.E., Sotocinal S.G., Tabaka J.M., Wong D., van den Maagdenberg A.M., Ferrari M.D., Craig K.D. and Mogil J.S. (2010) Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6):447-449. |
| Stereotypic behaviour | As noted in Section 6.2, "Housing Management", stereotypic behaviours are often associated with poor environments and must be considered a potential sign of stress<br><br>May be reduced if mice are provided with enrichment<br><br>Litter size has been shown to be positively correlated to stereotypic behaviour in females of some strains of mice | Bechard A., Meagher R. and Mason G. (2011) Environmental enrichment reduces the likelihood of alopecia in adult C57BL/6J mice. *Journal of the American Association of Laboratory Animal Science* 50(2):171-174.<br><br>Bechard A., Nicholson A. and Mason G. (2012) Litter size predicts adult stereotypic behavior in female laboratory mice. *Journal of the American Association of Laboratory Animal Science* 51(4):407-411.<br><br>Gross A.N., Richter S.H., Engel A.K. and Würbel H. (2012) Cage-induced stereotypies, perseveration and the effects of environmental enrichment in laboratory mice. *Behavioural Brain Research* 234(1):61-68. |
| Barbering | May indicate a welfare concern, particularly when it is self-inflicted | Tynes V.V. (2013) Behavioral dermatopathies in small mammals. *The Veterinary Clinics of North America. Exotic Animal Practice* 16(3):801-820. |
| Positive behaviour | Mice that are not fearful of human contact should not avoid the presentation of a hand in their cage, and may even approach it<br><br>This may also be seen with the use of tunnels or an open hand when picking up mice | Spangenberg E.M. and Keeling L.J. (2016) Assessing the welfare of laboratory mice in their home environment using animal-based measures – a benchmarking tool. *Laboratory Animals* 50(1):30-38 (supplementary material) (accessed on 2019-06-19).<br><br>Hurst J.L. and West R.S. (2010) Taming anxiety in laboratory mice. *Nature Methods* 7(10):825-826. |

# GLOSSARY

**Abnormal behaviours** – actions performed by an animal that are not part of the behavioural repertoire of that species in the wild.

**Affective state** – refers to the mental state of an animal that leads to subjective experiences and physiological and behavioural changes in the body.

**Analgesia** – decrease in response to noxious stimuli.

**Anesthesia** – a state caused by an external agent leading to loss of sensation and motor function.

**Barrier**– a means of separating areas of an animal facility to reduce or minimize cross-contamination; barriers are commonly used to separate animals of different or unknown health statuses.

**Bedding** – material spread on the bottom of a cage, pen, stall, etc. for the purpose of providing comfort to the animals and keeping them dry; also referred to as substrate.

**Cage components** – temporary or permanent additions to an animal's enclosure that address its needs or enrich the environment.

**Conspecifics** – animals belonging to the same species.

**Discomfort** – a mild form of distress.

**Distress** – a state where the animal must devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation; it is associated with invasive or restrictive procedures conducted on an animal, or other conditions which significantly compromise the welfare of an animal, which may or may not be associated with pain.

**Ear punch** – the removal of a piece of an animal's ear (generally a notch or small hole), which can be used for identification, and the tissue that is removed can be used for genotyping.

**Endpoint** – predetermined criteria for intervening in a procedure to terminate, minimize or reduce an animal's pain and/or distress, which takes into account the welfare of the animal (welfare endpoint) and the goal of the experiment (scientific endpoint).

**Environmental enrichment** – enhancements to an animal's environment that go beyond meeting its basic species-specific needs and further improve overall quality of life.

**Experimental design** – the process of planning a study to ensure the correct number of animals consistent with the scientific objectives, to use methods to reduce subjective bias, and to employ appropriate statistical analysis.

**Genetically modified** – a deliberate modification of the genome (the material responsible for inherited characteristics).

**Genotyping** – a process used to determine differences in the genetic makeup (genotype) of an individual animal by examining the individual's DNA sequence using biological assays and comparing it to another individual's sequence or a reference sequence.

**Homeostasis** – the process of internal regulation by which biological systems tend to maintain stability while adjusting to conditions that are optimal for survival.

**Husbandry** – all aspects of the care and management of animals in facilities: laboratory, farm and aquatic (these guidelines do not include care of animals in the field).

**Metabolic cage** – individual housing for animals to permit the easy measurement of food and fluid intake and collection of urine and feces.

**Pain** – an aversive, sensory experience associated with actual or potential tissue damage.

**Personal protective equipment (PPE)** – garments or equipment designed to protect personnel from injury, infection, or allergic reaction when working with animals; potential hazards include physical injury (bites, scratches, etc.), biohazards, and airborne particulate matter.

**Phenotype** – refers to the observable physical properties of an organism; these include the organism's appearance, development, and behaviour.

**Play** – voluntary interaction of animals with objects or other animals for purposes other than meeting their needs for survival or reproduction, which results in positive welfare.

**Quarantine** – confinement of animals which may carry an infectious disease, for a specified period to allow for evaluation.

**Quality of life** – the welfare of the animal throughout its entire lifespan.

**Refinement** – the modification of husbandry or experimental procedures to minimize pain and distress.

**Sedatives** – drugs which reduce an animal's agitation.

**Sentinel animal** – a specific pathogen-free (SPF) animal known to be susceptible to an infectious agent that is placed in the area suspected of being contaminated, for example in a new shipment of laboratory animals under quarantine; the sentinel animal is then tested for infection or development of antibodies to the infectious agent.

**Specific pathogen-free (SPF)** – a designation used to describe the health status of animals for which a specific list of potentially infectious organisms have been tested for and not found.

**Standard operating procedure** – written documents that describe in step-by-step detail how a procedure should be carried out.

**Stereotypies** – repetitive or unvarying behaviours that appear to have no purpose.

**Stress** – a state caused by factors external to an animal that displace homeostasis; stress can be beneficial (e.g., in triggering a flight response if the animal is threatened, thus helping it to cope with changes in its environment); however, prolonged stress can cause changes to an animal's endocrine system, leaving it less able to cope with its environment.

**Three Rs** – Replacement, Reduction and Refinement in animal-based science, as first explained by Russell and Burch in *Principles of Humane Experimental Technique* (1959).

**Welfare** – the physical health and mental well-being of the animal.