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Conseil canadien de protection des animaux



CCAC guidelines: Nonhuman primates

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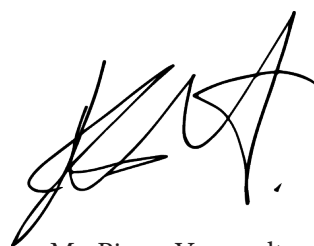
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Nonhuman primates

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: Nonhuman primates* provides information for investigators, animal care committees, facility managers, veterinarians and animal care staff to help facilitate improvement in both the care given to nonhuman primates 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) (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 nonhuman primates should keep abreast of the current literature.

For studies outside of Canada, Canadian investigators are subject to these guidelines as well as 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

Consideration must be given to providing the animals a complex environment with sufficient horizontal and vertical space for unhindered species-specific behaviours suited to the age and health status of the animals.

Subsection 2.2.1 Spatial Requirements, p.12

Guideline 2

Nonhuman primates must be provided with perching or elevated areas and other opportunities for species-typical behaviours, while avoiding harmful cage materials.

Subsection 2.2.2 Cage Design, p.13

4. PROCUREMENT

Guideline 3

When acquisition of nonhuman primates is necessary, they should be captive-bred and preferably of the second filial generation (i.e. their parents and grandparents were held in breeding facilities) or a subsequent generation.

Subsection 4.1 Source, p.17

Guideline 4

Before acquiring animals, institutions must require proof that the facilities where the animals were obtained have been inspected and were found to be in compliance with CCAC or other internationally recognized standards.

Subsection 4.1 Source, p.17

Guideline 5

Pre-shipment procedures must be in place for all transport of nonhuman primates, including transport between institutions within Canada.

Subsection 4.3 Pre-Shipment Procedures, p.19

Guideline 6

Receiving institutions must select a reputable supplier and/or transporter and select the route of the shortest time duration possible. They must also ensure appropriate equipment and animal care procedures are in place, and that there are plans for maintaining appropriate environmental conditions and for monitoring the animals during the transportation process.

Subsection 4.4 Transportation, p.20

Guideline 7

Prior to authorization of a nonhuman primate shipment, health records must be reviewed and approved by the receiving institution's veterinarian.

Subsection 4.5 Receiving Animals, p.21

5. BREEDING

Guideline 8

In-house breeding of nonhuman primates must be approved by the animal care committee and conducted in species-appropriate housing and conditions.

p.25

6. HUSBANDRY

Guideline 9

Any nonhuman primate injured as a result of the housing environment must be reported to the veterinarian and the animal care committee so that measures can be taken to address housing deficiencies and prevent recurrence of injury.

Subsection 6.2 Housing Management, p.28

Guideline 10

All animals of social species should be pair or group housed in conditions that allow full contact with compatible animals.

Subsection 6.2.1 Social Interaction, p.28

Guideline 11

Nonhuman primates should be provided with the opportunity to express foraging behaviour on a daily basis.

Subsection 6.3.1 Foraging, p.31

7. ANIMAL HANDLING, RESTRAINT AND TRAINING

Guideline 12

Any handling or restraint technique must be safe for the animal and the handler and minimize stress.

Subsection 7.1 Handling and Restraint, p.36

Guideline 13

Any handling or restraint technique should be introduced gradually through positive reinforcement training to minimize stress for the animals.

Subsection 7.1 Handling and Restraint, p.36

Guideline 14

Positive reinforcement techniques should be used.

Subsection 7.2 Animal Training, p.37

Guideline 15

A flexible training program should be developed for each animal following standard procedures and tailored to individual differences in learning.

Subsection 7.2.1 Training Programs, p.38

Guideline 16

Personnel conducting training must be competent, with demonstrated expertise.

Subsection 7.2.2 Personnel Involved in Training, p.38

8. WELFARE ASSESSMENT

Guideline 17

Nonhuman primates should be healthy; not be experiencing pain; and express a high prevalence and diversity of species-specific behaviours, with low prevalence and severity of chronic behavioural signs of anxiety, fear and aggression, and abnormal behaviours.

p.40

10. EXPERIMENTAL PROCEDURES

Guideline 18

The least invasive methods must be used, taking into consideration the particular animals involved and the research objectives.

p.45

Guideline 19

Category of Invasiveness E procedures must only be permitted for research related to public health emergencies.

p.45

Guideline 20

Animals should be trained for experimental procedures, including removal from housing and restraint where these are necessary components of the procedure.

p.46

Guideline 21

Welfare assessment, monitoring and compensatory measures should be in place to minimize any adverse welfare impacts of genetic modification.

Subsection 10.4 Genetically Modified Nonhuman Primates, p.47

Guideline 22

Food or fluid intake regulation should be used only if alternative means of motivation involving positive reinforcement (e.g., food treats or social stimuli) cannot meet the same experimental objectives of the study.

Subsection 10.5 Food and Fluid Intake Regulation, p.49

Guideline 23

For each food or fluid intake regulation protocol, the minimum level of regulation for each individual animal that will produce the required behavioural performance for the experiment and maintain the animal's health must be used.

Subsection 10.5 Food and Fluid Intake Regulation, p.49

Guideline 24

Food or fluid intake regulation must meet the animal's individual needs.

Subsection 10.5 Food and Fluid Intake Regulation, p.50

Guideline 25

Food or fluid regulation should be introduced gradually from ad libitum so that the animals have sufficient opportunity to adapt.

Subsection 10.5 Food and Fluid Intake Regulation, p.50

Guideline 26

Any animal on food or fluid regulation must be closely monitored.

Subsection 10.5 Food and Fluid Intake Regulation, p.51

Guideline 27

Prior to surgery for experimental procedures, animals should be healthy.

Subsection 10.7.2 Animal Preparation, p.54

Guideline 28

All members of the surgical team must be competent in their required tasks, be informed of the details of the surgery plan in advance, and rigorously adhere to the principle of asepsis to prevent surgical site infections.

Subsection 10.7.3 Surgical Team Preparation, p.54

Guideline 29

All surgical procedures must be well planned in advance, taking into account the individual physical characteristics of the animal involved.

Subsection 10.7.4 Surgery, p.55

Guideline 30

A plan for monitoring the animal during surgery must be in place.

Subsection 10.7.5 Intra-Operative Care and Monitoring, p.55

Guideline 31

Post-operative care and monitoring must be planned based on the invasiveness of the procedure and the individual needs of the animal, and adapted to unforeseen situations.

Subsection 10.7.6 Post-Operative Care and Monitoring, p.56

12. HUMAN SAFETY

Guideline 32

The risks of working with nonhuman primates must be identified, and all individuals working with nonhuman primates must be informed, trained and competent.

p.59

1 INTRODUCTION

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

This guidelines document has been developed to address the ethics and care of nonhuman primates maintained in Canadian scientific institutions. Great apes were not included in the development of the guidelines as these animals are not held in Canadian scientific institutions (see Section 1.1, “Taxonomy and Phylogeny”, for an explanation of the distinction). Hence, this document is not applicable for considerations regarding the ethics and care of great apes.

As with any animal-based science, application of the Three Rs (Replacement, Reduction, and Refinement) should guide decisions concerning the validity of scientific studies and the care of nonhuman primates (Burm et al., 2014). Replacement and reduction are important considerations in the design of animal-based studies. Consideration of replacement focuses on methods that avoid or replace animals, including absolute replacements, such as inanimate systems, and relative replacements, such as some invertebrates (which current interpretation of scientific evidence indicates have a significantly lower potential for pain perception). Consideration of reduction focuses on the fewest number of animals that provide valid information. Reduction through involvement of animals in multiple studies should be done carefully by assessing the impact on the welfare of the individual animals, considering their whole life experience (Wolfensohn et al., 2015).

The present guidelines focus primarily on implementation of refinement, both in terms of the care of animals in a facility and when a properly constituted animal care committee has approved a particular study involving nonhuman primates. Animals living in an environment where facilities and practices are oriented toward the promotion of good animal welfare are likely to be less stressed and exhibit normal behaviours and physiology (Poole, 1997).

Issues that are particular to nonhuman primates in science include: 1) their highly developed cognitive abilities and social interaction, and the associated needs; 2) their relatively long lifespan, which may result in a lengthy period of captivity associated with long-term studies or involvement in multiple studies; 3) their unpredictable actions, which have safety implications; and 4) the sources of the animals, which are typically far from the institution, resulting in lengthy travel and other welfare concerns associated with breeding, housing and quarantine prior to arrival (Prescott et al., 2010). Welfare concerns associated with capture are also a consideration for nonhuman primates that come from the wild or are studied in the field (see [CCAC guidelines on: the care and use of wildlife](#) (CCAC, 2003), for general considerations).

Behaviours important to nonhuman primate welfare are outlined in Section 1.1. Their sensory abilities (Section 1.2) and potential inter-animal behavioural variations (Section 1.3) also have an impact on welfare considerations.

This document focuses on applying refinement strategies to enable the expression of behaviours important to the welfare of nonhuman primates and to minimize any pain and distress. These strategies are generally applicable to all nonhuman primate species (excluding Hominidae (great apes)), commonly housed in Canadian scientific institutions; however, those caring for or working with nonhuman primates are responsible for any additional requirements for the particular species or the individual animal involved.

1.1 TAXONOMY AND PHYLOGENY

Primates form a diverse group of placental mammals represented by at least 488 species (Rylands and Mittermeier, 2014). Recent work in comparative genomics has generated a primate phylogeny that refines previous depictions of primate speciation (Pecon-Slattery, 2014; Pozzi et al., 2014; Raaum, 2015; Fleagle, 2013; Herlyn, 2016).

The order Primates comprises two suborders: Haplorhini and Strepsirrhini, whose divergence has been estimated to about 70 million years ago. Strepsirrhini includes the infraorder Lemuriformes (lemurs) and Lorisiformes (loris, galagos), while Haplorhini includes the infraorder Simiiformes and Tarsiiformes (tarsiers). The Simiiformes is composed of the clade Catarrhini and Platyrrhini, which diverged about 44 million years ago. Catarrhini include the family Cercopithecidae (Old World monkeys), Hylobatidae (gibbons), and Hominidae (great apes, humans); the Cercopithecidae diverged from the latter two families less than 30 million years ago.

Old World monkeys (Cercopithecidae) are a family of primates grouped into several genera under two subfamilies: Cercopithecinae (cheek-pouch monkeys) and Colobinae (leaf-eating monkeys with a multi-chamber stomach; sacculated stomach containing symbiotic microbes that aid in the digestion of high-fibre foods). For example, the Cercopithecidae include the genera *Macaca* (*Macaca mulatta* (rhesus), *Macaca fascicularis* (cynomolgus or crab-eating), *Macaca radiata* (bonnet), *Macaca fuscata* (Japanese), *Macaca nemestrina* (pigtailed), and *Macaca arctoides* (stumptail)), *Mandrillus* (mandrills), *Papio* (baboons), *Lophocebus* and *Cercocebus* (crested and white-eyelid mangabeys), as well as *Cercopithecus* (guenons), *Chlorocebus* (green and vervet monkeys), and *Erythrocebus* (patas). Colobinae include the genera *Colobus* (colobuses), *Nasalis* (proboscis), and *Semnopithecus* (langurs).

New World monkeys (Platyrrhini) are grouped into five families: Aotidae (owl monkeys), Atelidae (howler, spider, woolly spider, and woolly monkeys), Callitrichae (marmosets and tamarins), Cebidae (capuchins and squirrel monkeys), and Pitheciidae (titis, sakis, and uakaris).

The original basis for some of these classifications, first given by the French naturalists Buffon (1766) and Geoffroy Saint-Hilaire (1812a, b), was the shape of the external nose. The catarrhines are described as having narrow nostrils that turn downward, whereas the platyrrhines have broad nostrils that turn sideways. The term strepsirrhine refers to the sinuous aspect of the nostrils of these primates, in contrast to the simpler nose of the haplorhines (Pocock, 1918).

Among mammalian orders, Primates are more closely related to Dermoptera (colugos, flying lemurs) than Scandentia (tree shrews) (Mason et al., 2016; Melin et al., 2016). Together with Lagomorpha (rabbits and hares) and Rodentia (rodents), these orders form the Euarchontoglires.

1.2 SENSES

The care and involvement of nonhuman primates in scientific studies require consideration of their sensory abilities (Prescott, 2006a; Joint Working Group on Refinement, 2009). While the many species of primates vary in their behavioural adaptations within their arboreal habitats in tropical and subtropical forests, their senses constitute a unique combination among mammals (Martin, 1990, 2012; Dominy et al., 2001, 2004).

Vision

Primates are highly visual mammals, as their evolution is associated with visual specialization (Kirk, 2006; Ross and Martin, 2007; Cartmill, 2012; Sussman et al., 2013; Melin et al., 2016). Primates are among the mammals with the largest eyes (Ross and Kirk, 2007). Most importantly, they have well-developed visual acuity (Kirk and Kay, 2004; Souza et al., 2011), with the haplorhines being the only mammals whose retina possesses a fovea. Primates are also unique among mammals to possess trichromatic colour vision (Conway et al., 2010). This is true for all catarrhines and howler monkeys as well as the majority of female platyrrhines, as the genes for long-wavelength-sensitive photo-pigments are located on the X chromosome (Jacob, 2008; Kawamura, 2016). Lastly, primates are frontal-eyed animals with highly convergent orbits conferring binocular (stereoscopic) depth perception, the degree of which correlates with the size of the visual cortex and the expansion of primate brain volume (Heesy, 2009). These visual adaptations are associated with foraging for fruit and foliage, locomoting in an arboreal environment, avoiding predation, and social communication (Sussman et al., 2013; Kawamura, 2016).

Olfaction

The elaboration of the primate visual system was originally viewed to have evolved at the expense of olfaction, because primates exhibit some reduction of nasal anatomy (Smith et al., 2015). In contrast to a reduced reliance on olfaction, it is now recognized that primates have an acute sense of smell and that their olfactory systems have evolved to adapt to their respective ecology in a variety of behavioural contexts (Heymann, 2006; Drea, 2015; Laska and Salazar, 2015; Nevo and Heymann, 2015). The main olfactory system is particularly involved in foraging and food selection as well as predator avoidance, while the accessory olfactory system is specialized for processing social odours and thus important in reproduction and communication; scent-marking and urine-washing behaviours are common in strepsirrhines and platyrrhines.

Gustation

Food selection involves not only colour vision and olfaction but also the sense of taste, which is well developed in primates and dominated by sensitivity to sweet and bitter substances. Positive and negative responses to, respectively, sweet (sugars) and bitter taste (alkaloids and tannins) have been interpreted as taste perception being an opponent system to discriminate nutritious foods from potentially toxic and noxious substances (Hladik et al., 2003; Beauchamp, 2016). Sweet sensitivity, but not bitter sensitivity, has been found to correlate with body size (Simmen and Hladik, 1998) as well as with the density of fungiform papillae on the tongue (Alport, 2009). Lastly, sexual dimorphism in gustatory anatomy has been reported in humans, chimpanzees and capuchins (Muchlinski et al., 2011), suggesting that females have greater taste sensitivity that can affect dietary intake.

Audition

Primates have hearing abilities predicted by external and inner ear structures (Kirk and Gosselin-Ildari 2009; Coleman and Colbert, 2010; Coleman and Boyer, 2012). They are mammals with good low-frequency sensitivity, generally better than most laboratory mammals (Heffner and Heffner, 2007). This is especially true for catarrhines, to whom this enhanced low-frequency sensitivity may confer long-distance communication. In terms of high-frequency sensitivity, primates fit to the mammalian pattern. Firstly, they can hear much higher frequency sounds than birds and reptiles (Fay, 1992). Secondly, smaller primate species (with smaller heads and thus shorter maximal interaural time difference) can hear much higher frequency sounds than larger species, including humans (Heffner, 2004; Coleman, 2009). This enhanced high-frequency sensitivity may facilitate sound localization with intensity cues. Sound localization acuity correlates with visual acuity (Heffner and Heffner, 2016), allowing the highly visual primates to direct their gaze to sound sources, whether predators or conspecifics. Indeed, vocalizations are important communication signals for primates, and their auditory system is adapted for detecting and discriminating between such signals (Ghazanfar and Santos, 2004; Ramsey et al., 2012).

Somatosensation

In all extant primates except humans, the big toe (hallux) diverges widely from the other digits, permitting the foot to grasp powerfully, while the hand usually has at least some grasping capacity. The fingers typically bear blunt, flat nails rather than bilaterally flattened, pointed claws. The ventral surfaces of fingers and toes bear tactile pads with skin ridges (dermatoglyphs) that provide traction on arboreal substrates and, in association with Meissner's corpuscles, enhance the sense of touch (Martin, 1990).

Interestingly, anthropoid primates have several unique specializations, first in the density of digital mechanoreceptors, where there is an unusual emphasis on the hand as tactile organ, and then in the thalamus and cortex, where more subdivisions of the brain are devoted to somatosensory perception.

In monkeys, Meissner's corpuscles are located in the dermal papillae between the epidermal limiting and intermediate ridges, forming orderly rows, the contours of which follow the overlying fingerprint. (Bolanowski-Pawson 2003). The size of Meissner's corpuscles varies as a function of body mass (Verendeew et al., 2015).

1.3 BEHAVIOURS IMPORTANT TO NONHUMAN PRIMATE WELFARE

Addressing the welfare of nonhuman primates in the laboratory environment requires consideration of their natural behaviours and provision of opportunities for their expression where appropriate. Direct social contact with compatible animals is the most important factor in promoting species-typical activities and reducing abnormal behaviour¹ (such as excessive aggression, self-wounding, and stereotypical behaviours) (Lutz and Novak, 2005; NRC, 1998; Wolfensohn and Honess, 2005; Baker et al., 2012). In their natural environment, nonhuman primates are social animals (Lutz and Novak, 2005). Although social structures vary with species, nonhuman primates spend a large proportion of their time engaged in social interactions (Lutz and Novak, 2005). Tactile contact with other primates, exhibited through huddling, grooming, etc., is important to the well-being of both infants and adults (Joint Working Group on Refinement, 2009). When scientific or

¹ Abnormal behaviour is behaviour indicative of stress or anxiety (e.g., self-injury) or that diminishes the expression of positive natural behaviours.

veterinary reasons justify single housing of nonhuman primates, with the approval of the animal care committee, it should be for the shortest duration possible, and allow for visual, olfactory, auditory and tactile contact with other animals, in line with the scientific or veterinary constraints. Positive human contact can also be beneficial (see Section 6.6, “Human Contact and Handling”).

Nonhuman primates exhibit a diversity of locomotor activities, including walking, running, climbing, leaping, swinging and hanging (Schmidt, 2010). Many nonhuman primates use elevated areas, such as tree branches, as safe resting sites and to escape predators (Chopra et al., 1992). Preferences for particular activities vary with species, habitat and context (see Schmidt, 2010 for a review).

Foraging (procuring and processing food) is an important behaviour of nonhuman primates; in the wild they spend a great portion of their awake time engaged in foraging (Lutz and Novak, 2005). Therefore, foraging opportunities and other means of prolonging feeding time (e.g., puzzle feeders) should be provided to occupy time, stimulate manipulative and cognitive skills, and prevent negative behaviours.

In animal facilities, the structure of the animal’s environment plays a role in their ability to perform natural behaviours. For example, elevated areas are an important feature, and many nonhuman primates prefer to occupy higher vertical spaces when given an option (Clarence et al., 2006; MacLean et al., 2009; Reinhardt, 1992). For some species of macaque, the use of elevated structures is related to the animal’s dominance level, with higher-ranked animals occupying the highest elevations (Reinhardt, 1992). Height can also provide a feeling of safety to animals (MacLean et al., 2009).

In the laboratory environment, routines, such as for feeding and cage cleaning, provide an element of predictability and this may be important to the welfare of nonhuman primates (Gottlieb et al., 2013).

1.4 SOURCES OF VARIATION

It is important to know the social organization and behaviours of the species involved, as well as the characteristics of the individual animals. For example, marmosets typically live with family members, and some species require nests (owl monkeys and marmosets) while others do not (macaques). Some species scent mark and need branches or similar items to mark.

Nonhuman primates’ stress responses and aggressive behaviours vary with species, and may be further influenced by sex, age, reproductive status, social status, individual temperament, health status and the individual’s background (e.g., source, rearing conditions, previous housing, experimental procedures, training, and previous experience or contact with humans) (Honess and Marin, 2006).

2 FACILITIES

For general guidance on laboratory animal facilities, see the [CCAC guidelines on: laboratory animal facilities – characteristics, design, and development](#) (CCAC, 2003) and the *Canadian Biosafety Standards*, 2nd ed. (Government of Canada, 2015a). Additional guidelines and information of particular concern for nonhuman primates are presented in this section.

Facilities housing nonhuman primates must be secure and unauthorized access must be prevented.

2.1 ANIMAL ROOMS AND PROCEDURE ROOMS

Facilities should be designed to provide separation of groups in quarantine from established colonies, and separation of animals of different species, different health status, etc. following quarantine. Nonhuman primate rooms should be maintained under negative pressure. Ideally, there should be an anteroom, which helps to maintain pressure gradients.

Surfaces within the animal room must be easily sanitized (Government of Canada, 2015a). Drains and gutters are recommended for animal rooms.

Measures must be in place to prevent animals from accessing potentially hazardous equipment or furniture (including light fixtures, ventilation ducts, electrical wires and outlets, and sprinklers).

2.2 PRIMARY ENCLOSURE

Primary enclosures include cages, pens, corrals, etc. In facilities where the room is the primary enclosure, an anteroom is required.

2.2.1 Spatial Requirements

Guideline 1

Consideration must be given to providing the animals a complex environment with sufficient horizontal and vertical space for unhindered species-specific behaviours suited to the age and health status of the animals.

Cages must be designed for pair or group housing of animals, such that normal affiliative behaviour and avoidance behaviour can be expressed, and negative interactions reduced (see Section 6.2, “Housing Management”). Cages must provide sufficient space, both horizontally and vertically, to allow adequate freedom of movement for the animals to perform positive physical and social behaviours important to their welfare (e.g., grooming, resting, foraging, play, normal locomotor repertoire), while reducing the incidence of negative behaviours.

Species-specific needs and the size and age of the animals dictate the cage size and complexity required to address the animal's physical, psychological and behavioural well-being (Buchanan-Smith et al., 2004). Other factors to be considered include group size and the health status of the animals. There is currently little consensus on appropriate minimum enclosure sizes internationally, and space requirements can be influenced by the quality of the animal's environment. Standards for cage size cannot be based solely on the body weight of an animal (Buchanan-Smith et al., 2004). In-house experience, the experiences of other institutions, and behaviours that have been shown to be important to nonhuman primates in the wild (as noted in Section 1, "Introduction") should be considered in determining the best designs to meet the animals' needs.

Appendix 1 provides an assessment tool to assist with rating behaviours and elements that must be evaluated when determining the appropriate cage size. The assessment should be a team approach involving the veterinarian, trainer, facility manager, investigators, and research and animal care staff, who have knowledge of the unique characteristics and behaviour of the particular animals, in order to assess the animals' quality of life (Lambeth et al., 2013). The animal's needs must be met regardless of the length of time it will be held.

Cages should be of sufficient dimensions to allow the addition of furnishings that promote positive behaviours and can maximize use of the available space for the animals. Larger cages with complex features may stimulate locomotor activity, while eliminating unwanted stereotypic behaviours (Kitchen and Martin, 1996), and contribute to optimal growth patterns in young animals (Faucheux et al., 1978). Housing animals in groups in larger cages provides each animal with greater space to utilize and may help to stabilize social relationships, while also making efficient use of the room.

2.2.2 Cage Design

Guideline 2

Nonhuman primates must be provided with perching or elevated areas and other opportunities for species-typical behaviours, while avoiding harmful cage materials.

Perches or elevated areas are required for all nonhuman primates. Cage design should favour vertical space over horizontal space and include elevated resting areas and climbing structures so that the animals can fully utilize the vertical space, unless such features are detrimental to the animals (e.g., animals recovering from anesthesia or having a cognitive or physical impairment that would make these features unsafe); see Section 6.4, "Environmental Enrichment".

Cages should provide the ability to temporarily separate animals for procedures, treatments, feeding, training, safe introduction of new animals to a pair or group, and temporary incompatibility issues, and be flexible such that they can be combined to allow animals greater space or to accommodate larger groups. Cages should also be designed to offer structural barriers that allow some privacy and multiple escape routes to avoid attacks and intimidation from dominant individuals.

Cage materials should be easily cleaned and sanitized. Any nuts and bolts should not be accessible to animals and there should be no sharp surfaces or small openings, to prevent injuries and entrapment.

2.3 OUTDOOR FACILITIES

If nonhuman primates are housed outdoors, there must be a secondary enclosure to prevent escapes and intrusions. Shelter from the elements must be provided for each animal at all times. During cold weather, the animals must have free access to climate-controlled indoor housing.

Health problems can arise in some species exposed to low temperatures. For example, cynomolgus monkeys held at temperatures below 12°C can experience health problems, depending on the duration of exposure and age of the animal, and animals with access to outdoor housing can experience frostbite from perching on metal in cold weather.

3

FACILITY MANAGEMENT

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

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

3.1 LIGHT

It is preferable to use natural light in combination with artificial light and the use of shutters, as necessary, to maintain the photoperiod for animals housed indoors. A 12 hour/12 hour light/dark cycle is adequate for nonhuman primates and reduces changes in physiological variables associated with seasonality (Qin et al., 2015; Lemos et al., 2009). A dusk/dawn transition may be beneficial and can be accomplished through a gradual change in light intensity or, where that is not possible, signalled through a visual or auditory cue that lets the animals know about the upcoming light transition (e.g., a radio or a small light going on/off a few minutes before the lights change).

3.2 TEMPERATURE AND RELATIVE HUMIDITY OF INDOOR FACILITIES

Consideration should be given to the combined effects of temperature and humidity, and the impact on evaporative heat loss (Walters et al., 2004). Many nonhuman primate species originate from tropical or subtropical climates and prefer high temperatures and relative humidity. Temperature requirements are species specific and facilities should balance consideration of the climate where the species originates with the health and safety of the staff.

Variation in temperature must be avoided; however, nonhuman primates will adapt to a range of temperatures if given sufficient time (i.e. no rapid, abrupt fluctuations) and if provided with physical structures to facilitate thermoregulation (e.g., a nest box, a thermoneutral resting structure, contact bedding or a heat lamp or heated area). The degree of body contact between individuals can be an indicator of whether the temperature is affecting their welfare (Schino and Troisi, 1990).

Relative humidity is commonly kept between 40-70%. Nonhuman primates are more susceptible to low relative humidity than other species. Bloody nose syndrome (associated with *Moraxella catarrhalis*, found in the normal flora of the nasopharynx) is linked to a lack of humidity in the room (Sasseville and Ditters, 2008). It spreads among animals easily and is difficult to eradicate. Therefore all measures must be taken to prevent its introduction in the colony by maintaining an adequate humidity level. If detected, it must be reported to the veterinarian so that mitigation measures can be taken.

3.3 SOUND AND VIBRATION

While nonhuman primates can generate a lot of noise, consideration should be given to housing them where they will not be exposed to excessive sound levels, including ultrasound (Heffner, 2004; Coleman, 2009) and vibration. Additionally, incorporation of wood and plastics in conventional metal cages can help reduce noise levels.

4

PROCUREMENT

For general guidance on the acquisition, transportation and quarantine of animals, see the [CCAC guidelines on: the procurement of animals used in science](#) (CCAC, 2007). Additional guidelines and information of concern for nonhuman primates are provided in this section.

4.1 SOURCE

Guideline 3

When acquisition of nonhuman primates is necessary, they should be captive-bred and preferably of the second filial generation (i.e. their parents and grandparents were held in breeding facilities) or a subsequent generation.

Nonhuman primates involved in research should be purpose-bred with a defined health status (Joint Working Group on Refinement, 2009), and acquired from a location that minimizes transit time (see Section 4.4, “Transportation”). Acquiring animals that are habituated to a captive environment, the presence of humans and suited to the requirements of the study will result in better welfare for the animals and better scientific results (Joint Working Group on Refinement, 2009). The procurement of great apes for scientific studies is not appropriate.

In exceptional circumstances where wild-caught animals are required for a research project and there are no alternatives, sufficient scientific justification and the details of the capture method must be provided to the animal care committee for approval. Cost must not be a factor in the decision to acquire wild-caught animals. Wild-caught nonhuman primates should be in captivity for at least six months prior to their involvement in science to allow for acclimation, unless the goal of the project is to study wild animals and the study involves capture (i.e. field study; where the [CCAC guidelines on: the care and use of wildlife](#) (CCAC, 2003) apply). Where nonhuman primates have been in captivity for less than two years, a longer period of quarantine is required (see Section 4.6, “Quarantine and Acclimation After Receipt of Animals”).

Guideline 4

Before acquiring animals, institutions must require proof that the facilities where the animals were obtained have been inspected and were found to be in compliance with CCAC or other internationally recognized standards.

Institutions should promote refinement of living conditions and care at the nonhuman primate source by only procuring animals from facilities that are attentive to factors such as health status, husbandry and care, quarantine, transport and acclimatization (Joint Working Group on Refinement, 2009).

Breeding and holding facilities for nonhuman primates must have been inspected within the last three years and must be found to be in compliance with CCAC or other internationally recognized standards (e.g., Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), United Kingdom Home Office, European Directive). A written report of the inspection with reference to the standards used for assessment must be retained (e.g., proof of AAALAC accreditation). Where institutions do not have the resources to individually inspect facilities, they must ensure that a reputable third party has conducted such inspections.

The following aspects must be included in an inspection of breeding and holding facilities:

- facility management practices, including housing and husbandry procedures;
- veterinary care and oversight programs and appropriate medical supplies (vaccines and common treatments such as antibiotics, antidiarrheal medications, etc.);
- disease surveillance records and diagnostic tools;
- preventive medicine program;
- welfare and enrichment plan;
- clinical records of animals; and
- whether the colony is closed or receiving expansion or replacement breeders (for breeding facilities) (Abee et al., 2012).

Special consideration should be given to pedigree information and obtaining animals that have been previously paired or grouped, or at least have been socially housed with other primates, to increase the likelihood of compatibility.

4.2 REGULATIONS

Regulatory requirements for the importation of nonhuman primates are established by the Canadian Food Inspection Agency (CFIA, 2009). Documentation must be in compliance with CFIA import permits and the conditions specified in the CFIA permit. The CFIA must be notified of any movement of imported nonhuman primates between facilities or institutions and the conditions of carcass disposal at the time of death.

As a member of the [Convention on International Trade in Endangered Species of Wild Fauna and Flora \(CITES\)](#), Canada bans commercial trade of endangered species and regulates and monitors trade in other species that might become endangered. Institutions planning to import nonhuman primates must be aware of species listed by CITES (identified in CITES Appendices I or II). The importation of any species listed by CITES in Appendix II, or specimen of these species, requires an import permit from CITES Canada. For importation of species listed by CITES in Appendix I, or specimens of these species, a CITES export permit from the exporting country is also required in addition to the CITES import permit from Canada.

The World Organization for Animal Health (OIE) is referenced in CFIA import permits. Important information is provided in the OIE's [Terrestrial Animal Health Code](#) (OIE, 2016) Chapter 5.9, "Quarantine Measures Applicable to Non-Human Primates" and Chapter 6.11, "Zoonoses Transmissible from Non-Human Primates".

4.3 PRE-SHIPMENT PROCEDURES

Guideline 5

Pre-shipment procedures must be in place for all transport of nonhuman primates, including transport between institutions within Canada.

Pre-shipment procedures should include, but may not be limited to, measures to assess the health of the animals and to acclimate the animals to the conditions at their destination (including new housing conditions, social conditions, and experimental, testing and/or restraint procedures).

Pre-shipment procedures depend on:

- the type and duration of transport (e.g., terrestrial, aerial and/or by sea, and international export or between two institutions within Canada);
- how the animals are held prior to shipment (e.g., if held outdoors, more extensive evaluation is required before shipping); and
- the type of facility the animals have been held in (e.g., closed community with no tuberculosis).

The time required for pre-shipment isolation depends on prior husbandry and management practices, the health status of the colony, diseases endemic in the place of origin, the needs of the importer, and relevant regulations (Joint Working Group on Refinement, 2009). Pre-shipment isolation is generally for a minimum of 30 days (Abee et al., 2012). During this time, animals should be socially housed (Joint Working Group on Refinement, 2009) and cagemate identification should be provided to the receiving institution.

Information relevant to pre-shipment examination and testing is described in Abee et al. (2012) and CFIA (2009). Pre-shipment procedures for animals imported from other countries except the US must include tuberculosis testing (two times, at least two weeks apart), which should be performed no longer than three months before shipping. All animals must be healthy and fit for transport, and have had a physical exam no longer than two weeks prior to transport. The examination should include body weight, body condition, a complete blood count and serum chemistry, rectal culture, fecal examination, pelage examination, and viral serology (Abee et al., 2012). Pre-shipment treatments (e.g., parasiticides) may be required.

Animals testing positive for tuberculosis or *Macacine herpesvirus 1* (also known as herpes simian B virus) should not be accepted for transport (see Section 9, “Health and Disease Control”). It should be noted that nonhuman primates can carry infectious disease agents that diagnostic testing does not necessarily detect; this is a constant risk wherever humans and nonhuman primates interact.

Nonhuman primates imported into Canada must be permanently and uniquely identified as specified by CFIA (2009), using such methods as tattoos or microchips. Refinements for these identification methods are described by the Joint Working Group on Refinement (2009), as noted in Section 6.1, “Identification”.

4.4 TRANSPORTATION

Guideline 6

Receiving institutions must select a reputable supplier and/or transporter and select the route of the shortest time duration possible. They must also ensure appropriate equipment and animal care procedures are in place, and that there are plans for maintaining appropriate environmental conditions and for monitoring the animals during the transportation process.

Receiving institutions share responsibility for the well-being of the animals during transport with the shipping facilities. For the receiving institution, the responsibility for acquisition of animals includes ensuring: 1) the animals are obtained from a supplier meeting CCAC or internationally recognized standards, as described in Section 4.1, “Source”; and 2) all phases of the transport are carried out in a manner that minimizes discomfort and distress. As noted in the [CCAC guidelines on: procurement of animals used in science](#) (CCAC, 2007), “Institutions, in consultation with animal users...are responsible for selecting the method and timing of transportation of animals from the suppliers, and monitoring the transportation process.”

When transporting nonhuman primates by air, receiving institutions and shipping facilities are responsible for compliance with the International Air Transport Association (IATA) *Live Animal Regulations* (IATA, 2016), which provide information concerning documentation, containers and other requirements. While IATA specifically provides information for air transportation, this information is also useful for land transportation. Additional useful information is available in Swallow et al. (2005) and ILAR (2006), which are the two major references cited in the [CCAC guidelines on: procurement of animals used in science](#) (CCAC, 2007).

Those transporting nonhuman primates must ensure acceptable environmental conditions are maintained such that the temperature during transport is similar to that at the shipping facility. In addition, transportation plans must be developed that include:

- acceptable transportation equipment – the enclosure must restrict the animal’s movement to prevent the animal from hurting itself or causing injury to handlers, but be of a sufficient size for the animal to be comfortable;
- trained personnel – staff overseeing the care of the animals during transport must be trained to recognize signs of distress;
- care of the animals, including daily checks and the provision of food and water if transportation will last more than three hours;
- measures to maintain the biosecurity and safety of the animals;
- backup measures (e.g., critical equipment and supplies) to prevent situations that could affect the welfare of the animals;
- measures to prevent escape;
- contingency plans in case of transportation delays, accidents, etc.; and
- emergency contact information – a veterinarian must be available by phone in case of questions or concerns and emergencies during transport.

Ideally, fruits with high water content (e.g., watermelon, apples) should be provided to the animals during transport for longer transit times.

When an institution is planning to transport nonhuman primates, all phases of transport must be considered, including the periods between major events (e.g., moving an animal from an institution to a truck or between an airplane and truck). Attention to detail is important. For Canadian institutions, transport during the winter months can be particularly problematic. The animals should never be exposed to temperatures that could negatively affect their welfare, even for very short periods between transport phases.

Travel time and the quality of transport are important, and consideration must be given to the whole travel experience of the animal when planning to meet their needs during transport and upon arrival at the institution. For example, when importing animals, consideration of travel time must include the time required to transport the animals to the airport in the country of origin; in some cases this can take days. Minimizing the duration of travel is important to limit transport-induced stress for the animals, and the shortest and most direct route of transportation available must be used (OIE, 2016).

During transportation, animals must be easily accessible. It is common to confine animals individually; however, they must have some contact with conspecifics. Each cage must be firmly affixed to the transport vehicle. For driver health and safety, the cargo area of the transport vehicle must be physically separated from the driver.

4.5 RECEIVING ANIMALS

Guideline 7

Prior to authorization of a nonhuman primate shipment, health records must be reviewed and approved by the receiving institution's veterinarian.

For international travel, health records must also accompany the animal during transit.

Strict procedures for reception of animals are important to prevent cross-contamination within the facility. Institutions must ensure procedures and facilities are in place prior to arrival of the animals. An initial on-site evaluation of the animals must be performed on arrival to detect signs of illness or abnormal behaviour (see Section 4.5.1, “Examinations”). Nonhuman primates should be taken directly to the animal room.

Transport containers must be sanitized or incinerated. The receiving area and corridors must be sanitized after use.

The animals should be provided with water and food, including fruits and vegetables, upon arrival. Small and frequent meals may be more acceptable to the animals than large meals, depending on the length of transport, their level of stress, and any changes in their diet.

The animals must be observed to ensure they are able to access water from the water source provided.

4.5.1 Examinations

Examination of the animals upon arrival must be conducted by a veterinarian, or someone designated by the veterinarian, in two phases:

- initial exam – observation of the animal with no intervention unless necessary for the well-being of the animal; and
- full exam – conducted under sedation a minimum of two days and a maximum of four days after arrival if the likelihood of disease is high, in order to allow the animal time to rest after transport and ensure any health concerns are addressed in a timely fashion. Where disease is less likely and the animal's health condition is adequate based on the initial exam, this may be extended, depending on CFIA requirements.

4.6 QUARANTINE AND ACCLIMATION AFTER RECEIPT OF ANIMALS

The specific procedures for quarantine after receipt of animals are dependent on the source of the animals and the type and length of transport. Where CFIA permits are required, all aspects of husbandry, including quarantine, must comply with permit conditions.

The duration of quarantine is based on veterinary judgment of the length of time necessary to assess the health status of the animal. A period of 30 to 90 days is generally recommended; where 90 days refers to a feral animal that has been held in captivity for less than two years or an unfamiliar species. If multiple shipments of nonhuman primates are contained in the same room, the quarantine period should finish when the requirements for the last shipment have been met (Abee et al., 2012).

Strategies to prevent nonhuman primates from being quarantined alone must be considered (e.g., animals acquired in pairs or groups).

The need for sedation during quarantine depends on the procedures to be undertaken, the particular animal and its health status, and the level of quarantine required. Interventions on animals should be limited as much as possible to reduce the need for sedation. The number of staff entering the quarantine room should also be limited.

In determining the appropriate acclimation period, consideration must be given to the duration of transport, any change in the type of housing or diet, the age of the animals (i.e. older animals may require a longer acclimation period), the particular characteristics of the animals and the type of study they will be involved in (see Capitanio et al., 2006, for a review of factors affecting the acclimation period). For example, based on oxidative stress, cynomolgus macaques need a 21-day acclimation to recuperate from transportation (Pan et al., 2016).

Acclimation begins during quarantine and consists of two phases. The initial rest phase commonly lasts for three weeks; however, a shorter rest period may be acceptable if the animal has only undergone a local transfer and the housing conditions are similar to those where the animal was previously housed. Most animals do not eat their regular ration during transport due to stress, and a period of rest is needed to resume normal eating. In addition, any food deprivation prior to transport and any further deprivation when sedation has been used should be considered. During the initial phase, procedures are only conducted for veterinary purposes related to the health of the animals and quarantine procedures.

The second phase of acclimation may involve training, adaptation to procedures, pre-experimental activities (e.g., acquisition of baseline physiological data), etc. The amount of time for completion of this phase and initiation of a study will depend on the particular animals and their experiences, and the requirements of the study. For some animals transported by air, it may take over a month for their behaviour and levels of stress to return to those observed prior to transport (Honess et al., 2004).

4.6.1 Facilities and Equipment

Biocontainment infrastructure and practices must be in place (see Containment Level 2 in the most recent version of the *Canadian Biosafety Standards* (Government of Canada, 2015a)). Containment Level 3 procedures may be needed in cases of zoonotic disease risks. The specific requirements will depend on the history of the animal, the type of study and the conditions presented in a CFIA permit (see CFIA, 2009, Appendix I and II; and CFIA, 2017 for an overview), in addition to the *Canadian Biosafety Standards* (Government of Canada, 2015a).

Quarantine should take place in a separate building or at least in a room that is completely separated from the rest of the colony, with designated caging and equipment (including personal protective equipment) because of the potential risk of contamination (Abee et al., 2012).

Personnel must be provided with appropriate protection equipment, based on a risk assessment, including a long-sleeved gown or coverall, face mask suited to the risk, protective eyewear, gloves, and dedicated shoes or shoe covers (Abee et al., 2012) (see Section 12, “Human Safety”). All personal protective equipment must be removed for disposal or sterilization upon exiting quarantine facilities. As an added precaution, it is recommended that if staff cannot avoid moving from the quarantine to clean areas, they shower and put on new clothing before entering the resident colony (Abee et al., 2012).

4.6.2 Husbandry Practices During Quarantine

During quarantine, animals should be socially housed with compatible animals in a manner similar to the general housing of nonhuman primates outside quarantine. While there may be concerns about the spread of disease when socially housing animals, the treatment of any diseases detected is commonly performed on a group or room basis, regardless of whether the animals are singly or socially housed (Joint Working Group on Refinement, 2009).

If single housing is unavoidable and sufficiently justified to the animal care committee, the animals must be able to see and hear conspecifics, and single housing should be for the shortest duration possible (Joint Working Group on Refinement, 2009).

The environment provided must address the behavioural, psychological and physical needs of the animals (Joint Working Group on Refinement, 2009). In all types of housing, animals must be monitored for signs of stress or incompatibility with other animals, and visual barriers should be provided if necessary (Joint Working Group on Refinement, 2009).

When the primary enclosure is cleaned with water or steam, the animals must be removed unless the enclosure is large enough to ensure the animals will not be sprayed or distressed. Animals should be removed from the enclosure when disinfection products are used, and adequate rinsing is required after the use of potentially harmful chemical cleaning agents. If sanitizing items added to enclosures is difficult, disposable items such as hay, wood, cardboard and paper should be used (Joint Working Group on Refinement, 2009).

Consideration must be given to the frequency of cage changes necessary, in line with CFIA requirements when applicable. Any items that leave the quarantine area must be properly decontaminated.

4.6.3 Health Surveillance and Testing

A veterinarian or trained technician must observe the animals at least daily.

During quarantine, tuberculosis testing should be done 1-3 times, depending on where the animal originated, with at least 14 days between tests. It generally takes 4-6 weeks from the inoculation of tuberculosis to have a positive skin test (Capuano et al., 2003; Lin et al., 2006). When a CFIA permit is required, tuberculosis testing is defined in the regulations. A complete physical exam under sedation should generally be performed at the same time as tuberculosis testing throughout the quarantine period.

The animals should be tested for endoparasites and ectoparasites (fecal flotation or examination). Other tests to be considered include blood smears, serological testing, and rectal swab culture, with the animals treated appropriately and as needed, following veterinary recommendations. Preventative treatments will depend on the origin of the animal.

The administration of immunizations must not interfere with testing. For example, measles vaccinations interfere with tuberculosis responses. Therefore, if the animals have not been previously vaccinated for measles or there is no vaccination record, it may be preferable to provide immunization at the end of the quarantine period.

5

BREEDING

As stated in the [*CCAC guidelines on: procurement of animals used in science*](#) (CCAC, 2007), “in-house animal breeding colonies should only be established when absolutely necessary, and should be efficiently managed, consistent with anticipated need and the principle of reduction.”

Guideline 8

In-house breeding of nonhuman primates must be approved by the animal care committee and conducted in species-appropriate housing and conditions.

Breeding nonhuman primates in-house must be reviewed carefully by the institution’s animal care committee and approved only when: 1) it constitutes an improvement in the welfare of the animals over other sources; and 2) it is intended to meet very specific scientific objectives that would not be met otherwise. For example, in-house breeding may be a refinement over capture and transportation over long distances, or it may be necessary if the research requires very young animals or where the social structure is a component of the experiment. In the case of young animals, in-house breeding can permit studies to proceed without separating the young from their mothers. This may also be accomplished when conditions allow investigators to conduct their research at the location of the supplier.

In-house breeding must only be undertaken in institutions with appropriate infrastructure and with competent, experienced personnel who maintain high standards of nonhuman primate care. Breeding facilities must be suited to the ages of the animals and provide sufficient space, environmental complexity and control for the animals to be comfortable and have opportunity to express a range of species-typical behaviours (LASA and MRC, 2004). Successful breeding requires consideration of the species requirements and the nurturing experience of the breeding animals.

Breeding must be carefully managed to fulfill the objectives of the breeding program, prevent inbreeding, and ensure the numbers of animals are carefully controlled to meet the research requirements and prevent overpopulation. In-house breeding can provide greater control over housing and husbandry conditions than when animals are procured from other sources, and provide opportunities for socialization and training at a young age (Joint Working Group on Refinement, 2009). However, when facilities are too small or lack experienced personnel, problems can arise in balancing supply and demand (Joint Working Group on Refinement, 2009).

Nonhuman primates should be weaned without external intervention and allowed to remain in the colony until they become behaviourally independent (IPS, 2007; Prescott et al., 2012). The age of weaning varies with species (IPS, 2007); however, all nonhuman primates should be weaned as late as possible. Macaques should not be weaned before 10 months of age, depending on their weight, health and behaviour (Prescott et

al., 2012). In the wild, weaning is a gradual process. Weaning nonhuman primates too early can affect brain development and cognitive ability (Sanchez et al., 1998). It can lead to behavioural deficiencies (Prescott et al., 2012), negative behaviours indicative of fear and aggression (see Novak et al., 2012, for a review), and long-lasting changes in physiology and immunological responses (Prescott et al., 2012). The negative effects of early weaning can persist into adulthood (see Parker and Maestripieri, 2011, for a review).

HUSBANDRY

For general guidance on husbandry, see the [CCAC guidelines: Husbandry of animals in science](#) (CCAC, 2017). Additional guidelines and information of concern for nonhuman primates are provided in this section.

6.1 IDENTIFICATION

For importation of nonhuman primates into Canada, requirements for identification of animals specified by CFIA must be met (see Section 4.3, “Pre-Shipment Procedures”).

All nonhuman primates should be uniquely identified, using the least invasive method appropriate for the housing situation and intended studies. The advantages and disadvantages of various methods for identifying nonhuman primates are reviewed by the Joint Working Group on Refinement (2009). Methods, such as natural appearance, collar, dye and hair clipping, have minimal effects on the animals but are limited in their duration and reliability. Tattooing and microchipping are permanent methods; however, these are invasive and may require a second, more visible method of identification.

For invasive procedures, the use of local or topical anesthetic or general anesthesia must be considered. Additionally, training animals to present the body part where a microchip is to be read can eliminate the need for handling and restraint (Joint Working Group on Refinement, 2009).

6.2 HOUSING MANAGEMENT

The goal of housing management is to provide rich and stimulating conditions that will render the animals capable of adapting or modifying their environment to increase their resiliency, improve their psychological well-being, and reduce fear, anxiety and stress responses. This goal will be accomplished by providing opportunities for the animals to increase the time they spend in species-typical behaviours (thus reducing the amount of time spent in abnormal behaviours), while providing a safe environment to the animals and the humans working with them (see Section 12, “Human Safety”).

Housing management requires development of a management plan based on knowledge of the animal’s species-specific behavioural repertoire. Consideration must be given to such aspects as:

- social interactions and natural social groupings;
- control and complexity of their environment;
- feeding habits;
- space requirements; and
- use of physical objects.

Consideration must also be given to the effects of the position of the cage on the particular animals being housed in terms of social interaction, disturbance, etc. For example, some animals may benefit from having a view of activity in the corridor, while for others this may be a source of stress.

The effectiveness of the management program must be assessed regularly using quantitative measures to ensure species-typical behaviours are increased or sustained at a high level. There may also be a concurrent decrease in abnormal behaviours; however, this can be affected by other influences such as early social history. Examples of quantitative measures include time spent foraging and eating, incidence of stereotypic behaviours, and number and severity of incidents of aggression (also see Section 8, “Welfare Assessment”). Assessment of the housing management program should be a team approach, involving the veterinarian, behavioural specialists, facility manager, investigator, and research and animal care staff, as approved by the animal care committee.

Guideline 9

Any nonhuman primate injured as a result of the housing environment must be reported to the veterinarian and the animal care committee so that measures can be taken to address housing deficiencies and prevent recurrence of injury.

There should be an annual evaluation of the appropriateness of the housing system (including any environmental enrichment) by the veterinarian, as well as follow-up when incidents are reported.

6.2.1 Social Interaction

Guideline 10

All animals of social species should be pair or group housed in conditions that allow full contact with compatible animals.

Social housing is important for the welfare of nonhuman primates, and any other form of housing must be justified to, and approved by, the veterinarian and animal care committee. When animals are removed from social housing for a particular component of an experimental protocol (e.g., behavioural testing), it should be for the minimum duration required and approved by the animal care committee, and the animals should be returned to their housing promptly following completion of the session.

While there may be potential risks associated with social housing (e.g., disease transmission or problematic aggression), the benefits of social housing outweigh the risks. There is substantial evidence that social housing of nonhuman primates positively affects their welfare (e.g., Dettmer and Fragaszy, 2000; Reinhardt and Hurwitz, 1993; Reinhardt and Reinhardt, 1991; Eaton et al., 1994; Boccia et al., 1989; Coelho et al., 1991; Watson et al., 1998; and Gonzalez et al., 1982). The benefits of social housing include the ability to cope effectively with stress, demonstration of species-typical behaviours, absence of maladaptive behaviours, presence of a balanced temperament, and reduction of chronic stress (McNulty et al., 2004; Schapiro and Bushong, 1994; and Seelig, 2007 as cited in DiVincenti and Wyatt, 2011; Reinhardt, 1990). Social housing can have a positive impact on scientific studies, such as improving the sensitivity of detecting drug-induced effects (Xing et al., 2015). Experience has shown that the risks can be managed effectively, for example, by careful selection of compatible animals and providing sufficient space and environmental complexity.

Housing strategies, in order of preference, are generally group or family housing; pair housing; side-by-side caging that allows grooming contact; and single housing with visual, auditory and olfactory contact.

Anxious behaviour has been found to be lower in paired rhesus macaques that are allowed full contact, compared to singly housed controls (Baker et al., 2008). When full-time social housing is not possible, part-time social housing is preferred over single housing (Baker et al., 2014). Partial contact may provide some benefits of social housing, while allowing the animals choice and control (Lee et al., 2012; Baker et al., 2014). If animals are singly housed, they must not be housed in a room without conspecifics.

While social housing is very important to the welfare of nonhuman primates, it should be combined with additional housing management practices to further address the animals' needs and reduce the incidence of stereotypic behaviours (Eaton et al., 1994).

6.2.1.1 Establishing Compatible Pairs or Groups, or Introducing a New Animal

A detailed plan must be developed before creating a pair or group of nonhuman primates, or introducing an animal to an existing group, taking into account the natural social organization of the species and the characteristics of the individual animals (Capitanio et al., 2017; Truelove et al., 2017). In general, young animals (less than three years of age) will adapt to new cage mates, while older individuals may not accept new cage mates as easily.

For adults, the plan should include gradual introduction of the animals by housing them in close proximity with visual but no physical contact, and observation by staff for affiliative or aggressive behaviour. If the response is positive, the animals can be moved to a situation of protected physical contact (e.g., through wire mesh, grooming bars, perforated panels) with continued observation by staff. If the response remains positive, the animals can be introduced to full contact under direct observation by staff members who are ready to intervene and separate the animals if there are welfare concerns (minor displays of aggression should be monitored but may not require intervention). An animal should not be moved into the cage of another animal, but rather the animals should be introduced in a neutral environment or by opening a divider between their adjoining cages. See the Joint Working Group on Refinement (2009) for more information on developing an introduction strategy.

Under exceptional circumstances, to avoid single housing, compatible animals of the same genus but different species (e.g., *Macaca*) could be housed together (see DiVincenti et al., 2012; Rehrig et al., 2014).

Social housing of adult males with females or with other males raises consideration of strategies to prevent breeding and/or aggression. Males have shown a preference for female partners and the benefits of social housing can be provided without allowing breeding by administering birth control (e.g., implants or monthly prostaglandin injections to marmoset females) or by housing a vasectomized male with a female or group of females. Strategies to minimize aggression among males when housing them together include housing an intact male with a castrated male or group of castrated males, or housing an adult male with a juvenile male. The practice of cutting, blunting or filing canines must not be used as a means of addressing male aggression.

The location of animals held in separate cages within a room is important to limit the influence of dominant animals over more submissive animals. Visual barriers between certain animals may be helpful in minimizing this influence. Where nonhuman primates are group housed, they may require additional space per animal as well as multiple escape routes and structural barriers to allow opportunity to avoid any aggressive animals.

6.2.2 Feeding Habits

The housing management plan must accommodate the feeding habits of the animals and their health status. For example, if they normally forage on the ground, food should be provided in a manner that functionally simulates foraging on the ground (see Section 6.3.1, “Foraging”). The plan must also take into account the potential for dominance and competition among socially housed animals to ensure the needs of each animal are met.

6.2.3 Space Requirements

The management plan must also address the availability of space and furnishings to accommodate species-specific behaviours important to the animals. For example, appropriate space is provided for each animal to perform normal locomotor behaviour (e.g., walking, climbing, leaping) and sleep in a normal manner (e.g., animals that would normally sleep in trees should be provided with suitable perches).

6.2.4 Use of Objects and Control of Their Environment

The housing management plan should include the addition of objects for nonhuman primates to manipulate, suited to the species and age of the animals (e.g., puzzle feeders), as well as plans for rotation or replacement of objects to sustain interest (Lutz and Novak, 2005). Nonhuman primates respond favourably to objects that can be destroyed, such as pieces of wood (Lutz and Novak, 2005).

Any objects provided must be safe for the animals, and risks to the animals (e.g., injury, swallowing the object and intestinal obstruction) must be assessed.

Nonhuman primates benefit from the ability to exercise some degree of control over their situation (see a review by Rennie and Buchanan-Smith, 2006a). This includes control of their environment (e.g., noise), social interactions, and activities such as feeding.

6.3 NUTRITION, FEEDING AND WATER

Nonhuman primates must be fed a quality diet that is appropriate for the species and obtained from a reputable supplier. The quantity fed should be controlled as per recommendations of the National Research Council (NRC, 2003a) or other reliable source to ensure normal growth of young animals, maintenance of healthy body condition and prevention of obesity. Overweight animals are at greater risk of diabetes, cardiovascular diseases, muscle atrophy, and degenerative joint diseases (Schmidt, 2010). The use of feed should be based on manufacturer’s expiration and storage recommendations. Any change in diet should be done gradually.

Fresh fruits and vegetables should be given every day to supplement the basic ration, as appropriate for the species and age of the animals. Nonhuman primates should be given a daily source of vitamin C. Fruit juices are effective rewards during positive reinforcement training for most nonhuman primates.

The goal of the feeding strategy is to increase the amount of time animals spend foraging and eating, without increasing their caloric intake. Examples of such strategies include providing food that is difficult to access (e.g., feeding through bars, feeding treats frozen in ice, and using puzzle feeders), providing high-fibre/low-calorie forage items, and hiding small food items in the bedding substrate (Chamove et al., 1982; Joint Working Group on Refinement, 2009), in line with the health status, motor control and dexterity of the animal.

Food is a powerful motivator and can be used as a reward to positively reinforce desirable behaviours. Even the daily feeding routine can be incorporated as a training tool (e.g., having the animals come to the front of the cage to obtain food in order to accustom them to human presence in close proximity). In general, high calorie, high sugar foods should be avoided as treats for positive reinforcement.

Nonhuman primates should be provided with water *ad libitum* through either an automated system or water bottles (NRC, 2003a; Fortman et al., 2017), unless water is used as a reward during behavioural experiments (see Section 10.5, “Food and Fluid Intake Regulation”).

Animals that are new to an automated system or that may be experiencing physical difficulties must be carefully observed to ensure they are sufficiently able to access the water (Fortman et al., 2017).

For group housing, the feeding and watering strategy must be adapted to the social group so that submissive animals are not prevented from accessing food and water by dominant animals. For small groups, the number of access points for food and water should be the same as the number of animals. Consideration should also be given to the hierarchy in a group or room and whether the feeding schedule and delivery of food may induce aggression. Feeding structures and devices should be designed and situated to minimize food contamination.

6.3.1 Foraging

Guideline 11

Nonhuman primates should be provided with the opportunity to express foraging behaviour on a daily basis.

Feeding strategies that encourage nonhuman primates to spend long periods searching for, gathering and processing food are desirable. Nonhuman primates will spend a significant amount of time foraging if provided with the opportunity (McNulty et al., 2004), and there is evidence that this has a positive effect on their welfare (Bayne et al., 1991; Chamove et al., 1982).

Bedding and other floor substrates seeded with small foods can promote foraging behaviour among nonhuman primates (Doane et al., 2013). Removable drain baskets can be used to prevent the substrate from blocking drains (see [Husbandry](#) on the NC3Rs Macaque Website).

An animal's ability to forage may be influenced by its health status, and this should be considered in planning foraging opportunities. Additionally, any food consumed through foraging activities should be considered as part of the daily food allotment.

6.4 ENVIRONMENTAL ENRICHMENT

Environmental enrichment is a term used to refer to provision of stimulating and responsive environments (Shepherdson et al., 1998) above the basic conditions which meet the animal's physical, physiological and psychological needs. Modifications to an animal's environment should focus first on the species-specific needs of each animal (i.e. those physical and behavioural needs for which some degree of distress would occur in their absence), as well as on identifying any further improvements to the animal's environment that may provide positive welfare benefits (enrichment) (Weary 2012).

It is imperative to recognize and maintain standard conditions (i.e. the basic conditions that must be met for animals); however, further environmental enrichment must always be considered. Long-term housing should offer a variety of activities and resources for animals to explore.

An assessment must be made of the safety of any environmental enrichment in terms of the risk of injury, ingestion, etc. The animal's response to any initiatives should also be monitored to determine which initiatives the animal prefers, avoids or ignores.

Information on addressing the physical and sensory environment and cognitive capabilities of nonhuman primates is presented below. For additional examples, see [Enrichment](#) on the NC3Rs Macaque Website and Vernes and Louwerse (2010).

6.4.1 Addressing the Animal's Physical Environment

There is substantial evidence that nonhuman primates prefer the use of perches and elevated areas in cages (Clarence et al., 2006; MacLean et al., 2009; Reinhardt, 1992), and that these features can have positive implications for the animals' welfare (Neveu and Deputte, 1996). Sufficient perching space at different levels within the upper area of the housing unit must be provided to accommodate all of the animals (see Section 2.2.2, "Cage Design").

Additional items that may improve the animal's physical environment include:

- objects that can be manipulated and modified, mirrors, logs, branches, paper and cardboard boxes;
- swings, ladders, play and resting structures, porches, hammocks;
- pools;
- flooring substrate; and
- nest boxes.

Nonhuman primates have enhanced tactile sensitivity (see Hoffmann et al., 2004) and may benefit from objects and foraging opportunities that incorporate a variety of textures.

All objects must be safe and non-toxic. Natural substrates such as wood are preferred, and maple, beech and oak are all considered appropriate (Joint Working Group on Refinement, 2009). Any object or structure (e.g., perches) should be sanitized or replaced regularly to minimize risks associated with physical injury and microbiological burden, with consideration to their use for scent marking.

When providing such objects, the main goal is to sustain the animal's interest. Novelty is important; over time, animals may lose interest in items for which they had initially shown a preference (Taylor et al., 1997). Sustained interest can be achieved by:

- using objects the animals can manipulate and modify (e.g., destructible items such as paper, cardboard boxes or wood);
- introducing new objects regularly (rotation according to a schedule); and
- linking objects to strong natural behaviours, such as eating, foraging, grooming, or locomotion and postural movements.

6.4.2 Addressing the Animal’s Sensory Environment

At a minimum, the animals must be able to have visual, olfactory and auditory interaction with conspecifics (as noted in Section 6.2, “Housing Management”). Animals should also have the capacity to remove themselves from visual contact when they want by way of barriers and/or privacy panels (see Section 6.2, “Housing Management”). Submissive animals may require some privacy to comfortably eat and drink.

New World (South American) species rely on olfactory cues more than Old World (African and Asian) species. Natural material (e.g., wood) that will retain scent marks should be provided for New World species.

6.4.2.1 Music and Videos

The value of music and videos for nonhuman primates should be carefully evaluated. Some studies have shown that videos do not provide relevant benefits (Harris et al., 1999; Lee et al., 2011), while others have shown the animals to be attracted to videos (Andrews and Rosenblum, 2002; Bloomsmith and Lambeth, 2000; O’Neill-Wagner, 2005; Platt and Novak, 1997; Swartz and Rosenblum, 1980). Among the criteria to be considered are the species, sex, age and housing arrangement of the animals, as well as the type of video presented.

6.4.3 Stimulating the Animal’s Cognitive Capability

Cognitive tasks are an integral part of the management program, whether part of the research study or part of training for husbandry and veterinary care (see positive reinforcement training in Section 7.2, “Animal Training”). Further stimulating the cognitive capacities of nonhuman primates should be encouraged through tasks such as learning and memory tests and setting problems for animals to solve.

6.5 PHYSICAL ACTIVITY

Housing should offer the capability and stimulation for physical activity in the form of natural locomotor activities appropriate for the age of the animal and the animal model being studied. Cages should be of a sufficient size to allow exercise on an ongoing basis (see Section 2.2.1, “Spatial Requirements”). When nonhuman primates are housed in cages with limited space for extended periods, other opportunities for natural locomotor behaviour should be provided.

A larger and more complex play area may provide physical benefits and other behavioural improvements (Griffis et al., 2013); however, its impact must be assessed in terms of the effect on all nonhuman primates (i.e. those being given access to the area and those who remain in their home cages if the area is within sight). The larger area should be monitored to ensure it provides benefit to the animals, with modifications to the furniture, etc. as necessary to encourage physical activity (Joint Working Group on Refinement, 2009). The exercise area and training of the animals should be designed to facilitate the retrieval of the animals in a manner that is safe for the animals and personnel.

6.6 HUMAN CONTACT AND HANDLING

Animals should be approached in a calm, respectful, non-challenging and reassuring manner. This is particularly important when housing a new animal. Behaviour patterns in some nonhuman primates have been shown to vary in response to a direct or indirect gaze from people (Zou et al., 2015; Coleman and Pierre, 2014; Fox and Kalin, 2014; Capitanio, 1999).

It is important to understand affiliative and agonistic behaviours of the animals (e.g., lip smacking, avoiding direct eye contact) to encourage positive relationships between personnel and nonhuman primates. See [Behaviour](#) on the NC3Rs Macaque Website for examples of behaviours and facial expressions used by macaques in communication.

Habituation and desensitization methods are encouraged to help primates overcome fear of people and to prepare them for research and other procedures where they may be in close contact with humans (Clay et al., 2009). For example, treats can be distributed in a manner that leads to animals coming to the front of the cage when people are present. The use of training techniques with positive reinforcement can facilitate housing management (see Section 7.2, “Animal Training”).

6.7 ANIMAL MONITORING

All animals must be observed at least daily by competent staff² and any abnormalities must be reported to the veterinarian. Observations should be made of the animal’s behaviour, attitude and indirect measures of health, including feces, food intake and urine output.

For animals undergoing experimental procedures, plans must be in place to monitor the animals based on the particular procedures involved and the potential risks to the welfare of the animals (see Section 10, “Experimental Procedures”).

6.8 CLEANING AND SANITATION

All cages should be spot cleaned frequently and sanitized regularly to keep the environment dry and comfortable for the animals. The frequency of sanitizing will vary depending on factors such as the number, age and type of animals, the type of bedding and the size of the enclosure; cages are commonly sanitized every two weeks and pens every four weeks. The period for sanitizing can be extended if justified (for instance, during quarantine, or low housing density); however, it should not be extended on a regular basis, as doing so will make cages difficult to clean and sanitize.

Criteria for sanitizing pens depend on whether new animals will be introduced or the same animals will be returned to the pen. Thorough sanitization is required if pens will be used by different animals.

If it is necessary to have the animals present in the pens or cages during cleaning, the cleaning process (including the water temperature, cleaning agents, etc.) must not be harmful to the animals and care should be taken not to spray or otherwise disturb the animals. Precautions should be taken to minimize the creation of aerosols while spraying enclosures.

Objects within cages and pens should be sanitized with at least the same frequency as the enclosure. However, scent-marking is an important component of the natural behaviour of some species (e.g., marmosets), and cleaning procedures should be designed to minimize stress associated with disturbance of scent marks (Joint Working Group on Refinement, 2009).

² Competency is described in the [CCAC guidelines on: training of personnel working with animals in science](#) (CCAC, 2015).

The sanitation process should be monitored to ensure the required level of sanitation is achieved. It should be validated and verified regularly.

Objects and equipment leaving a nonhuman primate area must first be sanitized or covered. The sanitation method should be in accordance with the clinical situation. It should be designed to eliminate common parasites, gram-negative and gram-positive bacteria, and tuberculosis.

6.9 RECORD KEEPING

All animals must be clearly identified, with the protocol number to which they have been assigned, the name of the principal investigator, emergency contacts, and any other relevant information (including a brief summary of any experimental procedures) on sheets or cards posted on, or near, the enclosures.

Individual animal records must be kept, indicating such information as birth date, sex, origin, physical measurements, reproduction information, health information, medical history and nutritional history. Records of research activities must be kept, including complete records of all scientific and experimental procedures, as treatment in one study may influence the animals' response in a second study. A record of cage mates may also be useful to ensure the animal will be socially housed with animals that were confirmed to be compatible; however, these animals will still require monitoring (see Section 6.2.1, "Social Interaction").

Information gathered through welfare assessments, including details of health, behaviour and physiology (see Section 8, "Welfare Assessment") must be documented and retained in the animal's records.

7

ANIMAL HANDLING, RESTRAINT, AND TRAINING

7.1 HANDLING AND RESTRAINT

Handling or restraint should only be used when other options are not possible.

Guideline 12

Any handling or restraint technique must be safe for the animal and the handler and minimize stress.

The need to handle or restrain a nonhuman primate and the choice of method must be carefully assessed on a case-by-case basis. The following questions should be evaluated whenever handling or restraint is being considered:

- Is it necessary?
- What is the minimum degree of restraint required?
- What is the shortest duration of restraint required?
- What is the most appropriate technique for this particular situation?
- Is the technique safe for the animal and the handler?
- How can stress be minimized for the animals when using this technique?

The choice of technique will depend on the degree of training that the animal has undergone to become habituated to the technique, the size of the animal, and the particular situation for which handling or restraint is required. In all cases, stress for the animal must be minimized.

Guideline 13

Any handling or restraint technique should be introduced gradually through positive reinforcement training to minimize stress for the animals.

Habituation and/or desensitization with positive reinforcement will minimize stress for the animals and increase safety for personnel when handling or restraining techniques are required (Lee et al., 2012; Field et al., 2015). When prolonged physical restraint is required, periods of rest must be provided for the animal and the choice of procedure should ensure the animal is comfortable.

For animals that have not been trained to a restraint technique, chemical immobilization may be preferred over physical restraint.

7.2 ANIMAL TRAINING

The objectives of animal training are often to reduce stress experienced by the animals and decrease the risk of injury to both animals and personnel in relation to husbandry, veterinary and experimental procedures. Training can increase cognitive stimulation for the animals, increase the efficiency and ease of working with them, and be a source of enrichment.

Guideline 14

Positive reinforcement techniques should be used.

The use of positive reinforcement training is highly preferred over other forms of animal training. Positive reinforcement training is part of the operant conditioning learning model, which includes positive reinforcement, negative reinforcement, positive punishment and negative punishment.

This type of training uses positive reinforcement (introducing something positive (e.g., giving a food treat) on the performance of the correct behaviour to increase the frequency of its occurrence) and negative punishment (removing something positive (e.g., withholding the opportunity to earn a reward with a timeout) when an inappropriate behaviour is performed to reduce its frequency).

Training using positive reinforcement training is based on stimulus-response-reinforcement. The trainer asks for a behaviour using a signal (stimulus), the animal performs the requested behaviour (response), and then the animal gets something it wants (reinforcement). The stimulus can be a spoken word or a hand signal. This form of training can involve the use of a ‘secondary reinforcer’, such as a “click”, that is repeatedly paired with the primary reinforcement (e.g., food treat). For more information on positive reinforcement training for nonhuman primates, see Westlund (2015); Coleman et al. (2012); Whittaker and Laule (2012); Laule and Whittaker (2007); Rennie and Buchanan-Smith (2006b); Prescott et al. (2005); Prescott and Buchanan-Smith (2003); Laule et al. (2003).

Negative reinforcement (removing something negative on the performance of the correct behaviour to increase its frequency) teaches avoidance behaviour as the animal learns to avoid behaviours that elicit uncomfortable consequences. Because of the negative consequences involved, any use of this technique must be justified to, and approved by, the animal care committee. Positive punishment (introducing something negative when an inappropriate behaviour is performed to reduce its frequency) should not be used.

Positive reinforcement training has been used successfully to reduce the stress associated with procedures (Spiezio et al., 2015; Owen and Amory, 2011; Laule et al., 2003; Scott et al., 2003), to reduce stereotypic behaviours (Coleman and Maier, 2010; Baker et al., 2009; Bloomsmith et al., 2007; Laule, 1993), and to increase affiliative interactions between conspecifics (Schapiro et al., 2001).

7.2.1 Training Programs

Guideline 15

A flexible training program should be developed for each animal following standard procedures and tailored to individual differences in learning.

Training programs should allow progressive habituation to the environment and the practices and procedures. Training programs may contain several components: habituation to conditions, techniques and routine husbandry practices; and training for specific experimental, husbandry or clinical procedures or to influence behaviour in some other way (e.g., training for cooperative feeding). The training program should be developed based on the specific animal and the study requirements. Training programs should be documented and regularly reviewed (see Baker, 2016; Perlman et al., 2012; Prescott and Buchanan-Smith, 2007).

Habituation is thought to reduce stress for the animals and should be mandatory, except for very short protocols (i.e. if animals are to be kept less than one week). Basic training should aim to have the animal comfortable coming to the front of the cage, for example, to accept food treats from staff. However, more fearful animals will require a longer period of habituation to achieve this task (Clay et al., 2009).

Appropriate training for removing animals from a cage for husbandry and experimental procedures (McMillan et al., 2014; Bliss-Moreau et al., 2013; Bassett et al., 2003; McKinley et al., 2003) can increase the safety of personnel involved, and can make the use of larger cages easier to manage.

Training to cooperate during experimental procedures can provide an opportunity to greatly improve the welfare of animals (Rogge et al., 2013; Graham et al., 2012; Veeder et al., 2009; Scott et al., 2003). The type of study will determine the needs for specific training. For example, animals can be trained to come to the front of their cage, present a limb and remain stationary for dosing or blood removal procedures (see Coleman et al., 2008; Reinhardt, 2003).

There must be proper documentation of training, including individual records of progress. To be effective, training should be consistent. Repetition is key to learning in most cases (Fernstrom et al., 2009), and the patience of the staff during training is essential to ensure consistent progression for each animal (see Rennie and Buchanan-Smith, 2006b; Prescott et al., 2005; Laule et al., 2003).

There are individual differences in how fast animals can learn (Coleman, 2012; Coleman et al., 2005; McKinley et al., 2003) and these should be accommodated. If this is not possible within the limits of the scientific study, consideration should be given to moving the animal to another study.

7.2.2 Personnel Involved in Training

Guideline 16

Personnel conducting training must be competent, with demonstrated expertise.

To ensure animals are trained appropriately and effectively, personnel conducting training must be competent.³ It is expected that all personnel involved in handling animals are fully trained in positive reinforcement training techniques. Additionally, consultation with a professional trainer can improve the effectiveness of training programs.

In some cases, it may be appropriate to share training between research teams, veterinary staff and animal care staff. Training procedures should be guided by standard operating procedures to accommodate a team approach while maintaining consistency.

Training animals requires time and resources; however, time is often saved over the long term by having cooperative animals. For example, McKinley et al. (2003) showed that the time invested in training marmosets to voluntarily remain on a weighing scale was recovered within 8-20 sessions, Graham et al. (2012) showed that training nonhuman primates to cooperate reduced the time required for routine procedures such as blood collection, injection, urine collection, etc., and Veeder et al. (2009) showed that the efficiency of caring for mangabeys was increased when animals were trained to shift from one side of their enclosure to another to facilitate cleaning.

3 Competency is described in the [*CCAC guidelines on: training of personnel working with animals in science*](#) (CCAC, 2015).

WELFARE ASSESSMENT

Guideline 17

Nonhuman primates should be healthy; not be experiencing pain; and express a high prevalence and diversity of species-specific behaviours, with low prevalence and severity of chronic behavioural signs of anxiety, fear and aggression, and abnormal behaviours.

Welfare assessment evaluates the lifetime experiences of the animal, taking into account physical condition, psychological and behavioural well-being, environment, and experimental procedures (Wolfensohn et al., 2015), and should be performed throughout an animal's life. Welfare assessment should be conducted regularly (for instance, at the bi-annual physical exam) or when important changes occur (for instance, between successive studies or modification to the social housing).

Welfare assessment is a critical component of phenotyping nonhuman primate models of disease, recognizing that some disease models may impact socialization. It should also be used as part of humane endpoint monitoring. The assessment should inform any mitigation strategies that might be necessary to improve an animal's well-being and be used to determine whether an animal is appropriate for a particular research project.

The assessment should incorporate information noted in the following sections, which can be gathered from physical exams, daily observations, training and other activities. A team approach with good communication among those involved in the care and welfare assessment of the animals and the conduct of experimental procedures is important to assess the animal's quality of life (Lambeth et al., 2013). Wolfensohn et al. (2015) provide an example of qualitatively scoring the information for a number of the parameters identified, and Smith et al. (2006) provide examples of checklists that could be used.

8.1 HEALTH INDICATORS

Health indicators for nonhuman primates include, but are not limited to, the following: body weight, appetite, body condition score, coat condition, skin or mucosa condition, appearance of the eyes, gait pattern, presence of physical injuries/pathologies/diseases, presence of pain or inflammation, posture, body temperature, appearance of feces or urine, susceptibility to disease, and wound healing.

Body condition score provides a method of assessing the body fat and muscle of animals. Clingerman and Summers (2012) provide an example of a body condition score system in which a score of 1 indicates “emaciated”, 3 is “optimum”, and 5 indicates “grossly obese”; descriptions and illustrations are provided for each 0.5 increment on this 1-5 scale. When scoring body condition, it is important to be aware of variation between observers (Clingerman and Summers, 2012). While an optimum body condition score is the aim, in cases where animals are genetically lean a change in the body condition score may be a better indicator than a particular score.

Other indicators of health may be appropriate in particular situations. For example, growth rate can be used as an indicator of the health of young animals, and reproduction and infant care can be useful indicators of health when applicable. A number of [health indicators](#) are described on the NC3Rs Macaque Website.

8.2 BEHAVIOURAL INDICATORS

Behavioural indicators need to be considered on an individual level, relative to a baseline that has been predetermined for each animal following acclimation and prior to any study. A change in normal behaviour for an animal (e.g., a normally calm animal behaving aggressively) may indicate it is experiencing pain and/or distress.

Some animals are inherently more aggressive than others, and an assessment of aggressiveness should focus on the well-being of the individual animals involved (i.e. whether the aggression leads to anxiety, stress or injury), with an awareness that some aggression between animals may not affect the welfare of either animal. Honess and Marin (2006) review sources of variation in stress and aggression that should be taken into account when evaluating animal well-being.

Behavioural assessment requires that animals be observed both in the presence of an observer and remotely over a period of time. It is important to know if certain behaviours are induced or inhibited by the presence of an observer (for example, the gaze of an observer has been shown to affect behaviour patterns in cynomolgus monkeys (Zou et al., 2015)). Cameras can be useful for providing distance between the animal and observer or for making observations of the animal's behaviour after hours.

Each behavioural indicator should be evaluated on the basis of frequency, severity (mild, substantial, severe), and interference with other species-typical behaviours (Novak et al., 2012). Addressing the well-being of animals should increase the prevalence of affiliative behaviours, with a diversity of positive behaviours, and decrease the prevalence of negative behaviours. Novak et al. (2012) categorize negative behaviours as follows:

- whole-body stereotypies – bouncing, pacing, rocking, somersaulting and posturing;
- self-directed stereotypies – eye poking, over-grooming, self-grasping and self-sucking;
- appetitive stereotypies – coprophagy, regurgitation and urophagy; and
- self-injurious behaviours – hair-pulling and self-injury.

Negative emotional reactions, such as fear, anxiety, apathy, depression, and excessive aggression, can manifest as additional negative behavioural indicators. Fear and anxiety can be differentiated by behavioural expressions, postures and vocalizations (for example, intense fear is expressed through high-pitched screams).

Signs of pain in nonhuman primates include hunched or crouched posture, abnormal or slow gait, decrease in self-grooming, avoiding conspecifics, moaning, blank stare and/or glassy eyes, apprehensiveness or unresponsiveness, and refusing to eat and drink. As well, other animals are often attracted to animals experiencing pain.

Scoring systems have been developed to rank various behavioural indicators of animal well-being. For example, an evaluation system for hair loss can indicate compromised welfare through self-injury or negative behaviour with cage mates (Honess et al., 2005). See Bellanca et al. (2014) for an example of a scoring system

for hair loss. However, the scoring systems must be tailored to the species and be used on an individual basis, as indicators such as hair loss can have a number of physiological or psychological causes that need to be taken into consideration (Bellanca et al., 2014; Lutz et al., 2013; Novak and Meyer, 2009). The baseline can be employed to detect changes in the animal's well-being over time. When negative changes are observed, the veterinarian and/or behavioural specialist should be notified and a clinical case should be opened for the animal.

Scoring systems can also provide a useful measure of the effectiveness of an intervention or enrichment strategy. For example, a comparison with the animal's baseline score may indicate that a particular abnormal behaviour is less prevalent after an intervention. Scoring systems can also be useful in training new employees in recognition of changes in animal behaviour and relating observations to the baseline behaviour of the animal.

8.3 PHYSIOLOGICAL INDICATORS

Measurement of physiological indicators is generally more invasive than measurement of health or behavioural indicators, and should only be used to investigate welfare concerns or for situations where the measurements are being collected as a component of an animal care committee-approved study.

Physiological indicators may include body temperature, immunological functions (rates of lymphocyte proliferation or suppression of their activity, etc.), blood pressure, hematology and biochemistry blood analysis, heart rate, and levels of various 'stress hormones' (e.g., cortisol and noradrenaline). There is no single physiological indicator that can provide a clear measure of stress.

When physiological information is required, the least invasive method that will provide the necessary data should be used. Consideration should be given to obtaining samples from saliva (Hennessy et al., 1982 cited in Novak et al., 2012), hair (e.g., for cortisol (Davenport et al., 2006; Meyer et al., 2014)), urine and feces. Blood sampling from the ear, a low invasiveness procedure, is appropriate when a small amount of blood is needed (Lefevre et al., 2015).

HEALTH AND DISEASE CONTROL

9.1 CONTAINMENT OF ANIMALS

Nonhuman primates should be maintained in separate rooms on the following basis (Abee et al., 2012):

- species – New World species should be separated from Old World species; different species of New World nonhuman primates should be separated from each other; and different genera of Old World nonhuman primates should be separated; however, some species of macaques can be housed in the same room; and
- health – contagious animals or those suspected to be contagious should be separated from healthy animals, and animals with different health statuses should be separated.

Quarantine (see Section 4.6, “Quarantine and Acclimation After Receipt of Animals”) and sanitation (see Section 6.8, “Cleaning and Sanitation”) are important components of disease prevention.

9.2 IMMUNIZATIONS

Macaques should be vaccinated for measles prior to acquisition unless it will interfere with the intended research. If the animals were born in the institution, vaccination should occur at weaning and a booster administered at one year of age (i.e. within a few months).

If animals are housed outdoors, they should be vaccinated for rabies and tetanus; otherwise, these vaccinations are generally not necessary. Institutions should request that animals imported from outdoor facilities be vaccinated prior to acquisition.

Nonhuman primates may also be tested for hepatitis and vaccinated.

9.3 MONITORING ANIMAL HEALTH

All animals must be observed at least daily, as noted in Section 6.7, “Animal Monitoring”.

9.3.1 Physical Exams

Nonhuman primates must be examined upon arrival at an institution (see Section 4.5.1, “Examinations”). Routine physical examinations must be conducted at a minimum of once per year, and should be conducted two times per year (Weiss and Hampshire, 2015; Abee et al., 2012). Examinations may be required more frequently, depending on the condition of the animal (e.g., more frequently for animals on food and water regulation, aging animals). Physical examinations may be performed at the time of tuberculosis testing to minimize the number of interventions.

9.3.2 Testing

All animals should be tested for tuberculosis upon arrival at an institution, with follow-up testing every six months and a minimum of once per year. The frequency of testing should be based on the particular animal, conditions, and opportunity for tuberculosis to spread to other animals.

Appendix 2 lists tests that are available for use at the discretion of the veterinarian, depending on the species, condition and origin of the animals. Some of these tests are required by the Canadian Food Inspection Agency prior to importing a nonhuman primate.

9.4 PRECAUTIONS FOR PERSONNEL IN PREVENTION OF DISEASE TRANSMISSION TO ANIMALS

The risks associated with transmission of disease from personnel to animals should be evaluated by the veterinarian (for precautions to prevent disease transmission to humans, see Section 12, “Human Safety”).

To prevent disease transmission from humans to animals, personnel should wear gloves, a face mask, long-sleeved lab coat or coverall and dedicated shoes or shoe covers. Equipment dedicated to particular animals or within particular rooms or areas should be used strictly as intended. Additional precautions, such as increased respiratory protection (e.g., properly fitted N95 respirator), should be taken, based on risks to the animals.

Tuberculosis can have devastating effects on nonhuman primates. Staff should be assessed at least once per year for tuberculosis in consultation with the occupational health physician. The assessment will depend on the risk that the person has been exposed to tuberculosis, whether they have been vaccinated, etc. If a person has been vaccinated against tuberculosis, a skin test may yield a false positive and the quantiferon test is more appropriate (see the [Centers for Disease Control and Prevention](#) for more information).

Anyone potentially carrying a contagious disease (e.g., measles, influenza) should not enter the animal facility.

10

EXPERIMENTAL PROCEDURES

Animals involved in experimental procedures should be socially housed and have their needs addressed within the constraints of the study. As noted in Section 1, “Introduction”, promotion of good animal welfare leads to animals that are likely to be less stressed and more likely to exhibit normal behaviours and physiology (Poole, 1997). Animals that experience stress can have altered blood chemistry, behaviour, etc. (Shively and Willard, 2012; Parker and Maestripieri, 2011), and this can have significant impacts on research results.

Decisions on the involvement of individual animals in particular studies must consider the overall level of pain and distress experienced during the animal’s lifetime, and should ensure each animal is only assigned to one study with the potential to cause significant pain or distress.

Prior to conducting experimental procedures, endpoints must be established. The investigator should consult with the veterinarian and the animal care committee (see the [*CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing*](#) (CCAC, 1998) as well as the *Association of Primate Veterinarians Humane Endpoint Guidelines for Nonhuman Primates in Biomedical Research* (APV, 2010)). As noted in Section 8, “Welfare Assessment”, a team approach with good communication among those involved in the care and welfare assessment of the animals and in the conduct of experimental procedures is important to assess the animal’s quality of life, in consideration of the established endpoints (Lambeth et al., 2013).

Guideline 18

The least invasive methods must be used, taking into consideration the particular animals involved and the research objectives.

In designing experimental procedures, efforts must be made to minimize any pain and/or distress experienced by the animals.

Guideline 19

Category of Invasiveness E procedures must only be permitted for research related to public health emergencies.

Category of Invasiveness E procedures (i.e. procedures with the potential to cause severe pain) (CCAC, 1991) may be permitted only for research on infection and immunopathology associated with high-consequence pathogens, such as Ebola virus (Estes et al., 2018; Hart et al., 2015; Shurtleff and Bavari, 2015). Since the physiology and immune system of nonhuman primates are similar to humans, infection from these pathogens closely resembles disease progression in humans. Hence, they are highly recognized as the most

suitable model for studying pathogenesis and evaluating potentially effective treatments for these types of diseases.

Guideline 20

Animals should be trained for experimental procedures, including removal from housing and restraint where these are necessary components of the procedure.

Training animals can contribute to better scientific data. Animals that are trained exhibit fewer signs of stress and have different blood chemistry than those that have not been trained (Schapiro et al., 2005; Coleman et al., 2008; Coleman et al., 2012). Stress can also affect the immune system of the animals.

The acclimation period described in Section 4.6, “Quarantine and Acclimation After Receipt of Animals”, consists of two phases that occur prior to initiation of experimental procedures. The first phase is for rest and health assessment, while the second phase permits training and some preparation of animals for experiments. Training should be incorporated into daily activities and procedures, rather than only be conducted as a separate event.

10.1 ADMINISTRATION AND REMOVAL OF SUBSTANCES

Selection of procedures for the administration and removal of substances should be based on the particular substance involved, the frequency of administration and the period over which the procedure is to take place. For example, cage-side dosing of trained animals can be a less invasive alternative to gavage; however, it requires training and may not be appropriate for studies that involve dosing for a very short period. An osmotic pump or access port may be an alternative when administering substances over a long period, but their use will depend on the amount of substance to be delivered. Additionally, catheters may be appropriate for some studies where animals are dosed more than once per day, but their installation requires invasive surgery and tethering the animals.

Telemetry devices may provide an option for capturing physiological data (e.g., electrocardiogram, respiration rates, and blood pressure). However, their use is generally applied to long-term studies.

Only the minimum amount of substance required for the analysis that will be performed should be used. For considerations in administering and removing substances, including selection of appropriate routes and volumes, see Turner et al. (2011) and Diehl et al. (2001).

10.2 CRANIAL EXPLANTS AND IMPLANTS

Explants and implants must be made of biocompatible material and adapted to each animal. The number and invasiveness (level of penetration) of electrodes should be minimized in line with the objectives of the study, and justified to and approved by the animal care committee. The use of imaging technology to insert electrodes is encouraged to increase the accuracy of placement and minimize invasiveness.

Cranial explants require regular cleaning and routine maintenance of the margins; however, the animals can still be housed socially.

Electrophysiology implantable technology is advancing rapidly and potential new refinements should be evaluated in terms of effectiveness and implications on animal welfare through pilot studies.

10.3 DISEASE MODELS

Animals that are models for diseases must be given the same consideration for housing management as other animals, particularly for social interaction, including when they are housed under biocontainment conditions (see Section 6.2, “Housing Management”). In some cases, additional observations and/or modification of the animal’s environment, feeding regime, etc. may be required to address the needs of the individual animals. Welfare assessment (see Section 8) should be tailored to the particular animals involved and used to determine the welfare state of the animal and any mitigation strategies that may be required.

Endpoints must be developed based on the particular disease model and should be informed by the welfare assessments. A literature review for the particular model should be conducted, as several scoring systems have been developed that may assist in establishing endpoints, monitoring animals and designing mitigation strategies (e.g., see Henry et al., 1999, Imbert et al., 2000, and Doudet et al., 2004, for clinical rating scales of nonhuman primate models of Parkinson’s disease).

10.4 GENETICALLY MODIFIED NONHUMAN PRIMATES

Guideline 21

Welfare assessment, monitoring and mitigation measures should be in place to minimize any adverse welfare impacts of genetic modification.

General principles for generation, acquisition, welfare assessment and experimental procedures for all genetically modified animals are detailed in the [CCAC guidelines on: transgenic animals](#) (CCAC, 1997). Before genetically modified animals are brought into an institution, the protocol for their use and/or further propagation must be approved by the animal care committee.

As with any genetically modified animal, there should be documentation that includes the genotype and/or information about the means of identifying genetically modified animals from wild-type or other animal lines, along with information on welfare concerns and mitigation strategies, if available. Of particular concern for nonhuman primates is any potential impact of a modification on socialization and the rearing of offspring by the mother.

The selection of methods used to generate new genetically modified lines should be made with consideration of the Three Rs, as some techniques are more efficient. Procedures for the generation of genetically modified animals should be reviewed by the animal care committee, in keeping with the rapidly evolving nature of genetic modification and advances in research on animal welfare. Submission of protocols to the animal care committee for renewal should include a report on the efficiency of the methods used to produce new strains.

Advancements in gene-editing technology (i.e. zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9 (CRISPR/Cas9)) have improved the specificity and efficiency of genetic modification and

enabled the development of nonhuman primate models of disease (Izpisúa Belmonte et al., 2015; Jennings et al., 2016; Okano and Kishi, 2018; Sato and Sasaki, 2018). In particular, nonhuman primate models of disease are emerging in the fields of mental health (e.g., autism, schizophrenia, bipolar disorder) and neuro-degeneration (e.g., Alzheimer's disease and Parkinson's disease), as many clinical signs of these diseases are related to cognitive function and social behaviour, both of which are more developed in nonhuman primates than rodents (Jennings et al., 2016). Additionally, nonhuman primate models have the potential to address the limited translational success of rodent models and advance understanding of human brain disorders, because of greater similarities with humans in brain anatomy and physiology (Belmonte et al., 2015) and metabolism (O'Sullivan et al., 2013).

Sato and Sasaki (2018) note that desired phenotypes do not always appear due to mosaicism (a mixture of wild-type and mutant gene sequences resulting from partial genome editing in the embryo); the long lifespan of nonhuman primates makes the procedure used in mice to generate genetically modified animals from mosaics through backcrossing less feasible. Therefore to minimize the number of animals produced, it is important to verify gene editing tools prior to producing nonhuman primate models (Sato and Sasaki, 2018). The sampling method for genotyping should be the least invasive method that can provide the quantity and quality of tissue required for the particular genotyping method being used.

Close attention to phenotyping nonhuman primate models of disease is critical, and there needs to be awareness that such models may give rise to situations where socialization is difficult.

Genetically modified nonhuman primates may respond differently to drugs and feed, as well as a number of experimental conditions, when compared to nonhuman primates that have not been genetically modified. 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 use of the nonhuman primates for testing new drugs or in toxicity studies.

In addressing the Three Rs, it is important to maximize the information obtained from each animal and minimize unnecessary duplication of studies. This requires sharing data and cell or tissue samples where appropriate, and participation in archiving strategies at both national and international levels (Jennings et al., 2016). While this applies to all animal research and the need to minimize the numbers of animals, it is particularly important for primate research due to the logistics of maintaining primates and their relatively long lifecycle (Jennings et al., 2016).

A further challenge in working with genetically modified nonhuman primates is the limited opportunity for those involved to develop the necessary skills and expertise in the all areas related to the care of the animals and the conduct of procedures (Jennings et al., 2016).

10.5 FOOD AND FLUID INTAKE REGULATION

In some experimental protocols, food or fluid intake is regulated to motivate nonhuman primates to perform specific cognitive or behavioural tasks. However, there is no single procedure for food or fluid intake regulation suitable for all animals and all experiments. Each application of food or fluid intake regulation must be thoroughly described and scientifically justified, and be approved by the animal care committee. Records must be kept of the procedures (e.g., the time when regulation began and when it is due to end, after which time food or fluids must be provided) and the health and well-being of the animals.

Social housing of animals on protocols should be maintained. Animals should only be separated temporarily when receiving their ration.

Endpoints (when the food or fluid control will be relaxed or the animal removed from the study) must be approved by the animal care committee, and should be agreed upon in advance with the veterinarian.

For a review of experimental and animal welfare considerations, see Prescott et al. (2010), Toth and Gardiner (2000), and the National Research Council (2003b), from which the recommendations below have been taken.

Guideline 22

Food or fluid intake regulation should be used only if alternative means of motivation involving positive reinforcement (e.g., food treats or social stimuli) cannot meet the same experimental objectives of the study.

Regulation based on the timing of providing food or fluid is preferred to regulation based on reducing the intake of food or fluid, and food intake regulation is preferred to fluid intake regulation, as it has less potential for adverse health consequences.

For some training or experimental protocols, sufficient motivation may result from regulating the time when food or water is provided. For example, some animals may respond to training during the morning using a food reward as part of their daily ration, and then be provided with the remainder of their ration in the afternoon. However, animals should not be fasted during training.

For some studies, food rewards may not be suitable, for example, in studies involving electrophysiological recording where movement of the jaw from chewing can interfere with experimental results, or in studies where the time span of events is very short and requires timely delivery of rewards (Prescott et al., 2010). However, justification for any proposed use of fluid intake regulation must be provided to the animal care committee.

Guideline 23

For each food or fluid intake regulation protocol, the minimum level of regulation for each individual animal that will produce the required behavioural performance for the experiment and maintain the animal's health must be used.

Animals should be evaluated on an individual basis for reward preference, normal behaviour, capacity to perform a task, weight loss or weight gain, health status and food or fluid daily requirement.

Food or fluid intake regulation protocols must only involve healthy animals. The individual capacity of an animal to learn a specific task must be taken into consideration. A proper step-by-step training approach is essential (see Section 7.2, “Animal Training”). Steady progression through the steps of the training program is important to success; food or fluid regulation must not be used to force an animal to learn faster and omit some of the steps.

Three important aspects should be considered:

- the requirement for, and appropriate level of, regulation of food or fluid intake;
- any welfare consequences of food or fluid regulation on the animals; and
- the need for physical and psychological health assessments of the animals, and continuous daily monitoring.

Guideline 24

Food or fluid intake regulation must meet the animal's individual needs.

Caloric and other nutritional requirements (i.e. vitamins, minerals) should be met daily. Body weight and hydration status should be monitored, with the food or fluid schedule adjusted as necessary. Work on the control of drinking has shown that blood osmolality is a good predictor of a nonhuman primate's hydration state, and some estimates on the level of fluid at which an animal's thirst is abolished is available (e.g., Madison et al; 1980; Wood et al., 1980, 1982; Yamada et al., 2011).

The amount of food or fluid sufficient for each individual animal should be determined over a period of several days. Ad libitum food and fluid intake varies greatly between animals (e.g., Feldman et al., 1960; Robbins and Gavan, 1965; Hamilton, 1972; Oikawa et al., 1982; Suzuki et al., 1989; see also Newsome and Stein-Aviles, 1999). In addition, the amount of fluid taken ad libitum is generally greater than the amount needed for the animal to stay healthy (see Prescott et al., 2010).

Establishing the amount of food and water animals consume can be difficult, especially in social housing, and close monitoring of the animals is critical. For pair-housing, an average consumption level for the pair is often used. Calculations also need to take into account the tendency for particular animals to waste water.

Re-evaluation of basic nutritional and water requirements of growing monkeys should be done according to a defined schedule based on the species and age of the animal. Food or water regulation should not negatively impact growing individuals.

Fresh fruit and/or vegetables should be provided on a daily basis regardless of whether an animal is on food or fluid restriction. When feasible, the amount provided should be kept constant when an animal is on food or fluid restriction.

Guideline 25

Food or fluid regulation should be introduced gradually from ad libitum so that the animals have sufficient opportunity to adapt.

Transfer from ad libitum to regulation of food or fluid intake should be performed gradually over several days on a schedule for endogenous homeostasis to take place, to allow the animal to adapt and to minimize any health effects.

Guideline 26

Any animal on food or fluid regulation must be closely monitored.

Individual medical records must be maintained in the animal facility and include the following measures of hydration:

- body weight;
- skin turgor;
- food and water consumption data and trends;
- daily observation sheets, including fecal and urine output and consistency; and
- behavioural assessments with deviations from normal noted.

Prior to a planned major surgery, animals should be returned to normal feeding and fluid intake. In the event of minor or emergency anesthesia or surgery, fluids should be given.

An animal suffering from an illness (e.g., fever, diarrhea) or meeting the endpoint criteria established prior to the study must be removed from the food or fluid regulation program until recovery.

Supplementary information on implementation, monitoring and termination for food and fluid intake regulation is provided in Appendix 3.

10.6 ANESTHESIA AND ANALGESIA

Records must be kept each time an animal is sedated. Decisions about the number and frequency of sedations performed on an animal should be made in consultation with the veterinarian and take into consideration the health and well-being of the individual animal. To minimize the number of sedations, it may be appropriate to conduct some elective health assessments when animals are sedated for experimental procedures.

10.6.1 Anesthesia

10.6.1.1 Pre-Anesthesia

The type and dosage of anesthetic must be tailored to the need for restraint and/or pain mitigation, the objectives of the research, examination, etc., and the species and body weight of the particular animal, so that it provides sufficient relief for the animal. The minimum dosage required to induce and subsequently maintain a sufficient level of sedation or anesthesia to perform the procedure safely should be used. Local anesthetic agent infiltration should be used for minor procedures and used in combination with general anesthesia during more invasive surgeries.

Prior to administering anesthetics for experimental procedures, animals should undergo a thorough health assessment and an accurate determination of body weight. Medications should be dosed based on the animal's most recent weight (Oliveira and Dimitrov, 2008). An emergency kit should be available close at hand, and doses ideally pre-calculated to minimize errors in the event of an emergency.

Fasting requirements must take into consideration the size, age and health status of the animal, as well as the time of day the procedure will take place and the anesthetic to be used. For most nonhuman primates, food should be withheld overnight or for a minimum of four hours prior to anesthesia to reduce the risk of vomiting and aspiration. Special consideration should be given to young animals and small species such as marmosets and squirrel monkeys, which can rapidly become hypoglycemic, to ensure the period of fasting is not too long.

Water should not be withheld. Animals on fluid intake regulation should be gradually brought to ad libitum intake and their hydration status should be determined prior to pre-anesthesia.

After pre-anesthetic agent injection, the animal should be kept in a restricted area to prevent the risk of injuries caused by falling.

10.6.1.2 Monitoring

Suitable equipment must be available to monitor the animals' vital signs; however, trained personnel should also be present to provide careful observation of the animal's breathing, heartbeat, etc. and to complete monitoring records.

Monitoring anesthetized animals in a magnetic resonance imaging environment will require particular consideration. The anesthesia and monitoring equipment must be compatible with the magnetic environment and/or be located at a safe distance to avoid malfunction and risk of injuries to the animals and personnel.

An anesthetized animal should be visually monitored continually until recovered from anesthesia (i.e. able to assume and maintain an upright position).

Animals should be kept warm by providing a heat source until they recover from anesthesia.

10.6.1.3 Applications for Anesthesia

Anesthesia is used to chemically restrain an animal and to alleviate pain. For chemical restraint, the aim is to control the movement of the animal, not to alleviate pain. This may be required for physical examinations, tuberculosis testing, imaging purposes, etc.

The use of neuromuscular blocking agents must be scientifically justified and approved by the animal care committee. They must always be used in conjunction with general anesthesia. Their use is generally limited to experiments for which total immobility or the control of the breathing with a respirator is required (e.g., for magnetic resonance imaging). The general anesthesia technique, dose and regimen and the non-motor parameters used to monitor the anesthesia depth (e.g., blood pressure, heart rate, electroencephalography) must be validated first without the use of the neuromuscular blocking agent. The validation must be for a period that exceeds the maximum duration of the effect of the neuromuscular blocking agent, to ensure the animals will always remain under general anesthesia.

For surgery, the aim of anesthesia is to alleviate pain and render the animal unconscious. The concept of balanced anesthesia is applied to provide a combination of drugs, often at lower doses than if each was used alone, that maintains anesthesia for a defined period with reduced undesirable side effects.

Injectable anesthesia is usually used alone for short, non-invasive procedures or prior to use of gaseous anesthesia for smooth and rapid induction and to facilitate intubation.

An endotracheal tube is recommended for maintaining open airways. Preparation and procedures for intubation must be determined in advance based on the particular animal. Cuffed endotracheal tubes are preferred as they reduce the possibility of aspiration of saliva or stomach contents; however, inflation of the cuff of the endotracheal tube must be kept to the minimum needed to obtain an airtight fitting, to avoid wounding the trachea. A local anesthetic agent should be applied on the arytenoids prior to intubation. The endotracheal tube should be removed (after deflating its cuff, if used) as soon as the animal shows signs of impending arousal. Endotracheal tubes should be sterilized between animals to prevent disease transmission.

The use of gaseous anesthetic requires well-maintained equipment with a gas scavenging system or filter. Procedures should ensure appropriate flow rates are used and precautions are taken, such as the use of ophthalmic ointment to prevent dryness of the eyes and damage to the cornea.

10.6.2 Analgesia

Analgesia should be used for any procedure that would be expected to be painful; if there is uncertainty regarding whether a procedure may be painful, analgesia should be used. Analgesia should be used pre-emptively for procedures that are expected to cause pain. For surgical procedures, preemptive analgesia should be applied pre-operatively and until 72 hours after the surgery; additional analgesia should be provided as required to keep a stable level of comfort for the animal. Using a combination of analgesics is often more effective than a single agent (e.g., combining meloxicam and tramadol, or carprofen and buprenorphine with local anesthetic). The use of local analgesia (e.g., local infiltration of lidocaine and bupivacaine) is safe and generally encouraged in nonhuman primates to ensure a multimodal approach.

Animals must be monitored a minimum of once a day, and more frequently with invasive surgeries, by a veterinarian or veterinary technician to detect changes in behaviour indicative of pain and distress (see Section 8, “Welfare Assessment”). Animals that appear to be in pain must be reported to the veterinary staff and investigator.

10.6.3 Controlled Substances

Some anesthetics and analgesics are controlled substances and their use requires adherence to the *Controlled Drugs and Substances Act* (Government of Canada, 1996) and applicable regulations.

10.7 SURGERY AND POST-OPERATIVE CARE

10.7.1 Surgical Facilities

Major surgeries⁴ must take place in an aseptically prepared surgical suite with appropriate layout and adequate temperature, ventilation rate and pressure differentials (see the [CCAC guidelines on: laboratory animal facilities—characteristics, design and development](#) (CCAC, 2003), Section 3.3, “Surgery”, and Section 12, “Environment”).

⁴ Major surgeries involve invasion of a body cavity or involve procedures with the potential for significant complications.

Minor surgeries, such as wound repair, can be performed in a procedure room.

In defining endpoints in relation to a surgical procedure, consideration must be given to the recognition of pain and wound infection (Fante et al., 2007).

10.7.2 Animal Preparation

Guideline 27

Prior to surgery for experimental procedures, animals should be healthy.

Preparation of animals for major surgeries should be done in the preparation room of the surgical suite at the time of the procedure (Hranjec et al., 2010; Mangram et al., 1999; NICE, 2008).

The prepared area must be large enough to allow extension of the incision if necessary (Mangram et al., 1999). Hair should be removed using clippers and the site should be thoroughly cleansed with an appropriate surgical preparation procedure (Mangram et al., 1999; NICE, 2008; Hemani and Lepor, 2009).

Analgesics should be administered prior to surgery, allowing enough time to reach maximum effect before the surgical stimuli. Prophylactic antibiotic therapy could be used if recommended by the veterinarian.

10.7.3 Surgical Team Preparation

Guideline 28

All members of the surgical team must be competent in their required tasks, be informed of the details of the surgery plan in advance, and rigorously adhere to the principle of asepsis to prevent surgical site infections.

Training and assessment on the theory and practical aspects of surgical procedures are required to ensure competency of individuals (see the [CCAC guidelines on: training of personnel working with animals in science](#) (CCAC, 2015)).

Members of the surgical team must perform a pre-operative surgical scrub using proper technique and appropriate antiseptic procedures (see Mangram et al., 1999; NICE, 2008; and Tanner et al., 2016), prior to putting on a sterile gown and gloves. Surgeons should wear clean scrub suits, surgical masks or N95 respirators (according to risk), protective eyewear, surgical caps or bonnets, surgical gloves and sterile surgical gowns (Mangram et al., 1999; NICE, 2008). Surgical gloves must be changed between animals, and surgical gowns, etc. changed when they are considered non-sterile.

10.7.4 Surgery

Guideline 29

All surgical procedures must be well planned in advance, taking into account the individual physical characteristics of the animal involved.

Surgeries must be planned and performed in accordance with the following guiding principles:

- the least traumatic approach (based on anatomical structures) should be used (i.e. the smallest incisions enabling minimal disruption of normal tissues, organs, blood vessels, and nerves);
- aseptic technique should be used;
- appropriate equipment should be used;
- the most biocompatible material that will promote healing and reduce inflammation, dehiscence, necrosis, or infection should be used; and
- surgical and anesthesia time should be the shortest practical, which requires proper training of the surgeons, pre-surgery review of the anatomy and surgical approach, and preparation of all the necessary equipment and supplies.

Selection and preparation of surgical instruments must be undertaken prior to the day of surgery. The instruments that will be used in surgery must be sterilized by steam or gas sterilization, or by immersion in liquid sterilant prior to surgery and between animals. At time of use, instruments must be innocuous for the tissue (e.g., not too hot to avoid burning and well aerated or rinsed to avoid chemical burns). All required medications should be prepared in advance of the surgery (see Section 10.6.1, “Anesthesia”).

During surgery, measures should be taken to ensure:

- tissues are handled gently and are not allowed to dry;
- homeostasis (including cardiopulmonary stability) is effectively maintained;
- devitalized tissue and foreign bodies (i.e. sutures, charred tissues, necrotic debris) are minimized; and
- there is good apposition of tissues at the surgical site to ensure proper healing and reduce the risk of a seroma (accumulation of interstitial fluid) and post-operative infection.

Investigators must consult the veterinarian and keep up-to-date with the literature describing advancements in techniques and materials, and evaluate their application. The general standards for surgery outlined in the *CALAM Standards of Veterinary Care* (CALAM, 2007) must be followed.

10.7.5 Intra-Operative Care and Monitoring

Guideline 30

A plan for monitoring the animal during surgery must be in place.

The level of invasiveness and the length of the procedure will dictate the necessary precautions and monitoring required. It is important to maintain the animal's core body temperature near normal (Hranjec et al., 2010). Hydration and blood pressure must be maintained by intra-operative administration of physiological fluids.

A trained person should be dedicated to maintaining and monitoring an anesthetized animal. Monitoring of these animals could include body temperature, mucosa colour, heart rate, respiratory rate, oximetry, carbon dioxide, blood pressure, and electrocardiogram, depending on the type of surgery (see Section 10.6, “Anesthesia and Analgesia”). Monitoring should be continuous and recorded for later consultation, if needed.

10.7.6 Post-Operative Care and Monitoring

Guideline 31

Post-operative care and monitoring must be planned based on the invasiveness of the procedure and the individual needs of the animal, and adapted to unforeseen situations.

Animals under anesthesia must be monitored constantly until they recover from the anesthetic and are able to sit by themselves. After surgery, animals should also be carefully monitored for cardiopulmonary stability and maintenance of body temperature, as well as other health indices particular to the procedures performed (e.g., the occurrence of seizures in the case of cranial surgery (Oliveira and Dimitrov, 2008)), and an intervention plan should be in place to address any concerns.

The recovery area should be safe and comfortable for the animals. Animals should be returned to their social groups as soon as possible, or at least have visual, olfactory and auditory contact with conspecifics. The first meal after anesthesia should be approximately half of the regular portion to prevent bloating.

Following surgery, sterile saline can be used to cleanse the wound. Topical antimicrobial agents should not be used for cleaning surgical wounds that are healing by primary intention. See NICE (2008) and Mangram et al. (1999) for further information on post-operative incision care.

Proper anesthesia and/or analgesia must be maintained during the post-operative period to minimize pain and/or distress (see Section 10.6, “Anesthesia and Analgesia”). A veterinarian must be consulted to adjust the dose or provide other treatments. Prophylactic antibiotics can be continued post-operatively, as recommended by the veterinarian.

The animal should be monitored closely for appetite, water intake and general behaviour, at a frequency appropriate to the extent of the surgery and condition of the animal to ensure the animal's pain level is managed until the animal becomes stable.

11 END OF STUDY

11.1 EUTHANASIA

As noted in the [*CCAC guidelines on: euthanasia of animals in science*](#) (CCAC, 2010), “methods for euthanasia of animals, including emergency euthanasia, must be submitted for review and approval by an animal care committee”. In addition, the use of each animal should be maximized, for example by harvesting blood and tissues and coordinating their distribution to interested researchers in advance of euthanasia. Where there is not an immediate use for blood and tissues, they should be frozen for future studies and their availability communicated through research networks (Joint Working Group on Refinement, 2009).

All personnel responsible for carrying out euthanasia must be knowledgeable and competent to perform the procedure in a compassionate, professional, and appropriate manner that avoids distress to the animals. See the [*CCAC guidelines on: euthanasia of animals used in science*](#) (CCAC, 2010) for guiding principles and recommendations on euthanasia. Acceptable methods of euthanasia for nonhuman primates are intravenous injection of an overdose of barbiturates following sedation, and overdose of inhalant anesthetics followed by another method to ensure death (CCAC, 2010).

The death of any nonhuman primate imported into Canada (including during transit or in quarantine) must be reported to CFIA with a request for a licence to transport and dispose of the carcass. As with other animals, the institution is responsible for ensuring suitable carcass disposal methods and services are available and comply with federal, provincial or territorial and municipal regulations.

11.2 REHOMING

If nonhuman primates are to be moved to another research institution or a retirement facility, there must be assurance that the housing and husbandry requirements set out in this document will be met in full through a CCAC or other internationally recognized accreditation process.

Transportation of a nonhuman primate that was imported into Canada to another facility requires a licence from CFIA.

11.2.1 Relocation to Another Research Institution

In line with the Three Rs, reduction of animals in research may be achieved by assigning animals to other studies when appropriate and approved by the animal care committee. Such decisions must consider the overall level of pain and distress experienced during the animal’s lifetime, and should ensure each animal is only assigned to one study with the potential to cause significant pain or distress.

Any plans to relocate an animal to another research institution must consider whether the animal’s welfare will be compromised by the transportation process or the conditions at the new facility.

11.2.2 Relocation to a Retirement Facility

Decisions to relocate nonhuman primates to retirement facilities must be undertaken on a case-by-case basis and be based on the welfare of each individual animal. Relocation must follow an established procedure that takes into account the animal's age, health and previous experience, as well as the conditions under which the animal will be kept (Joint Working Group on Refinement, 2009). Nonhuman primates should never be moved to a facility where there is not a compatible conspecific (Prescott, 2006b).

There must be assurance that any proposed retirement facility is financially secure and able to properly care for the animal throughout its lifespan (Prescott, 2006b). Institutions must follow up on any relocation process to ensure the animal's welfare has not been compromised (Prescott, 2006b).

12

HUMAN SAFETY

Guideline 32

The risks of working with nonhuman primates must be identified, and all individuals working with nonhuman primates must be informed, trained and competent.

Institutions are required to support occupational health and safety for personnel working with nonhuman primates, including personnel working in nonhuman primate rooms or with nonhuman primate equipment and not specifically with the animals. It is important that employees are well informed of the health and safety risks and requirements associated with the particular nonhuman primates they are working with and the activities or situations involved. This includes awareness of proper handling and restraint techniques, proper wound decontamination procedures, relevant zoonotic diseases, appropriate use of personal protective equipment, policies, individual responsibilities, and reporting requirements for potential exposure to pathogens (Joint Working Group on Refinement, 2009).

As noted in Section 6, “Husbandry” and Section 7, “Animal Handling, Restraint and Training”, staff must be competent in their required tasks, as described in the [CCAC guidelines on: training of personnel working with animals in science](#) (CCAC, 2015). Additionally, Section 9.4, “Staff Training and Protection”, in the [CCAC guidelines: Husbandry of animals in science](#) (CCAC, 2017) provides guidance on staff training for the well-being and safety of the animals and for the safety of personnel (e.g., good personal hygiene; adherence to requirements for personal protective equipment; and awareness of the emotional cost of caring for and working with animals in research and any potential negative impacts on the person and their ability to properly care for and handle the animals).

Each institution housing nonhuman primates must have effective risk-based biosafety and occupational health programs with identification of hazards and implementation of preventive measures, such as vaccination and personal protective equipment. Consideration must be given to the species, source of the animals and the availability of information on their history, the type of holding facilities, and the procedures that will be conducted. The main risks for people working with nonhuman primates are tuberculosis, *Macacine herpesvirus 1* (not carried by marmosets and vervets), pathogens from feces (*Salmonella*, *Shigella*, *Campylobacter*, endoparasites, etc.) and physical injuries.

There should be clear delineation of areas and equipment that are considered contaminated and those that are clean, and procedures should be in place to maintain barriers. The type of personal protective equipment required depends on the level of risk, as determined through a risk assessment conducted by the occupational health and safety staff in conjunction with the veterinarian.

It is important to minimize health risks in the event of scratches, bites and splashes, and strict procedures must be in place and implemented. The *Canadian Biosafety Handbook* (Government of Canada, 2015b) states the following recommendations and precautions for those working with nonhuman primates:

- “When appropriate, arm-length reinforced leather gloves and long-sleeved gowns or coveralls should be worn to prevent scratches” (Section 13.1, “Animal Characteristics”);
- “Protection against aerosol exposure and splashes onto mucous membranes (e.g., with surgical mask, face shield, eye goggles) should be provided for handlers and anyone entering animal cubicles where NHPs are housed” (Section 13.9, “Working with Non-Human Primates”);
- “Protective clothing that has been in contact with animals should be decontaminated before being sent to laundry; laundering equipment located inside the containment zone is only suitable for decontamination when it has been proven to be effective for decontamination of the pathogen(s) present or suspected (i.e. validated)” (Section 13.1, “Animal Characteristics”);
- “Animal handlers should immediately and thoroughly cleanse all bites, scratches, and abraded skin, and rinse all splashes that result in contact with mucous membranes. Such exposures are to be reported without delay and post-exposure procedures implemented in accordance with the established emergency response plan and the medical surveillance program” (Section 13.1, “Animal Characteristics”); and
- “An emergency medical contact card must be issued to containment zone personnel handling NHPs” (Section 13.9 “Working with Non-Human Primates”).

If leather gloves will be required, it may be useful to habituate the animals to the gloves, so there is not a perceived association with capture.

N95 respirators should be used when working with known or suspected tuberculosis-positive animals and in other high-risk situations (e.g., post-mortem evaluation). To be effective, masks and respirators need to be properly fitted, with a fit test on a regular basis. In high-risk situations, additional measures are necessary as per the occupational health and safety physician’s recommendations.

All sores, cuts or other lacerations must be adequately covered while in primate rooms, and the dressings changed upon leaving the area.

Several species of nonhuman primate are particularly susceptible to tuberculosis. All nonhuman primate handlers and investigators should be assessed at least once per year for tuberculosis, in consultation with the occupational health physician. If a person has been vaccinated against tuberculosis, a skin test may yield a false positive and the quantiferon test is more appropriate (see CDC, 2016).

Captive macaques in Canada may be positive for *Macacine herpesvirus 1* and all macaque colonies should be treated as infected (Government of Canada, 2015b). Transmission of *Macacine herpesvirus 1* to humans occurs through animal bites or scratches or mucosal contact with body fluid or tissue (CDC, 2014). While *Macacine herpesvirus 1* infection in people is rare, it can result in brain damage or death (CDC, 2014). For information on prevention, detection and treatment of *Macacine herpesvirus 1*, see the Centers for Disease Control and Prevention (CDC, 2014).

Risks to human health and safety can be reduced by using purpose-bred animals of defined health status, ensuring animals are well-habituated and socialized to humans, and ensuring facilities are safe for staff and animals, with good observation panels and barriers to prevent escape (Joint Working Group on Refinement,

2009). Training nonhuman primates for husbandry and experimental procedures will reduce the stress experienced by the animals and improve the safety of the procedures for both the animals and personnel. The manner in which nonhuman primates perceive personnel is also important. People should move slowly, use a calm, quiet voice, and avoid direct eye contact; whenever possible, the animals should be the ones to initiate any interaction with people (Joint Working Group on Refinement, 2009).

Care must be taken to avoid injury from sharp materials (e.g., scalpels, needles). Needles should never be recapped. Double gloving is a good practice to increase protection against laceration, particularly for procedures with a higher risk of exposure (e.g., mouth examination or dentistry, endotracheal tube placement).

Cleaning and sanitizing primate facilities and equipment (see Section 6.8, “Cleaning and Sanitation”) also poses a risk to human health, and standard operating procedures must be developed and followed. Standard operating procedures must cover the sanitization of handling and restraint equipment, the disposal of excreta and bedding, and the disinfection of cages, instruments and other equipment used with nonhuman primates, including the protective outerwear of personnel prior to being sent to laundry.

Personnel working with nonhuman primates may be restricted from donating blood and organs for their entire life, and should check with the Canadian Blood Bank for restrictions.

REFERENCES

- Abee C., Mansfield K., Tardif S. and Morris T. (eds.) (2012) *Nonhuman Primates in Biomedical Research*, 2nd ed. American College of Laboratory Animal Medicine Series. Academic Press.
- Alport L.J. (2009) [Lingual fungiform papillae and the evolution of the primate gustatory system](#) (dissertation). Austin TX: University of Texas at Austin (accessed on 2019-02-19).
- Andrews M.W. and Rosenblum L.A. (2002) Response patterns of bonnet macaques following up to 75 weeks of continuous access to social-video and food rewards. *American Journal of Primatology* 57(4):213-218.
- Association of Primate Veterinarians – APV (2010) [Association of Primate Veterinarians Humane Endpoint Guidelines for Nonhuman Primates in Biomedical Research](#) (accessed on 2019-02-19).
- Baker K.C. (2016) [Survey of 2014 behavioral management programs for laboratory primates in the United States](#). *American Journal of Primatology* 78(7):780-796 (accessed on 2019-02-19).
- Baker K.C., Bloomsmith M., Neu K., Griffis C., Maloney M., Oettinger B., Schoof V.A.M. and Martinez M. (2009) [Positive reinforcement training moderates only high levels of abnormal behavior in singly housed rhesus macaques](#). *Journal of Applied Animal Welfare Science* 12(3):236-252 (accessed on 2019-02-19).
- Baker K.C., Bloomsmith M., Neu K., Griffis C., Oettinger B., Schoof V.A.M., Clay A. and Maloney M. (2008) Benefits of isosexual pairing of rhesus macaques (*Macaca mulatta*) vary with sex and are limited by protected contact but not by frequent separation of pairs. *American Journal of Primatology* 70(S1):44.
- Baker K.C., Bloomsmith M.A., Oettinger B., Neu K., Griffis C. and Schoof V.A.M. (2014) Comparing options for pair housing rhesus macaques using behavioral welfare measures. *American Journal of Primatology* 76(1):30-42.
- Baker K.C., Bloomsmith M.A., Oettinger B., Neu K., Griffis C., Schoof V. and Maloney M. (2012) [Benefits of pair housing are consistent across a diverse population of rhesus macaques](#). *Applied Animal Behaviour Science* 137(3-4):148-156 (accessed on 2019-02-19).
- Bassett L., Buchanan-Smith H.M., McKinley J. and Smith T.E. (2003) Effects of training on stress-related behavior of the common marmoset (*Callithrix jacchus*) in relation to coping with routine husbandry procedures. *Journal of Applied Animal Welfare Science* 6(3):221-233.
- Bayne K., Mainzer H., Dexter S., Campbell G., Yamada F. and Suomi S.T. (1991) The reduction of abnormal behaviors in individually housed rhesus monkeys (*Macaca mulatta*) with a foraging/grooming board. *American Journal of Primatology* 23(1):23-35.
- Beauchamp G.K. (2016) [Why do we like sweet taste: A bitter tale?](#) *Physiology & Behavior* 164:432-437 (accessed on 2019-02-19).
- Bellanca R.U., Lee G.H., Vogel K., Ahrens J., Kroeker R., Thom J.P. and Worlein J.M. (2014) [A simple alopecia scoring system for use in colony management of laboratory-housed primates](#). *Journal of Medical Primatology* 43(3):153-161 (accessed on 2019-02-19).

- Berrios-Torres S.I., Umscheid C.A., Bratzler D.W., Leas B., Stone E.C., Kelz R.R., Reinke C.E., Morgan S., Solomkin J.S., Mazuski J.E., Dellinger E.P., Itani, K.M.F., Berbari E.F., Segreti J., Parvizi J., Blanchard J., Allen G., Kluytmans, J.A.J.W., Donlan R., Schechter W.P., (2017) [Centers for Disease Control and Prevention Guidelines for the Prevention of Surgical Site Infection](#). *JAMA Surgery* 152(8):784-791 (accessed on 2019-02-19).
- Bliss-Moreau E., Theil J.H. and Moadab G. (2013) [Efficient cooperative restraint training with rhesus macaques](#). *Journal of Applied Animal Welfare Science* 16(2):98-117 (accessed on 2019-02-19).
- Bloomsmith M.A. and Lambeth S.P. (2000) Videotapes as enrichment for captive chimpanzees (*Pan troglodytes*). *Zoo Biology* 19(6):541-551.
- Bloomsmith M.A., Marr M.J. and Maple T.L. (2007) Addressing nonhuman primate behavioral problems through the application of operant conditioning: Is the human treatment approach a useful model? *Applied Animal Behaviour Science* 102(3-4):205-222.
- Boccia M.L., Reite M. and Laudenslager M. (1989) On the physiology of grooming in a pigtail macaque. *Physiology & Behavior* 45(3):667-670.
- Bolanowski S.J. and Pawson L. (2003) [Organization of Meissner corpuscles in the glabrous skin of monkey and cat](#). *Somatosensory & Motor Research* (accessed on 2019-02-19).
- Buchanan-Smith H.M., Prescott M.J. and Cross N.J. (2004) [What factors should determine cage size for primates in the laboratory?](#) *Animal Welfare* 13:S197-201 (accessed on 2019-02-19).
- Buffon G.L.L., Comte de (1766) *Histoire naturelle, générale et particulière*, tome 14.
- Burm S.M., Prins J.-B., Langermans J. and Bajramovic J.J. (2014) [Alternative methods for the use of non-human primates in biomedical research](#). Workshop Report. *ALTEX* 31(4):520-529 (accessed on 2019-02-19).
- Canadian Council on Animal Care – CCAC (1997) [CCAC guidelines on: transgenic animals](#). Ottawa ON: CCAC (accessed on 2019-02-19).
- Canadian Council on Animal Care – CCAC (1998) [CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing](#). Ottawa ON: CCAC (accessed on 2019-02-19).
- Canadian Council on Animal Care – CCAC (2003) [CCAC guidelines on: laboratory animal facilities—characteristics, design, and development](#). Ottawa ON: CCAC (accessed on 2019-02-19).
- Canadian Council on Animal Care – CCAC (2003) [CCAC guidelines on: the care and use of wildlife](#). Ottawa ON: CCAC (accessed on 2019-02-19).
- Canadian Council on Animal Care – CCAC (2007) [CCAC guidelines on: procurement of animals used in science](#). Ottawa ON: CCAC (accessed on 2019-02-19).
- Canadian Council on Animal Care – CCAC (2010) [CCAC guidelines on: euthanasia of animals used in science](#). Ottawa ON: CCAC (accessed on 2019-02-19).
- Canadian Council on Animal Care – CCAC (2015) [CCAC guidelines on: training of personnel working with animals in science](#). Ottawa ON: CCAC (accessed on 2019-02-19).

- Canadian Council on Animal Care – CCAC (2017) [*CCAC guidelines: Husbandry of animals in science*](#). Ottawa ON: CCAC (accessed on 2019-02-19).
- Canadian Food Inspection Agency (CFIA) (2009) [*Requirements for Non-Human Primates Imported into Canada*](#). Canadian Food Inspection Agency site (accessed on 2019-02-19).
- Canadian Food Inspection Agency (CFIA) (2017) [*Criteria for Quarantine Facilities*](#). Canadian Food Inspection Agency site (accessed on 2019-02-19).
- Capitanio J.P. (1999) Personality dimensions in adult male rhesus macaques: Prediction of behaviors across time and situation. *American Journal of Primatology* 47(4):299-320.
- Capitanio J.P., Blozis S.A., Snarr J., Steward A. and McCowan B.J. (2017) [*Do “birds of a feather flock together” or do “opposites attract”? Behavioral responses and temperament predict success in pairings of rhesus monkeys in a laboratory setting*](#). *American Journal of Primatology* 79(1):1-11 (accessed on 2019-02-19).
- Capitanio J.P., Kyes R.C. and Fairbanks L.A. (2006) [*Considerations in the selection and conditioning of Old World monkeys for laboratory research: Animals from domestic sources*](#). *ILAR Journal* 47(4):294-306 (accessed on 2019-02-19).
- Capuano S.V.III, Croix D.A., Pawar S., Zinovik A., Myers A., Lin P.L., Bissel S., Fuhrman C., Klein E. and Flynn J.L. (2003) [*Experimental Mycobacterium tuberculosis infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection*](#). *Infection and Immunity* 71(10):5831-5844 (accessed on 2019-02-19).
- Cartmill M. (2012) Primate origins, human origins, and the end of higher taxa. *Evolutionary Anthropology* 21(6):208-220.
- Centers for Disease Control and Prevention – CDC (2014) [*B Virus \(herpes B, monkey B virus, herpesvirus simiae, and herpesvirus B\)*](#) (accessed on 2019-02-19).
- Centers for Disease Control and Prevention – CDC (2016) [*Tuberculosis \(TB\)*](#) (accessed on 2019-02-19).
- Chamove A.S., Anderson J.R., Morgan-Jones S.C. and Jones S.P. (1982) [*Deep woodchip litter: Hygiene, feeding, and behavioral enhancement in eight primate species*](#). *International Journal for the Study of Animal Problems* 3(4):308-318 (accessed on 2019-02-19).
- Chopra P.K., Seth P.K. and Seth S. (1992) Behavioral profile of free-ranging rhesus monkeys. *Primate Report* 32:75–105.
- Clarence W.M., Scott J.P., Dorris M.C. and Paré M. (2006) [*Use of enclosures with functional vertical space by captive rhesus monkeys \(Macaca mulatta\) involved in biomedical research*](#). *Journal of the American Association for Laboratory Animal Science* 45(5):31-34 (accessed on 2019-02-19).
- Clay A.W., Bloomsmith M.A., Marr M.J. and Maple T.L. (2009) Habituation and desensitization as methods for reducing fearful behavior in singly-housed rhesus macaques. *American Journal of Primatology* 71(1):30-39.
- Clingerman K.J. and Summers L. (2012) [*Validation of a body condition scoring system in rhesus macaques \(Macaca mulatta\): Inter- and intrarater variability*](#). *Journal of the American Association for Laboratory Animal Science* 51(1):31-36 (accessed on 2019-02-19).

- Coelho A.M.Jr., Carey K.D. and Shade R.E. (1991) Assessing the effects of social environment on blood pressure and heart rates of baboons. *American Journal of Primatology* 23(4):257-267.
- Coleman M.N. (2009) What do primates hear? A meta-analysis of all known nonhuman primate behavioral audiograms. *International Journal of Primatology* 30(1):55-91.
- Coleman K. (2012) Individual differences in temperament and behavioral management practices for nonhuman primates. *Applied Animal Behaviour Science* 137(3-4):106-113.
- Coleman M.N. and Boyer D.M. (2012) Inner ear evolution in primates through the cenozoic: Implications for the evolution of hearing. *The Anatomical Record* 295(4):615-631.
- Coleman M.N. and Colbert M.W. (2010) Correlations between auditory structures and hearing sensitivity in non-human primates. *Journal of Microbiology* 271:511-532.
- Coleman K., Bloomsmit M.A., Crockett C.M., Weed J.L. and Schapiro S.J. (2012) Behavioral management, enrichment, and psychological well-being of laboratory nonhuman primates. In: *Nonhuman Primates in Biomedical Research*, vol. 1, 2nd ed. (Abee C., Mansfield K., Tardif S. and Morris T., eds.) Chapter 6. pp. 149-176. Academic Press.
- Coleman K. and Maier A. (2010) [The use of positive reinforcement training to reduce stereotypic behavior in rhesus macaques](#). *Applied Animal Behaviour Science* 124(3-4):142-148 (accessed on 2019-02-19).
- Coleman K. and Pierre P.J. (2014) [Assessing anxiety in nonhuman primates](#). *ILAR Journal* 55(2):333-346 (accessed on 2019-02-19).
- Coleman K., Pranger L., Maier A., Lambeth S.P., Perlman J.E., Thiele E. and Schapiro S.J. (2008) [Training rhesus macaques for venipuncture using positive reinforcement techniques: A comparison with chimpanzees](#). *Journal of the American Association for Laboratory Animal Science* 47(1):37-41 (accessed on 2019-02-19).
- Coleman K., Tully L.A. and McMillan J.L. (2005) Temperament correlates with training success in adult rhesus macaques. *American Journal of Primatology* 65(1):63-71.
- Conway B.R., Chatterjee S., Field G.D., Horwitz G.D., Johnson E.N., Koida K. and Mancuso K. (2010) [Advances in color science: From retina to behavior](#). *Journal of Neuroscience* 30(45):14955-14963 (accessed on 2019-02-19).
- Davenport M.D., Tiefenbacher S., Lutz C.K., Novak M.A. and Meyer J.S. (2006) Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General and Comparative Endocrinology* 147(3):255-261.
- Dettmer E. and Fragaszy D. (2000) [Determining the value of social companionship to captive tufted capuchin monkeys \(Cebus apella\)](#). *Journal of Applied Animal Welfare Science* 3(4):293-304 (accessed on 2019-02-19).
- Diehl K.-H., Hull R., Morton D., Pfister R., Rabemampianina Y., Smith D., Vidal J.-M. and van de Vorstenbosch C. (2001) [A good practice guide to the administration of substances and removal of blood, including routes and volumes](#). *Journal of Applied Toxicology* 21(1):15-23 (accessed on 2019-02-19).
- DiVincenti L. Jr., Rehrig A. and Wyatt J. (2012) [Interspecies pair housing of macaques in a research facility](#). *Laboratory Animals* 46(2):170-172 (accessed on 2019-02-19).

- DiVincenti L. Jr. and Wyatt J.D. (2011) [Pair housing of macaques in research facilities: A science-based review of benefits and risks](#). *Journal of the American Association for Laboratory Animal Science* 50(6):856-863 (accessed on 2019-02-19).
- Doane C.J., Andrews K., Schaefer L.J., Morelli N., McAllister S. and Coleman K. (2013) [Dry bedding provides cost-effective enrichment for group-housed rhesus macaques \(*Macaca mulatta*\)](#). *Journal of the American Association for Laboratory Animal Science* 52(3):247-252 (accessed on 2019-02-19).
- Dominy N.J., Lucas P.W., Osorio D. and Yamashita N. (2001) The sensory ecology of primate food perception. *Evolutionary Anthropology* 10(5):171-186.
- Dominy N.J., Ross C.F. and Smith T.D. (2004) Evolution of the special senses in primates: Past, present and future. *The Anatomical Record Part A, Discoveries in Molecular Cellular and Evolutionary Biology* 281(1):1078-1082.
- Doudet D.J., Cornfeldt M.L., Honey C.R., Schweikert A.W. and Allen R.C. (2004) PET imaging of implanted human retinal pigment epithelial cells in the MPTP-induced primate model of Parkinson's disease. *Experimental Neurology* 189(2):361-368.
- Drea C.M. (2015) D'scent of man: A comparative survey of primate chemosignaling in relation to sex. *Hormones and Behavior* 68:117-133.
- Eaton G.G., Kelley S.T., Axthelm M.K., Iliff-Sizemore S.A. and Shiigi S.M. (1994) Psychological wellbeing in paired adult female rhesus (*Macaca mulatta*). *American Journal of Primatology* 33(2):89-99.
- Estes J.D., Wong S.W. and Brenchley J.M. (2018) Nonhuman primate models of human viral infections. *Nature Reviews: Immunology* 18(6):390-404.
- Fante F., Boldrin M., Polito L., Ravarotto L., Castagnaro M., Hutabba S., Cozzi E. and Ancona E. (2007) [Refinement of a transplantation project in the nonhuman primate by the use of a humane endpoint](#). *Laboratory Animals* 41(4):456-469 (accessed on 2019-02-19).
- Faucheux B., Bertrand M. and Boulière F. (1978) Some effects of living conditions upon the pattern of growth in the stump-tail macaque (*Macaca arctoides*). *Folia Primatologica* 30(3):220-236.
- Fay R.R. (1992) Structure and function in sound discrimination among vertebrates. In: *The Evolutionary Biology of Hearing*, pp. 229-263 (Webster D.B., Popper A.N. and Fay R.R., eds.). New York: Springer-Verlag.
- Feldmahn A.L., Smith W.K. and Leventhal C.M. (1960) Diurnal patterns of micturition and drinking in rhesus monkeys. *Annals of the New York Academy of Sciences* 85:828-841.
- Fernström A.L., Fredlund H., Spångberg M. and Westlund K. (2009) Positive reinforcement training in rhesus macaques—training progress as a result of training frequency. *American Journal of Primatology* 71(5):373-379.
- Field A.E., Jones C.L., Kelly R., Marko S.T., Kern S.J. and Rico P.J. (2015) [Measurement of fecal corticosterone metabolites as a predictor of the habituation of rhesus macaques \(*Macaca mulatta*\) to jacketing](#). *Journal of the American Association for Laboratory Animal Science* 54(1):59-65 (accessed on 2019-02-19).

- Fleagle J.G. (2013) *Primate Adaptation and Evolution*, 3rd ed. Elsevier, Academic Press.
- Fortman J.D., Hewett T.A. and Bennett B.T. (2017) *The Laboratory Nonhuman Primate*, 2nd ed. New York NY: CRC Press.
- Fox A.S. and Kalin N.H. (2014) [A translational neuroscience approach to understanding the development of social anxiety disorder and its pathophysiology](#). *American Journal of Psychiatry* 171(11):1162-1173 (accessed on 2019-02-19).
- Geoffroy Saint-Hilaire M. (1812a) Tableau des quadrumanes, ou des Animaux composant le premier Ordre de la Classe des Mammifères. *Annales du muséum d'histoire naturelle*, tome 19, pp. 85-122.
- Geoffroy Saint-Hilaire M. (1812b) Suite au tableau des quadrumanes. *Annales du muséum d'histoire naturelle*, tome 19, pp. 156-170.
- Ghazanfar A.A. and Santos L.R. (2004) Primate brains in the wild: the sensory bases for social interactions. *Nature Reviews: Neuroscience* 5(8):603-616.
- Gonzalez C.A., Coe C.L. and Levine S. (1982) Cortisol responses under different housing conditions in female squirrel monkeys. *Psychoneuroendocrinology* 7(2-3):209-216.
- Gottlieb D.H., Coleman K. and McCowan B. (2013) [The effects of predictability in daily husbandry routines on captive rhesus macaques \(*Macaca mulatta*\)](#). *Applied Animal Behaviour Science* 143(2-4):117-127 (accessed on 2019-02-19).
- Government of Canada (1996) [Controlled Drugs and Substances Act](#) (S.C. 1996, c. 19). Minister of Justice (accessed on 2019-02-19).
- Government of Canada (2015a) [Canadian Biosafety Standard](#), 2nd ed. (accessed on 2019-02-19).
- Government of Canada (2015b) [Canadian Biosafety Handbook](#), 2nd ed. (accessed on 2019-02-19).
- Graham M.L., Rieke E.F., Mutch L.A., Zolondek E.K., Faig A.W., Dufour T.A., Munson J.W., Kittredge J.A. and Schuurman H.J. (2012) [Successful implementation of cooperative handling eliminates the need for restraint in a complex nonhuman primate disease model](#). *Journal of Medical Primatology* 41(2):89-106 (accessed on 2019-02-19).
- Griffis C.M., Martin A.L., Perlman J.E. and Bloomsmith M.A. (2013) [Play caging benefits the behavior of singly housed laboratory rhesus macaques \(*Macaca mulatta*\)](#). *Journal of the American Association for Laboratory Animal Science* 52(5):534-540 (accessed on 2019-02-19).
- Hamilton C.L. (1972) Long term control of food intake in the monkey. *Physiology & Behavior* 9(1):1-6.
- Harris L.D., Briand E.J., Orth R. and Galbicka G. (1999) Assessing the value of television as environmental enrichment for individually housed rhesus monkeys: A behavioral economic approach. *Contemporary Topics in Laboratory Animal Science* 38(2):48-53.
- Heffner R.S. (2004) [Primate hearing from a mammalian perspective](#). *The Anatomical Record, Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology* 281(1):1111-1122 (accessed on 2019-02-19).

- Heffner H.E. and Heffner R.S. (2007) Hearing ranges of laboratory animals. *Journal of the American Association for Laboratory Animal Science* 46(1):20-22.
- Heffner H.E. and Heffner R.S. (2016) [The evolution of mammalian sound localization](#). *Acoustics Today* 12:20-25 (accessed on 2019-02-19).
- Hemani M.L. and Lepor H. (2009) [Skin preparation for the prevention of surgical site infection: Which agent is best?](#) *Reviews in Urology* 11(4):190-195 (accessed on 2019-02-19).
- Hennessy M.B., Mendoza S.P. and Kaplan J.N. (1982) Behavior and plasma cortisol following brief peer separation in juvenile squirrel monkeys. *American Journal of Primatology* 3:143-151 (cited in Novak et al., 2012).
- Henry B., Fox S.H., Peggs D., Crossman A.R. and Brotchie J.M. (1999) The alpha2-adrenergic receptor antagonist idazoxan reduces dyskinesia and enhances anti-parkinsonian actions of L-dopa in the MPTP-lesioned primate model of Parkinson's disease. *Movement Disorder* 14(5):744-753.
- Herlyn H. (2016) The phylogenetic system of primates—character evolution in the light of a consolidated tree. *Organisms Diversity & Evolution* 16(4):689-713.
- Hessy C.P. (2009) Seeing in stereo: The ecology and evolution of primate binocular vision and stereopsis. *Evolutionary Anthropology* 18(1):21-35.
- Heymann E.W. (2006) Introduction. [The neglected sense – olfaction in primate behavior, ecology, and evolution](#). *American Journal of Primatology* 68(6):519-524 (accessed on 2019-02-19).
- Hladik C-M., Pasquet P., Danilova V. and Hellekant G. (2003) [The evolution of taste perception: Psychophysics and taste nerves tell the same story in human and non-human primates](#). *Comptes Rendus Palevol* 2:281-287 (accessed on 2019-02-19).
- Hoffmann J.N., Montag A.G. and Dominy N.J. (2004) [Meissner corpuscles and somatosensory acuity: The prehensile appendages of primates and elephants](#). *The Anatomical Record, Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology* 281(1):1138-1147 (accessed on 2019-02-19).
- Honess P.E., Johnson P.J. and Wolfensohn S.E. (2004) [A study of behavioural responses of non-human primates to air transport and re-housing](#). *Laboratory Animals* 38(2):119-132 (accessed on 2019-02-19).
- Honess P.E., Gimpel J.L., Wolfensohn S.E. and Mason G.J. (2005) [Alopecia scoring: The quantitative assessment of hair loss in captive macaques](#). *ATLA* 33(3):193-206 (accessed on 2019-02-19).
- Honess P.E. and Marin C.M. (2006) Behavioural and physiological aspects of stress and aggression in non-human primates. *Neuroscience and Biobehaviour Reviews* 30(3):390-412.
- Hranjec T., Swenson B.R. and Sawyer R.G. (2010) [Surgical site infection prevention: How we do it](#). *Surgical Infections* 11(3):289-294 (accessed on 2019-02-19).
- Imbert C., Bézard E., Guitraud S., Boraud T. and Gross C.E. (2000) Comparison of eight clinical rating scales used for the assessment of MPTP-induced parkinsonism in the Macaque monkey. *Journal of Neuroscience Method* 96(1):71-76.

- Institute for Laboratory Animal Research – ILAR (2006) *Guidelines for the Humane Transportation of Research Animals*. Washington DC: National Academies Press.
- International Air Transport Association – IATA (2016) [Live Animal Regulations](#) (accessed on 2019-02-19).
- International Primatological Society – IPS (2007) [IPS International Guidelines for the Acquisition, Care and Breeding of Nonhuman Primates](#), 2nd ed. IPS (accessed on 2019-02-19).
- Izpisúa Belmonte J.C., Callaway E.M., Caddick S.J., Churchland P., Feng G., Homanics G.E., Lee K-F, Leopold D.A., Miller C.T., Mitchell J.F., Mitalipov S., Moutri A.R., Movshon J.A., Okano H., Reynolds J.H., Ringach D.L., Sejnowski T.J., Silva A.C., Strick P.L., Wu J. and Zhang F. (2015) Brains, genes, and primates. *Neuron* 86(3):617-631.
- Jacobs G.H. (2008) Primate color vision: A comparative perspective. *Visual Neuroscience* 25(5-6):619-633.
- Jennings C.G., Landman R., Zhou Y., Sharma J., Hyman J., Moyshon J.A., Qiu Z., Roberts A.C., Roe A.W., Wang X., Zhou H., Wang L., Zhang F., Desimone R. and Feng G. (2016) Opportunities and challenges in modelling human brain disorders in transgenic primates. *Nature Neuroscience* 19(9):1123-1130.
- Joint Working Group on Refinement (2009) [Refinements in husbandry, care and common procedures for non-human primates: Ninth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement](#) (Jennings M. and Prescott M.J., eds.). *Laboratory Animals* 43(Suppl 1):S1:1-S1:47 (accessed on 2019-02-19).
- Kawamura S. (2016) [Color vision diversity and significance in primates inferred from genetic and field studies](#). *Genes Genomics* 38:779-791 (accessed on 2019-02-19).
- Kirk E.C. (2006) [Visual influences on primate encephalization](#). *Journal of Human Evolution* 51:76-90 (accessed on 2019-02-19).
- Kirk E.C. and Gosselin-Ildari A.D. (2009) Cochlear labyrinth volumes and hearing abilities in primates. *The Anatomical Record* 292:765-776.
- Kirk E.C. and Kay R.F. (2004) The evolution of high visual acuity in the Anthroidea. In: *Anthropoid Origins: New Visions*, pp.539-602 (Ross C.F. and Kay R.F., eds.). New York: Kluwer Academic/Plenum Publishing.
- Kitchen A.M. and Martin A.A. (1996) [The effects of cage size and complexity on the behaviour of captive common marmosets, *Callithrix jacchus jacchus*](#). *Laboratory Animals* 30(4):317-326 (accessed on 2019-02-19).
- Laboratory Animal Science Association – LASA and Medical Research Council – MRC (2004) [Key Considerations in the Breeding of Macaques and Marmosets for Scientific Purposes](#) (accessed on 2019-02-19).
- Lambeth S.P., Schapiro S.J., Bernacky B.J. and Wilkerson G.K. (2013) [Establishing ‘quality of life’ parameters using behavioural guidelines for humane euthanasia of captive non-human primates](#). *Animal Welfare* 22(4): 429-435 (accessed on 2019-02-19).
- Laska M. and Hernandez Salazar L.T. (2015) Olfaction in Nonhuman primates. In: *Handbook of Olfaction and Gustation*, 3rd ed. (Ed. R.L. Doty.) Chapter 27. pp 605-620. John Wiley & Sons, Inc.
- Laule G. (1993) The use of behavioral management techniques to reduce or eliminate abnormal behavior. *Animal Welfare Information Centre Newsletter* 4(1-2):8-11.

- Laule G.E., Bloomsmith M.A. and Schapiro S.J. (2003) The use of positive reinforcement training techniques to enhance the care, management, and welfare of primates in the laboratory. *Journal of Applied Animal Welfare Science* 6(3):163-173.
- Laule G. and Whittaker M. (2007) Enhancing nonhuman primate care and welfare through the use of positive reinforcement training. *Journal of Applied Animal Welfare Science* 10(1):31-38.
- Lee G.H., Thom J.P., Chu K.L. and Crockett C.M. (2012) [Comparing the relative benefits of grooming-contact and full-contact pairing for laboratory-housed adult female *Macaca fascicularis*](#). *Applied Animal Behaviour Science* 137(3-4):157-165 (accessed on 2019-02-19).
- Lee G.H., Yi M.J. and Crockett C.M. (2011) [Assessing video presentation as enrichment for captive male pig-tailed macaques \(*Macaca nemestrina*\)](#). *Laboratory Primate Newsletter* 50(4):7-9 (accessed on 2019-02-19).
- Lefevre A., Ballesta S., Pozzobon M., Charieau J.L., Duperrier S., Sirigu A. and Duhamel J.R. (2015) Blood microsampling from the ear capillary in non-human primates. *Laboratory Animals* 49(4):349-352.
- Lemos D.R., Downs J.L., Raitiere M.N. and Urbanski H.F. (2009) [Photoperiodic modulation of adrenal gland function in the rhesus macaque: Effect on 24-hour plasma cortisol and dehydroepiandrosterone sulfate rhythms and adrenal gland gene expression](#). *Journal of Endocrinology* 201(2):275-285 (accessed on 2019-02-19).
- Lin P.L., Pawar S., Myers A., Pegu A., Fuhrman C., Reinhart T.A., Capuano S.V., Klein E. and Flynn J.L. (2006) [Early events in *Mycobacterium tuberculosis* infection in cynomolgus macaques](#). *Infection and Immunity* 74(7):3790-3803 (accessed on 2019-02-19).
- Lutz C.K., Coleman K., Worlein J. and Novak M.A. (2013) [Hair loss and hair-pulling in rhesus macaques \(*Macaca mulatta*\)](#). *Journal of the American Association for Laboratory Animal Science* 52(4):454-457 (accessed on 2019-02-19).
- Lutz C.K. and Novak M.A. (2005) Environmental enrichment for nonhuman primates: Theory and application. *ILAR Journal* 46(2):178-191.
- MacLean E.L., Prior S.R., Platt M.L. and Brannon E.M. (2009) [Primate location preference in a double-tier cage: The effects of illumination and cage height](#). *Journal of Applied Animal Welfare Science* 12(1):73-81 (accessed on 2019-02-19).
- Maddison S., Wood R.J., Rolls E.T., Rolls B.J. and Gibbs J. (1980) Drinking in the rhesus monkey: Peripheral factors. *Journal of Comparative and Physiological Psychology* 94(2):365-374.
- Martin R.D. (1990) *Primate Origins and Evolution*. Princeton NJ: Princeton University Press.
- Martin R.D. (2012) [Primates](#). *Current Biology* 22(18):R785-R790 (accessed on 2019-02-19).
- Mason V.C., Li G., Minx P., Schmitz J., Churakov G., Doronina L., Melin A.D., Dominy N.J., Lim N.T., Springer M.S., Wilson R.K., Warren W.C., Helgen K.M. and Murphy W.J. (2016) [Genomic analysis reveals hidden biodiversity within colugos, the sister group to primates](#). *Science Advances* 2(8):e1600633 (accessed on 2019-02-19).

- McKinley J., Buchanan-Smith H.M., Bassett L. and Morris K. (2003) Training common marmosets (*Calithrix jacchus*) to cooperate during routine laboratory procedures: Ease of training and time investment. *Journal of Applied Animal Welfare Science* 6(3):209-220.
- McMillan J.L., Perlman J.E., Galvan A., Wichmann T. and Bloomsmith M.A. (2014) [Refining the pole-and-collar method of restraint: Emphasizing the use of positive training techniques with rhesus macaques \(*Macaca mulatta*\)](#). *Journal of the American Association for Laboratory Animal Science* 53(1):61-68 (accessed on 2019-02-19).
- McNulty J.A., Iskander E. and Kyes R.C. (2004) Time budgets of long-tailed macaques (*Macaca fascicularis*) on Tinjil Island, Indonesia. *American Journal of Primatology* 62(Suppl):65-66.
- Melin A.D., Wells K., Moritz G.L., Kistler L., Orkin J.D., Timm R.M., Bernard H., Lakim M.B., Perry G.H., Kawamura S. and Dominy N.J. (2016) [Euarchontan opsin variation brings new focus to primate origins](#). *Molecular Biology and Evolution* 33(4):1029-1041 (accessed on 2019-02-19).
- Meyer J., Novak M., Hamel A. and Rosenberg K. (2014) [Extraction and analysis of cortisol from human and monkey hair](#). *Journal of Visualized Experiments* (83):e50882 (accessed on 2019-02-19).
- Muchlinski M.N., Docherty B.A., Alport L.J., Burrows A.M., Smith T.D. and Paesani S.M. (2011) Behavioral and ecological consequences of sex-based differences in gustatory anatomy in *Cebus apella*. *Anatomical Record* (Hoboken) 294(12):2179-2192.
- National Institute for Health and Clinical Excellence (NICE) (2008) [Surgical Site Infection: Prevention and Treatment of Surgical Site Infection](#). NICE Clinical Guidelines, No. 74. London UK: National Collaborating Centre for Women's and Children's Health (accessed on 2019-02-19).
- National Research Council – NRC (1998) [The Psychological Well-Being of Nonhuman Primates](#). Committee on Well-Being of Nonhuman Primates, Institute for Laboratory Animal Research, National Research Council. Washington DC: National Academy Press (accessed on 2019-02-19).
- National Research Council – NRC (2003a) *Nutrient Requirements of Nonhuman Primates*, 2nd ed. Committee on Animal Nutrition, Ad Hoc Committee on Nonhuman Primate Nutrition. Washington DC: National Academies Press.
- National Research Council – NRC (2003b) [Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research](#). Institute for Laboratory Animal Research. Washington DC: National Academies Press (accessed on 2019-02-19).
- Neveu H. and Deputte B.L. (1996) [Influence of availability of perches on the behavioral well-being of captive, group-living mangabeys](#). *American Journal of Primatology* 38(2):175-185 (accessed on 2019-02-19).
- Nevo O. and Heymann E.W. (2015) Led by the nose: Olfaction in primate feeding ecology. *Evolutionary Anthropology* 24:137-148.
- Newsome W.T. and Stein-Aviles J.A. (1999) [Nonhuman primate models of visually based cognition](#). *ILAR Journal* 40(2):78-91 (accessed on 2019-02-19).

- Novak M.A. and Meyer J.S. (2009) [Alopecia: Possible causes and treatments, particularly in captive nonhuman primates](#). *Comparative Medicine* 59(1):18-26 (accessed on 2019-02-19).
- Novak M.A., Kelly B.J., Bayne K. and Meyer J.S. (2012) Behavioral disorders of nonhuman primates. In: *Nonhuman Primates in Biomedical Research*, vol. 1, 2nd ed. (Abee C., Mansfield K., Tardif S. and Morris T., eds.) Chapter 7. pp.177-196. Academic Press.
- Oikawa H., Yamashita T., Muto M. and Sawai M. (1982) Water intake and urinary output in rhesus (*Macaca mulatta*) and cynomolgus monkeys (*Macaca fascicularis*) (article in Japanese). *Jikken Dobutsu (Experimental Animals)* 31(4):279-286.
- Okano H. and Kishi N. (2018) Investigation of brain science and neurological/psychiatric disorders using genetically modified non-human primates. *Current Opinion in Neurobiology* 50:1-6.
- Oliveira L.M.O. and Dimitrov D. (2008) [Surgical techniques for chronic implantation of microwire arrays in rodents and primates](#). In: *Methods for Neural Ensemble Recordings*, 2nd ed. (Nicollelis M.A.L., ed.) Chapter 2. Boca Raton FL: CRC Press (accessed on 2019-02-19).
- O'Neill-Wagner P.I. (2005) Video entertainment may facilitate recovery for monkeys in a clinical setting. In: *Play & Culture Studies* (McMahon F.F., Lytle D.E. and Sutton-Smith B., eds.), vol. 6. Lanham MD: University Press of America, pp.43-51.
- O'Sullivan A., He X., McNiven E.M.S., Hinde K., Haggarty N.W., Lönnerdal B. and Slupsky C.M. (2013) Metabolomic phenotyping validates the infant rhesus monkey as a model of human infant metabolism. *Journal of Pediatric Gastroenterology and Nutrition* 56(4):355-363.
- Owen Y. and Amory J.R. (2011) A case study employing operant conditioning to reduce stress of capture for red-bellied tamarins (*Saguinus labiatus*). *Journal of Applied Animal Welfare Science* 14(2):124-137.
- Pan X., Lu L., Zeng X., Chang Y. and Hua X. (2016) [Effects of transportation on antioxidant status in cynomolgus macaques \(*Macaca fascicularis*\)](#). *Journal of the American Association for Laboratory Animal Science* 55(5):534-540 (accessed on 2019-02-19).
- Parker K.J. and Maestripietri D. (2011) [Identifying key features of early stressful experiences that produce stress vulnerability and resilience in primates](#). *Neuroscience and Biobehavioral Reviews* 35(7):1466-1483 (accessed on 2019-02-19).
- Pecon-Slatery J. (2014) Recent advances in primate phylogenomics. *Annual Review of Animal Biosciences*. 2:41-63.
- Perlman J.E., Bloomsmith M.A., Whittaker M.A., McMillan J.L., Minier D.E. and McCowan B. (2012) Implementing positive reinforcement animal training programs at primate laboratories. *Applied Animal Behaviour Science* 137(3-4):114-126.
- Platt D.M. and Novak M.A. (1997) Videostimulation as enrichment for captive rhesus monkeys (*Macaca mulatta*). *Applied Animal Behaviour Science* 52(1-2):139-155.

- Pocock R.I. (1918) On the external characters of the Lemurs and of Tarsius. *Proceedings of The Zoological Society of London* 1918:19-53.
- Poole T. (1997) [Happy animals make good science](#). *Laboratory Animals* 31(2):116-124 (accessed on 2019-02-19).
- Pozzi L., Hodgson J.A., Burrell A.S., Sterner K.N., Raaum R.L. and Disotell T.R. (2014) Primate phylogenetic relationships and divergence dates inferred from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution* 75:165-183.
- Prescott M.J. (2006a) [Primate sensory capabilities and communication signals: Implications for care and use in the laboratory](#). NC3Rs (accessed on 2019-02-19).
- Prescott M.J. (2006b) [Finding new homes for ex-laboratory and surplus zoo primates](#). *Laboratory Primate Newsletter* 45(3):5-8 (accessed on 2019-02-19).
- Prescott M.J., Howell V.A. and Buchanan-Smith H.M. (2005) Training of laboratory-housed non-human primates, Part 2: resources for developing and implementing training programmes. *Animal Technology and Welfare* 4(3):133-148.
- Prescott M.J., Brown V.J., Flecknell P.A., Gaffan D., Garrod K., Lemon R.N., Parker A.J., Ryder K., Schultz W., Scott L., Watson J. and Whitfield L. (2010) Refinement of the use of food and fluid control as motivational tools for macaques used in behavioral neuroscience research: Report of a working group of the NC3Rs. *Journal of Neuroscience Methods* 193(2):167-188.
- Prescott M.J. and Buchanan-Smith H.M. (2003) [Training nonhuman primates using positive reinforcement techniques](#). *Journal of Applied Animal Welfare Science* 6(3):157-161 (accessed on 2019-02-19).
- Prescott M.J. and Buchanan-Smith H.M. (2007) [Training laboratory-housed non-human primates, part 1: A UK survey](#). *Animal Welfare* 16:21-36 (accessed on 2019-02-19).
- Prescott M.J., Nixon M.E., Farningham D.A.H., Naiken S. and Griffiths M.-A. (2012) Laboratory macaques: When to wean? *Applied Animal Behaviour Science* 137(3-4):194-207.
- Qin D., Chu X., Feng X., Li Z., Yang S., Lü L., Yang Q., Pan L., Yin Y., Li J., Xu L., Chen L. and Hu X. (2015) The first observation of seasonal affective disorder symptoms in Rhesus macaque. *Behavioural Brain Research* 292:463-469.
- Raaum R.L. (2015) Molecular evidence on primate origins and evolution. In: *Handbook of Paleoanthropology*, 2nd ed., vol. 2 (Henke W. and Tattersall I., eds). pp. 1083-1135. Berlin, Heidelberg: Springer-Verlag.
- Ramsey M.A., Cunningham A.J., Finneran J.J., and Dominy N.J. (2012) [Social drive and the evolution of primate hearing](#). *Philosophical Transactions of the Royal Society B* 367:1860-1868 (accessed on 2019-02-19).
- Rehrig A., DiVincenti L. Jr. and Schery L.A. (2014) Social housing of non-human primates in a research facility: Socialisation across macaque species and sexes. *Animal Welfare* 23(4):387-389.
- Reinhardt V. (1990) [Social enrichment for laboratory primates: A critical review](#). *Laboratory Primate Newsletter* 29(3):7-11 (accessed on 2019-02-19).

- Reinhardt V. (1992) [Space utilization by captive rhesus macaques](#). *Animal Technology* 43(1):11-17 (accessed on 2019-02-19).
- Reinhardt V. (2003) Working with rather than against macaques during blood collection. *Journal of Applied Animal Welfare Science* 6(3):189-197.
- Reinhardt V. and Hurwitz S. (1993) [Evaluation of social enrichment for aged rhesus macaques](#). *Animal Technology* 44(1):53-57 (accessed on 2019-02-19).
- Reinhardt V. and Reinhardt A. (1991) [Impact of a privacy panel on the behavior of caged female rhesus monkeys living in pairs](#). *Journal of Experimental Animal Science* 34(2):55-58 (accessed on 2019-02-19).
- Rennie A.E. and Buchanan-Smith H.M. (2006a) [Refinement of the use of non-human primates in scientific research. Part II: Housing, husbandry and acquisition](#). *Animal Welfare* 15(3):215-238 (accessed on 2019-02-19).
- Rennie A.E. and Buchanan-Smith H.M. (2006b) [Refinement of the use of nonhuman primates in scientific research. Part I: The influence of humans](#). *Animal Welfare* 15(3):203-213 (accessed on 2019-02-19).
- Robbins R.C. and Gavan J.A. (1965) Influence of diet on the qualitative amino acid patterns in the urine of rhesus monkeys (*Macaca mulatta*). *Human Biology* 37(2):137-147.
- Rogge J., Sherenco K., Malling R., Thiele E., Lambeth S., Schapiro S. and Williams L. (2013) [A comparison of positive reinforcement training techniques in owl and squirrel monkeys: Time required to train to reliability](#). *Journal of Applied Animal Welfare Science*. 16(3):211-220 (accessed on 2019-02-19).
- Ross C.F. and Kirk E.C. (2007) [Evolution of eye size and shape in primates](#). *Journal of Human Evolution* 52(3):294-313 (accessed on 2019-02-19).
- Ross C.F. and Martin R.D. (2007) The role of vision in the origin and evolution of primates. In J. H. Kaas & T. M. Preuss (Eds.), *Evolution of Nervous Systems* (Vol. 4, pp. 59-78). London: Elsevier.
- Russell W.M.S. and Burch R.L. (1959) [The Principles of Humane Experimental Technique](#). Potters Bar UK: Universities Federation for Animal Welfare (UFAW) (accessed on 2019-02-19).
- Rylands A.B. and Mittermeier R.A. (2014) Primate taxonomy: Species and conservation. *Evolutionary Anthropology* 23(1):8-10.
- Sánchez M.M., Hearn E.F., Do D., Rilling, J.K. and Herndon J.G. (1998) Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys. *Brain Research* 812(1-2):38-49.
- Sasseville V.G. and Diters R.W. (2008) [Impact of infections and normal flora in nonhuman primates on drug development](#). *ILAR Journal* 49(2):179-190 (accessed on 2019-02-19).
- Sato K. and Saski E. (2018) [Genetic engineering in nonhuman primates for human disease modeling](#). *Journal of Human Genetics* 63:125-131 (accessed on 2019-02-19).

- Schapiro S.J. and Bushong D. (1994) [Effects of enrichment on veterinary treatment of laboratory rhesus macaques \(*Macaca mulatta*\)](#). *Animal Welfare* 3(1):25-36 (accessed on 2019-02-19).
- Schapiro S.J., Perlman J.E. and Boudreau B.A. (2001) Manipulating the affiliative interactions of group-housed rhesus macaques using positive reinforcement training techniques. *American Journal of Primatology* 55(3):137-149.
- Schapiro S.J., Perlman J.E., Thiele E. and Lambeth S. (2005) Training nonhuman primates to perform behaviors useful in biomedical research. *Lab Animal* 34(5):37-42.
- Schino G. and Troisi A. (1990) Behavioral thermoregulation in long-tailed macaques: Effect on social preference. *Physiology & Behavior* 47(6):1125-1128.
- Schmidt M. (2010) [Locomotion and postural behaviour](#). *Advances in Science and Research* 5:23-39 (accessed on 2019-02-19).
- Scott L., Pearce P., Fairhall S., Muggleton N. and Smith J. (2003) [Training nonhuman primates to cooperate with scientific procedures in applied biomedical research](#). *Journal of Applied Animal Welfare Science* 6(3):199-207 (accessed on 2019-02-19).
- Seelig D. (2007) A tail of 2 monkeys: Social housing for nonhuman primates in the research laboratory setting. *Journal of Applied Animal Welfare Science* 10(1):21-30 (cited in DiVincenti and Wyatt, 2011).
- Shepherdson D.J., Mellen J.D. and Hutchins M. (ed.) (1998) *Second Nature: Environmental Enrichment for Captive Animals*. Washington DC: Smithsonian Institution Press.
- Shively C.A. and Willard S.L. (2012) [Behavioral and neurobiological characteristics of social stress versus depression in nonhuman primates](#). *Experimental Neurology* 233(1):87-94 (accessed on 2019-02-19).
- Shurtleff A.C. and Bavari S. (2015) Animal models for ebolavirus countermeasures discovery: What defines a useful model? *Expert Opinion on Drug Discovery* 10(7):685-702.
- Simmen B. and Hladik A. (1998) Sweet and bitter taste discrimination in primates: Scaling effects across species. *Folia Primatologica* 69(3):129-138.
- Smith J.J., Hadzic V., Li X., Liu P., Day T., Utter A., Kim B., Washington I.M. and Basso M.A. (2006) Objective measures of health and well-being in laboratory rhesus monkeys (*Macaca mulatta*). *Journal of Medical Primatology* 35(6):388-396.
- Smith T.D., Eiting T.P. and Bhatnagar K.P. (2015) Anatomy of the nasal passages in mammals. In: *Handbook of Olfaction and Gustation*, 3rd ed. (ed R.L. Doty) Chapter 2: pp 39-61. John Wiley & Sons Inc.
- Souza G.S., Gomes B.D. and Silveira L.C.L. (2011) [Comparative neurophysiology of spatial luminance contrast sensitivity](#). *Psychology & Neuroscience* 4(1):29-48 (accessed on 2019-02-19).
- Spiezio C., Piva F., Regaiolli B. and Vaglio S. (2015) Positive reinforcement training: A tool for care and management of captive vervet monkeys (*Chlorocebus aethiops*). *Animal Welfare* 24(3):283-290.

- Sussman R.W., Rasmussen D.T. and Raven P.H. (2013) Rethinking primate origins again. *American Journal of Primatology* 75(2):95-106.
- Suzuki M.T., Hamano M., Cho F. and Honjo S. (1989) Food and water intake, urinary and fecal output, and urinalysis in the wild-originated cynomolgus monkeys (*Macaca fascicularis*) under the indoor individually-caged conditions [article in Japanese]. *Jikken Dobutsu [Experimental Animals]* 38(1):71-74.
- Swallow J., Anderson D., Buckwell A., Harris T., Hawkins P., Kirkwood J., Lomas M., Meacham S., Peters A., Prescott M.J., Owen S., Quest R., Sutcliffe R. and Thompson K. (2005) [Guidance on the transport of laboratory animals](#). Report of the Transport Working Group established by the Laboratory Animal Science Association (LASA). *Laboratory Animals* 39(1):1-39 (accessed on 2019-02-19).
- Swartz K.B. and Rosenblum L.A. (1980) Operant responding by bonnet macaques for color videotape recordings of social stimuli. *Animal Learning & Behaviour* 8(2):311-321.
- Tanner J., Dumville J.C., Norman G. and Fortnam M. (2016) [Surgical hand antisepsis to reduce surgical site infection](#). *The Cochrane database of systematic reviews* 22(1):CD004288 (accessed on 2019-02-19).
- Taylor W.J., Brown D.A., Davis W.L. and Laudenslager M.L. (1997) [Novelty influences use of play structures by a group of socially housed bonnet macaques \(*Macaca radiata*\)](#). *Laboratory Primate Newsletter* 36(1):4-6 (accessed on 2019-02-19).
- t'Hart B.A., Bogers W.M., Haanstra K.G., Verreck F.A. and Kocken C.H. (2015) The translational value of non-human primates in preclinical research on infection and immunopathology. *European Journal of Pharmacology* 759: 69-3.
- Toth L.A. and Gardiner T.W. (2000) [Food and water restriction protocols: Physiological and behavioral considerations](#). *Contemporary Topics in Laboratory Animal Science* 39(6):9-17 (accessed on 2019-02-19).
- Truelove M.A., Martin A.L., Perlman J.E., Wood J.S. and Bloomsmith M.A. (2017) Pair housing of macaques: A review of partner selection, introduction techniques, monitoring for compatibility, and methods for long-term maintenance of pairs. *American Journal of Primatology* 79(1):1-15.
- Turner P.V., Brabb T., Pekow C. and Vasbinder M.A. (2011) [Administration of substances to laboratory animals: Routes of administration and factors to consider](#). *Journal of the American Association for Laboratory Animal Science* 50(5):600-613 (accessed on 2019-02-19).
- Veeder C.L., Bloomsmith M.A., McMillan J.L., Perlman J.E. and Martin A.L. (2009) [Positive reinforcement training to enhance the voluntary movement of group-housed sooty mangabeys \(*Cercocebus atys atys*\)](#). *Journal of the American Association for Laboratory Animal Science* 48(2):192-195 (accessed on 2019-02-19).
- Verendeev A., Thomas C., McFarlin S.C., Hopkins W.D., Philips K.A. and Sherwood C.C. (2015) [Comparative analysis of Meissner's corpuscles in the fingertips of primates](#). *Journal of Anatomy* 227(1):72-80 (accessed on 2019-02-19).
- Vernes M.K. and Louwerse A.L. (eds.) (2010) *BPRC's Enrichment Manual for Macaques and Marmosets*. Rijswijk, The Netherlands: Biomedical Primate Research Center.

- Walters T.J., Ryan K.L. and Constable S.H. (2004) Thermoregulation by rhesus monkeys at different absolute humidities. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 174(6):481-487.
- Watson S.L., Shively C.A., Kaplan J.R. and Line S.W. (1998) Effects of chronic social separation on cardiovascular disease risk factors in female cynomolgus monkeys. *Atherosclerosis* 137(2):259-266.
- Weary D.M. (2012) [A good life for laboratory animals – How far must refinement go?](#) *Altex Proceedings*, 1/12, Proceedings of WC8, pp. 11-13 (accessed on 2019-02-19).
- Weiss D. and Hampshire V. (2015) Primate wellness exams. *Lab Animal* 44(9):342-344.
- Westlund K. (2015) Training laboratory primates – benefits and techniques. *Primate Biology* 2(1):119-132.
- Whittaker M. and Laule G. (2012) Training techniques to enhance the care and welfare of nonhuman primates. *Veterinary Clinics of North America: Exotic Animal Practice* 15(3):445-454.
- Wolfensohn S. and Honess P. (2005) *Handbook of Primate Husbandry and Welfare*. Oxford UK: Blackwell Publishing.
- Wolfensohn S., Sharpe S., Hall I., Lawrence S., Kitchen S., Dennis M. (2015) Refinement of welfare through development of a quantitative system for assessment of lifetime experience. *Animal Welfare* 24(2):139-149.
- Wood R.J., Maddison S., Rolls E.T., Rolls B.J. and Gibbs J. (1980) Drinking in rhesus monkeys: Role of pre-systemic and systemic factors in control of drinking. *Journal of Comparative and Physiological Psychology* 94(6):1135-1148.
- Wood R.J., Rolls E.T. and Rolls B.J. (1982) Physiological mechanisms for thirst in the nonhuman primate. *American Journal of Physiology* 242(5):R423-R428.
- World Organisation for Animal Health – OIE (2018) [Terrestrial Animal Health Code](#). Paris: OIE (accessed on 2019-02-19).
- Xing G., Lu J., Hu M., Wang S., Zhao L., Zheng W., Schofield J., Oldman K., Adkins D., Yu H., Platz S., Ren J. and Skinner M. (2015) Effects of group housing on ECG assessment in conscious cynomolgus monkeys. *Journal of Pharmacological and Toxicological Methods* 73:72-79.
- Yamada H., Louie K. and Glimcher P.W. (2011) Controlled water intake: A method for objectively evaluating thirst and hydration state in monkeys by the measurement of blood osmolality. *Journal of Neuroscience Methods* 191(1):83-89.
- Zou H., Luan Y., Liu M., Agre L.A., Buyske S., Xie Q., Cheng Z., Zhao G., Jin M., Guo N., Jin G.J. and Yu L. (2015) Differential behavior patterns in cynomolgus monkey *Macaca fascicularis* in home cage in response to human gaze. *Journal of Medical Primatology* 44(1):1-11.

APPENDIX 1

NONHUMAN PRIMATE HOUSING SYSTEM ASSESSMENT TOOL

The housing system must have the following features:

- allow for social housing;
- provide high perching space for each animal;
- contain sufficient and proper space for each animal to sleep in a normal manner;
- enable proper access to food and water;
- allow for proper sanitation; and
- be safe for the animals and for humans.

Outdoor housing must also have the following:

- secure perimeter to prevent escapes and intrusions;
- shelter from elements; and
- indoor access during cold weather.

An evaluation of the housing system also includes the elements listed in the table below. “Maximal use of the available room vertical space” is the most important element and is scored on a scale of 1-10 and other elements are rated on a scale of 1-5, where a score of “1” indicates the worst possible state. A total score is then calculated to determine if the housing system is optimal, acceptable or inadequate.

Table 1 Nonhuman Primate Housing System Assessment Tool

Housing Elements	Score									
	1	2	3	4	5	6	7	8	9	10
Maximal use of the available room vertical space										
Provides privacy areas to hide										
Allows escape from aggressor										
Prevents the monopolizing of food, water, and resting areas by dominant individuals										
Allows for foraging										
Animals can walk, run, climb, leap, swing, hang										
Optimal use of the 3-dimensional space by incorporating shelves, logs, ladders, climbing structures, branches, hammocks, swings, ropes, pool, etc.										
Wooden structures are provided										
Flooring substrate (bedding) is provided										
Presence of objects such as toys, mirrors, logs, branches, paper, cardboard boxes, etc.										
Allows separation of animals for treatments, feeding, training, and the introduction of new animals to a pair or group										

Housing System Status	Total Score
Optimal	>43
Acceptable	33-43
Inadequate	<33

APPENDIX 2

ANIMAL HEALTH TESTS

Health screening should be tailored to the particular animals and colony.

Tests	Details	Frequency of Testing
Tuberculosis	Mammalian old tuberculin (MOT) ¹	<p>All animals – upon arrival and then preferably every six months, with a minimum of once per year. Also used as a diagnostic tool when animals show clinical signs of disease.</p> <ul style="list-style-type: none"> • frequency depends on the particular animal, conditions, number of animals in the room, and the opportunity for tuberculosis to spread to other animals • done in conjunction with the regular physical exam as it requires chemical immobilization to ensure proper injection • suspected tuberculosis cases should undergo re-testing, followed by another diagnostic means if necessary for confirmation
Clinical pathology		<p>All animals – upon arrival and then preferably every six months, with a minimum of once per year. Also used as a diagnostic tool when animals show clinical signs of disease.</p> <ul style="list-style-type: none"> • done in conjunction with the regular physical exam. • particularly important for animals on water regulation, animals with explants or implants, and animals older than 12 years of age
Serology	<i>Macacine herpesvirus 1</i>	All macaques – annually
	Simian retroviruses (SRV)	<p>All Old World monkeys – annually</p> <ul style="list-style-type: none"> • can be discontinued for a closed colony that has been tested for three years with no positive results
	Simian immunodeficiency virus (SIV)	<p>All Old World monkeys – annually</p> <ul style="list-style-type: none"> • can be discontinued for a closed colony that has been tested for three years with no positive results
	Simian T-cell lymphotropic virus (STLV)	<p>All Old World monkeys – annually</p> <ul style="list-style-type: none"> • can be discontinued for a closed colony that has been tested for three years with no positive results

¹ Mammalian old tuberculin (MOT) is CFIA's preferred method of tuberculosis testing at the time of publication of this guidelines document (CFIA, 2009). It should be performed intradermally on the abdomen (marmosets and tamarins) or on the eyelid (other species) (CFIA, 2009). Purified protein derivative (PPD) is another method of testing, but is not recommended and should only be used when MOT is not available.

Tests	Details	Frequency of Testing
Microbiology		<p>All animals – prior to import in accordance with CFIA requirements, upon arrival and then preferably every six months, with a minimum of once per year. Also used as a diagnostic tool when animals show clinical signs of disease (e.g., diarrhea lasting more than 3-5 days, blood in feces).</p> <ul style="list-style-type: none"> positive tests may not necessitate treatment; the appropriate response to positive results should be determined by the veterinarian prior to testing
Parasitology	Parasites	<p>All animals – upon arrival and then preferably every six months, with a minimum of once per year. Also used as a diagnostic tool when animals show clinical signs of disease (test by fecal smears and flotation).</p> <ul style="list-style-type: none"> if tests are positive, animals should be monitored, but not necessarily treated, to avoid resistance problems
	Malaria	<p>Animals from areas with malaria – at least once during pre-shipment quarantine and during quarantine via a blood smear and/or polymerase chain reaction.</p> <ul style="list-style-type: none"> malaria is easily treated but the drugs may have side effects

References

Canadian Food Inspection Agency (CFIA) (2009) [*Requirements for Non-Human Primates Imported into Canada*](#). Canadian Food Inspection Agency site (accessed on 2019-02-19).

APPENDIX 3

FOOD AND FLUID INTAKE REGULATION

1. FOOD INTAKE REGULATION

Implementation of Food Intake Regulation

Where regulation of food intake is required for a study, a food intake baseline should be calculated on an individual animal basis, taking into account the anticipated amount required to support maintenance and growth (APV, n.d.). Food intake regulation should be implemented gradually (e.g., 5% decrease per month) to determine the minimum level of regulation necessary for the animal to perform the required task. It is generally recommended that the total amount of food provided should not be less than 85% of the full ration recommended by NRC (2003a) (APV, n.d.).

Monitoring

Animals must be monitored closely on a daily basis and body weight must be recorded at least once a week. Suggested criteria for monitoring are described in Table 1.

Table 1 Monitoring Criteria for Food Intake Regulation

Food consumption (total)	<ul style="list-style-type: none"> if an animal does not get the total ration in training, the remaining allotment should be given afterwards
Performance on test	<ul style="list-style-type: none"> there should be a qualitative determination whether the animal's performance is consistent
Body weight (preferably measured at the same time each day)	<ul style="list-style-type: none"> weight loss should not exceed 15% or result in a body weight less than 85% of the optimum baseline or normal growth curve for each particular animal for growing monkeys, the anticipated normal growth curve of the animal should be used as a reference, with extra attention focused on years 3 and 4 where there is usually a growth "burst" in macaque species (Prescott et al., 2010) weight loss over a certain period may not be a major concern in an obese animal, as determined by body condition scoring
Behaviour	<ul style="list-style-type: none"> observations should be made of any change in behaviour, depressive behaviour, etc.

Hematology and blood biochemistry can be used to assess health status of an individual showing clinical signs that do not resolve after stopping the food intake regulation.

Termination of Food Intake Regulation

In general, food intake regulation should be temporarily stopped for any animal that exhibits any of the following (APV, n.d.):

- weight loss >15% of their projected optimal body weight;
- body condition score of 2/5 or less (see Clingerman and Summers, 2012);
- development of new significant abnormal behaviours; and
- abnormal laboratory data.

Animals may be returned to the study when conditions improve; however, they should be permanently removed if the problem persists after the animals have been removed from the study more than twice (APV, n.d.).

2. FLUID INTAKE REGULATION

Implementation of Fluid Intake Regulation

Where regulation of fluid intake is required for a study, a stable ad libitum baseline must be established. Fluid intake regulation should be implemented gradually to determine the minimum level of regulation necessary for the animal to perform the required task. If at any stage the animal does not consume the total volume of fluid available during testing, supplemental water must be given following testing to meet the daily minimal total volume. The animal's weight and hydration status must be assessed daily.

There is no single metric or minimum daily fluid requirement that has proven ideal for all animals in all research situations (APV, n.d.). The following is an example of a strategy for determining the smallest degree of fluid intake regulation required; however, it should be adapted to the particular animal and situation. Initially, the amount of fluid provided should be the ad libitum baseline. The amount of fluid consumed by the animal per day should then be gradually reduced at a rate based on the weight of the animal and the animal's response to performing the required task (for instance, up to a reduction of 14mL/kg per day) until the total volume of fluid consumed has been reduced by up to 50 mL/kg. If the animal does not perform the required task, the total volume of available water can be further decreased under veterinary surveillance, but at a lesser rate (for example by 5mL/kg per day and up to a total of 20 mL/kg), providing the animal does not exhibit weight loss exceeding 15% of its baseline body weight or show any clinical signs of dehydration.

Once the animal is fully trained to perform the task, it should be allowed to work for as much fluid as it wants every working day. This is the new testing baseline. However, experiments should be tailored on a daily basis in accordance with the animal's needs and response. When more trials are to be performed, the amount of the reward per trial should be reduced, but only to the point where the animal continues to perform effectively.

Investigators are encouraged to offer alternative rewards (e.g., fruit juices), allowing the animal to increase the testing baseline while still maintaining task performance.

On days when animals are not required to work (e.g., weekends), an amount of water equivalent to the testing baseline is to be provided to avoid large fluctuations. If animals will not be tested for more than three days, the amount of fluids they receive should be gradually increased.

Monitoring

Animals must be monitored closely on a daily basis and body weight must be recorded at least once a week. Suggested criteria for monitoring are described in Table 2.

Table 2 Monitoring Criteria for Fluid Intake Regulation

Fluid consumption (total)	<ul style="list-style-type: none"> if an animal does not get the total ration in training, the remaining allotment should be given afterwards animals on fluid intake regulation should also be monitored for food consumption, as it may decrease with dehydration or sickness; however, an initial small decrease in consumption is not unusual
Performance on test	<ul style="list-style-type: none"> there should be qualitative determination of whether the animal's performance is consistent
Body weight (preferably measured at the same time of day)	<ul style="list-style-type: none"> if weight loss exceeds 10%, veterinary staff must be consulted; however, weight loss of less than 10% should still be monitored an initial weight loss up to 15% is not unusual, followed by weight gain and eventual stabilization of body weight for adults, baseline body weight is the weight before the start of the study for short-term studies, and after a break in the study for long-term studies for growing monkeys, the anticipated normal growth curve of the animal should be used as a reference, with extra attention focused on years 3 and 4 where there is usually a growth "burst" in macaque species (Prescott et al., 2010)
Hydration	<ul style="list-style-type: none"> observations should be made of the animal's activity, skin elasticity, and fecal pellet size and texture where there is indication of possible dehydration, the hydration status should be verified through blood osmolality, which should be <320 mOsmol/kg H₂O
Behaviour	<ul style="list-style-type: none"> observations should be made of any change in behaviour, anorexia, depressive behaviour, urine drinking, etc.

Hematology and blood biochemistry can be used to assess the health status of an individual showing clinical signs that do not resolve after stopping the fluid intake regulation.

Termination of Fluid Intake Regulation

The following criteria will necessitate evaluation by the veterinarian:

- significant weight loss (i.e. exceeding 15% of baseline body weight) at any time during the study;
- apathy, lack of interest, depressive behaviour, abnormal behaviour, anorexia or reduced appetite that has not improved despite veterinary intervention; and
- abnormal blood parameters such as hemoconcentration, azotemia and electrolyte imbalances.

Upon consultation with the veterinarian, fluid intake regulation may be reduced or stopped. If terminated because of animal health, fluid intake regulation can only resume with approval from the veterinarian.

Withdrawal of fluid intake regulation occurs when testing is no longer required for extended periods. In such cases, the following procedure should be applied: starting from the testing baseline, increase water availability daily at increments of 20mL/kg/day until the animal is receiving its ad libitum baseline. After reaching the ad libitum baseline, the animal can then have free access to unlimited water.

References

Association of Primate Veterinarians – APV (n. d.) [*Food Restriction Guidelines for Nonhuman Primates in Biomedical Research*](#) (accessed on 2019-02-19).

Clingerman K.J. and Summers L. (2012) [*Validation of a body condition scoring system in rhesus macaques \(Macaca mulatta\): inter- and intrarater variability*](#). *Journal of the American Association for Laboratory Animal Science* 51(1):31-36 (accessed on 2019-02-19).

National Research Council – NRC (2003b) [*Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research*](#). Institute for Laboratory Animal Research. Washington DC: National Academies Press (accessed on 2019-02-19).

Prescott M.J., Brown V.J., Flecknell P.A., Gaffan D., Garrod K., Lemon R.N., Parker A.J., Ryder K., Schultz W., Scott L., Watson J. and Whitfield L. (2010) Refinement of the use of food and fluid control as motivational tools for macaques used in behavioral neuroscience research: Report of a working group of the NC3Rs. *Journal of Neuroscience Methods* 193(2):167-188.

GLOSSARY

Affective state – refers to the mental state of an individual (or 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, resulting in depression of the nervous system, leading to loss of sensation and motor function.

Basic conditions – conditions that are required to meet the essential needs of animals for good health and well-being (e.g., food, water, secure space, space to perform species-specific movements, social interaction appropriate for the species).

Biocontainment – the quarantine or isolation of biohazards such as bacteria, viruses, fungi or other infectious agents that may be pathogenic to humans, animals or other forms of life.

Chemical restraint – the use of sedatives or anesthetics to control an animal's activity and thereby allow certain procedures to be performed with minimal stress to the animal.

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.

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.

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

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

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

New World nonhuman primates – South American species.

Old World nonhuman primates – African and Asian species.

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

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

Positive reinforcement training – animal training based on stimulus-response-reinforcement; the trainer asks for a behaviour using a signal (stimulus), the animal performs the requested behaviour (response), and then the animal gets something it wants (reinforcement).

Pre-conditioning – training or other preparation of an animal to be subject to confined housing or experimental procedures (including restraint).

Puzzle feeders – feeding devices which require some manipulation by the animal to gain access to food.

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; sedatives may be appropriate for some situations where pain and distress are anticipated.

Serology – diagnostic examination of blood serum, especially with regard to the response of the immune system to pathogens.

Standard operating procedure – written document that describes in detail how a procedure should be carried out.

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 W.M.S. Russell and R.L. Burch in 1959 in *Principles of Humane Experimental Technique*.

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