



Canadian Council on Animal Care
Conseil canadien de protection des animaux



CCAC guidelines:
Husbandry of animals in science

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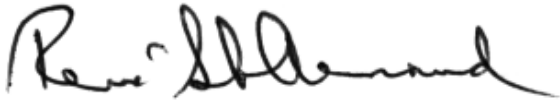
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Husbandry of animals in science

PREFACE

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

The *CCAC guidelines: Husbandry of animals in science* is part of a series of general guidelines documents that outline guiding principles for the ethics and care of all animals in science. This series streamlines information for investigators, animal care committees (ACCs), facility managers, veterinarians, technicians and animal care personnel to help facilitate improvement in both the care given to animals and the manner in which experimental procedures are carried out.

The *CCAC guidelines: Husbandry of animals in science* applies to husbandry practices for all animals held in institutions for scientific purposes. This includes wild animals brought into laboratory animal facilities for short or long term holding, but does not apply to wild animals in the field. Field studies are covered in the *CCAC guidelines on: the care and use of wildlife* (CCAC, 2003a).

These guidelines describe the standards that are expected to be met. The standards have been developed based on expert peer advice and current interpretation of scientific evidence.

In addition, CCAC guidelines are intended to provide a framework for the implementation of Russell and Burch's Three Rs (Replacement, Reduction and Refinement) (Russell and Burch, 1959), primarily the principle of Refinement. More specific information on husbandry practices can be found in the CCAC guidelines developed for specific types of animals and on the [CCAC website](#). These practices are constantly evolving and attention to this field should result in continual improvement in animal welfare.

SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT

Guideline 1:

The method for identifying animals should be appropriate for the type of animal and the requirements of the research project.

Section 2. Identification of Animals, p.6

Guideline 2:

Identification and marking techniques must be performed by trained personnel.

Section 2. Identification of Animals, p.7

Guideline 3:

Housing for animals in science should meet the physical and behavioural needs of the animals.

Section 3. Housing Management; Subsection 3.1 General Considerations, p.8

Guideline 4:

Sufficient good quality food must be provided to all animals, and be suited to the species, age of the animals and research requirements.

Section 4. Food, p.11

Guideline 5:

Fresh, clean drinking water should be supplied in a manner that makes it available to all animals at all times.

Section 5. Water, p.14

Guideline 6:

Species-appropriate bedding materials should be provided.

Section 6. Bedding, Nesting Material and Shelter, p.16

Guideline 7:

Measures should be sought to improve the welfare of the animals by enriching their environment in a manner that further addresses their species-specific and individual physical and behavioural needs.

Section 7. Environmental Enrichment, p.18

Guideline 8:

Animals should have opportunities to perform physical activities that allow the expression of species-specific behaviours.

Section 8. Exercise, p.21

Guideline 9:

Any contact, handling or restraint must take into account species and individual animal differences, and be conducted in a manner that minimizes stress for the animals and risks to the animals and personnel.

Section 9. Human Contact, Handling and Restraint, p.22

Guideline 10:

Health and behavioural monitoring must be in place to ensure that sick or injured animals are identified quickly and treated, as appropriate, and that the health status of the animals is maintained.

Section 10. Animal Monitoring; Subsection 10.1 Health and Behavioural Monitoring, p.26

Guideline 11:

Records appropriate to the species and animal-based procedures must be accessible.

Section 12. Record Keeping; Subsection 12.1 Access to Records and Record Retention, p.31

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 ACC.

Husbandry practices must aim to provide a good quality of life for the animals. Any approved research study should demonstrate husbandry plans that consider the normal physical, social and behavioural requirements of the animals.

Husbandry practices should be based on current practical experience, veterinary advice and scientific knowledge. The Five Freedoms (freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury or disease; freedom to express normal behaviour; and freedom from fear and distress) (FAWC, n.d.) provide a framework for assessing the welfare implications of husbandry practices.

Consideration of animal welfare implications must encompass the whole life of the animal, with appropriate husbandry provided from birth to end of life. The psychological well-being of animals can be enhanced by attention to their environment, which ideally should facilitate activity and rest, and incorporate appropriate complexity and opportunity to achieve behavioural goals, while providing stability and security (Poole, 1997).

The assessment of animal welfare is an evolving field¹, and the relevant literature should be consulted for a good understanding of the principles and current evidence-based practices (e.g., Mellor and Beausoleil, 2015; Hemsworth et al., 2015; the Welfare Quality Network’s *Welfare Quality Information Resource*; the CCAC website).

If animals are not provided with appropriate husbandry, this can have detrimental effects on both the welfare of the animals and the reliability of the scientific outcomes. Poole (1997) noted that poor welfare conditions can be associated with physiological, immunological and neurological changes in animals that can affect their reliability in scientific studies. For example, rodents raised in cages where the environment does not allow the expression of a range of normal behaviours have been shown to have altered brain functions (Wolfer et al., 2004; Würbel, 2001), as well as increased stereotypic behaviours and behaviours indicative of stress (Wolfer et al., 2004; Würbel and Garner, 2007), and handling has been shown to affect behaviour, reproduction and corticosteroids in pigs (Hemsworth et al., 1986). Furthermore, inconsistency in housing, environment (e.g., lighting, sound, etc.), application of procedures such as restraint, and interactions with

¹ For example, see Fraser et al. (1997) for earlier work in this field. Also see Mellor and Beausoleil (2015) for a review of how knowledge and understanding of animal welfare has changed since the introduction of the Five Freedoms.

animal care personnel (including changes in personnel) can increase experimental variability in animal-based research and affect the likelihood of achieving research goals (Reinhardt, 2004).

Standard Operating Procedures (SOPs) are an important component in ensuring proper husbandry practices are performed consistently, for the benefit of the animals and research. SOPs are written documents that provide step-by-step instructions for particular routine tasks or for particular procedures that are to be reproduced to give the same desired outcome. The US Environmental Protection Agency (EPA) provides details on developing SOPs and emphasizes that they should be readily accessible to all individuals responsible for carrying out such tasks (EPA, 2007). SOPs should be specific to each institution; however, sharing of SOPs among institutions and collaboration in the development of SOPs is a valuable way to apply expert opinion and experience for improvement of animal care and research. Examples of SOPs are provided on the [CCAC website](#).

Good record keeping and reporting of housing and husbandry activities are also essential elements of high quality research. Guidelines for reporting animal-based studies in journals encourage the inclusion of details on housing and husbandry (Kilkenny et al., 2010; NIH, 2016).

The guidelines that follow provide general information to assist institutions in the establishment of good husbandry practices for animal-based science.

2 IDENTIFICATION OF ANIMALS

Guideline 1:

The method for identifying animals should be appropriate for the type of animal and the requirements of the research project.

All animal enclosures (e.g., cages, pens, tanks, pastures, etc.) should be clearly marked, indicating the sex, date of birth, source and number of animals; specific instructions for their care; protocol number; and the investigator responsible for the animals. This should be done in a manner that cannot be tampered with by the animals, particularly in the case of nonhuman primates, or affected by environmental conditions such as humidity, as in the case of fish tanks. Where providing this information is not practical (e.g., accurate counting and sexing of newly hatched fish, or where the exact birth date of an animal is not known), estimates may be acceptable, at the discretion of the ACC.

In situations where individual identification is not necessary for the study or not possible (as in tanks of zebrafish), identification at the enclosure or group level may be sufficient. Where individual identification is required, the method should be the least invasive means of providing quick and unambiguous identification of the individual animal for the entire time it is housed. The choice of identification method should also take into consideration any potential impacts on the research, such as the need for blinding of research personnel.

In determining the most appropriate identification method to use, investigators should minimize any pain, distress and discomfort, including that associated with any restraint of the animal, using anesthetics and analgesics as appropriate.

Use of sedatives or local anesthetics and analgesics must always be considered in consultation with a veterinarian, and must be used if deemed necessary (i.e., if the pain and/or distress that the animal would experience from the identification procedure is greater than what would be experienced from the application of sedatives, anesthetics or analgesics and the associated restraint). Application of these substances must follow SOPs addressing associated human health and safety concerns.

Where genotyping is required, some methods of identification (e.g., ear punch) may provide appropriate tissue samples; however, the method that causes the least pain, distress and discomfort for both identification and genotyping should be used whenever it is possible. Non-invasive methods of acquiring samples for genotyping (e.g., mouth swabs, fur or feces collection) may be suitable for some studies, and in these situations, they should be the preferred methods.

Guideline 2:

Identification and marking techniques must be performed by trained personnel.

As noted in the *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015), training must be provided to personnel so that they have the necessary knowledge and technical skills to carry out required procedures. For identification, this includes training in handling and restraint, as well as training to conduct the relevant identification method. Information on potential methods of identification for various species is provided in the relevant CCAC guidelines developed for specific types of animals.

HOUSING MANAGEMENT

3.1 GENERAL CONSIDERATIONS

This section focuses on husbandry requirements related to the management of animals within facilities to address the animals' health, need for social contact, and expression of typical behaviours. While guidance on the physical aspects of facilities is provided in the *CCAC guidelines on: laboratory animal facilities – characteristics, design and development* (CCAC, 2003b), the ability to maintain appropriate animal environments through good husbandry practices is closely linked to the infrastructure and operation of the facility.

For guidance on special husbandry needs of animals during transport or quarantine/isolation, see the *CCAC guidelines on: procurement of animals used in science*, Section 5, “Transportation” and Section 6.3, “Acclimatization and Quarantine” (CCAC, 2007).

Guideline 3:

Housing for animals in science should meet the physical and behavioural needs of the animals.

Housing must address the basic needs of the animals, for example, the provision of sufficient food and water of good quality; space for normal species-specific postures and freedom of movement; clean areas to eat, hide and rest; appropriate temperature and humidity; and safety from injury and predation. Housing management should take into account the species, strain/breed, age, sex, physical development, temperament, hierarchy, previous experience of the particular animals involved, etc. Meeting the basic needs of the animals is critical to their well-being and normal development, and for the maintenance of homeostasis and normal physiology to ensure that the animals are “normal” for research purposes (for example, see Karp, 2012).

It is important to consider housing requirements when designing experiments, and any variations from standard conditions must be justified to the ACC. For example, metabolic cages for rodents and livestock or battery cages for poultry (instead of enriched cages or other systems that allow more freedom) must be justified. Normal housing conditions should be kept uniform within a study. Conditions which depart from the normal conditions should be as short in duration as possible. All housing conditions should be documented so that details can be reported in publications.

Additional information on enriching the environment is provided in Section 7, “Environmental Enrichment”.

3.2 SEPARATION OF ANIMALS ACCORDING TO SPECIES AND HEALTH STATUS

Different species should normally not be housed in enclosures that share the same ventilation or water circulation system. However, exceptions may apply, for example livestock barns or housing for mammalian,

avian or aquatic species where species interaction is desirable or may provide enrichment (e.g., community aquariums). Similarly, animals of different health status should be housed under separate ventilation or water circulation systems.

Prey and predator species should be separated such that there is no tactile, visual, olfactory or auditory contact (for example, see Arndt et al., 2010). Animals housed in the same room should also be compatible with regard to requirements for photoperiod, light intensity, room temperature, humidity, etc.

3.3 SPACE REQUIREMENTS

Animals require sufficient space to rest and exercise, access food and water, and freely express normal postures and behaviours. Information on space requirements for various species is provided in the relevant CCAC guidelines for specific types of animals.

Space requirements should be assessed by the ACC and veterinarian, in consultation with other experts when needed, to ensure they are appropriate for the species. For group-housed animals, the size of the social group in relation to its available space should be regularly assessed; as animals grow and mature, group size or enclosure size may require adjustment to ensure adequate space is available.

Rabbits and larger animals that are not able to meet their needs for physical activity within the constraints of their cages/stalls must be given access to an exercise area on a regular basis (see Section 8, “Physical Activity”).

3.4 SOCIAL HOUSING

Appropriate housing arrangements should be based on the normal social behaviour of the species and an assessment of the particular behaviour of the animals involved. Most experimental animals are social and benefit from the company of conspecifics or humans. Social animals should be housed in compatible groups to allow for basic social contact, which can help them cope with the constraints of confinement.

Group-housed animals must be monitored and the grouping regularly reassessed to ensure members of the group remain compatible (see Section 10.2, “Monitoring Responses to Changes in Housing and Husbandry”). Changes in daily routines, limited resources (e.g., food, water or resting spaces), grouping incompatible animals, frequent addition/removal/reintroduction of individuals from the group, or changes in sexual maturity of group members may disrupt hierarchies and lead to an increase in aggressive interactions. Special care must be taken when the social environment changes, particularly when animals are remixed, because of serious welfare concerns related to aggression and fighting. Maintaining group stability is important, but where the welfare of some members is compromised, incompatible individuals may need to be separated.

3.5 SINGLE HOUSING

Animals should not be singly housed unless there is sufficient justification based on medical or behavioural needs or the requirements of the study. In such cases, the animals should be returned to group housing as soon as practical, with careful monitoring (see Section 10.2, “Monitoring Responses to Changes in Housing and Husbandry”).

All protocols requiring single housing must justify its need, specify the duration of the single housing period, and describe proposed measures for meeting the social requirements of the animals. Where animals are singly housed, they should be provided with some degree of social contact whenever possible (e.g., visual, olfactory and/or auditory contact with conspecifics, or increased contact time with people as appropriate). For some animals, the illusion of contact with other animals (e.g., through the use of mirrors) may provide some benefit where social contact is not possible.

For many species, the negative effects of social isolation can be reduced by providing environmental enrichment (see Section 7, “Environmental Enrichment”). However, some species will always be stressed when housed alone, regardless of any enrichment provided (e.g., sheep (Carbajal and Orihuela, 2001); nonhuman primates (Dettmer and Fragaszy, 2000; Eaton et al., 1994); and horses (Yarnell et al., 2015)) and the need to singly house such species requires special justification.

3.6 OUTDOOR ENVIRONMENTS

Animals kept outdoors must be provided with, or have access to, shelter, protected feeders/waterers and resting areas, that are suited to the climate, the numbers of animals and the needs of the species and breed/strain, such that competition for resources is minimized. Management programs should be in place to address the special risks associated with outdoor environments, such as terrestrial and aerial predators, parasitic insects and arachnids, toxic plants, and adverse weather events. Animals that are new to an outdoor environment may need to undergo a period of acclimation.

4 FOOD

Guideline 4:

Sufficient good quality food must be provided to all animals, and be suited to the species, age of the animals and research requirements.

Food includes the base diet needed for maintaining the normal physiological and health status of the animals, as well as any food used for positive reinforcement and/or environmental enrichment. All food provided must meet the nutritional needs of the particular animals, as the different types of food available vary considerably in the content of their nutrients (such as protein, carbohydrate, fat, fiber, minerals and vitamins), digestibility, and the degree of control over ingredients, quality and other variables. Where the research requires exceptions to this, justification must be provided and approved by the ACC. The food provided to an animal should be adjusted as its physiological conditions change (e.g., during gestation, lactation, or maturation-phase for egg-bearing animals such as fish) or in response to sickness or dental problems, to meet its specific nutritional requirements.

4.1 FOOD AND FOOD INGREDIENTS

Food and food ingredients used in in-house formulations must be purchased from a supplier whose practices ensure quality and consistency and minimize the potential for exposure to vermin and contaminants during production, storage and transport. Institutions should periodically request that suppliers provide reports on laboratory analysis of feed to ensure the nutrient content remains constant throughout a study.

It is important to maintain the quality and consistency of the diet for the duration of a study and for related studies, and, where possible, the same supplier should be used. Consideration should always be given to whether the source of feed could introduce unknown or uncontrolled variables for particular research projects. Investigators should be aware that there is the potential for changes in feed supply to introduce variability, particularly for long-term studies.

Feed shipments should be examined upon receipt, and records should be kept as noted in Section 12.3.2, “Records for Food, Water and Bedding”. Where expiry dates are given for feed, these should be followed. For custom formulated and small-run mill diets, consideration should be given to retaining a sample in case further testing is required.

Food used for environmental enrichment (see Section 7, “Environmental Enrichment”) should also be of high quality, not contaminated or a source of vermin, and be suited to the species and experimental protocol.

4.2 FOOD STORAGE

Commercially prepared feed should be stored according to the manufacturer's recommendations. Feed bags should be placed off the floor on easily cleaned pallets, preferably plastic or metal, in an arrangement that ensures the oldest bags are used first. Feed in opened bags should be transferred to vermin-proof containers, and feed containers should be emptied and cleaned before refilling to ensure complete turnover of feed.

Feed must be stored in designated storage areas, with the exception of quantities of feed sufficient for a reasonable period (based on the type of feed) that may be kept in animal holding rooms in covered, vermin-proof containers, providing that conditions in the holding room meet feed manufacturer's requirements for temperature, humidity, etc.

Records of milling date, food ingredients and food decanting should be kept as part of a quality assurance program to maintain the quality of the food (see Section 12.3.2, "Records for Food, Water and Bedding").

Foods that are not commercially prepared (e.g., hay, silage, vegetables) should be stored according to standard practices for temperature, humidity, expiration date, etc.

Animals should not be fed food that is past the expiry date or manufacturer's recommendations for shelf life. Freezing can prolong the expiration of certain foods; however, potential deterioration of micronutrients from conditions and/or storage time should be evaluated by facility personnel, in consultation with the investigator, as appropriate. Information on freezing times for some foods is available on the [Canadian Partnership for Consumer Food Safety Education's website](#), and may also be obtained from the manufacturer. Processing of food using methods such as autoclaving should only be done if the food is certified for that type of processing (e.g., autoclavable).

Institutions should have SOPs in place to maintain the cleanliness of the storeroom, including a program for vermin control. Where cold storage is used, stored food supplies of pellet feed (e.g., for fish) should be checked for growth of mold, and records of checks should be kept. Compounds, such as detergents or chemicals that may compromise the safety or quality of feed, should not be stored with feed.

The use of live vertebrates as food is discouraged for ethical reasons, and alternatives should be found. However, if live vertebrates are required for the appropriate care of a particular species or are an integral part of the research, and approval has been given by the ACC, those animals should be provided with the same housing and husbandry standards as other animals in science.

4.3 FEEDING

Food must be provided in a manner that makes it available to each animal. The principal method of feeding should minimize deterioration and contamination of the food. However, this does not preclude supplementary feeding for environmental enrichment purposes (see Section 7, "Environmental Enrichment"), or methods to address an animal's needs during postoperative care or at other times where supplementary feeding may benefit the animal's welfare.

Food should normally be available according to the species-specific needs of the animal. Animals should be provided a sufficient, but not excessive, quantity of food, according to study requirements and monitoring.

Determining the appropriate amount of food should take into account the energy and nutrient content of both the base diet and any food given as part of the environmental enrichment program, and the condition of the animals (e.g., mass, developmental stage, etc.).

Where fasting is a component of a scientific study, it must be described and justified in an ACC-approved protocol. The duration of the fasting period must be suited to the species and always be as short as possible.

Drastic changes in diet should be minimized to avoid digestive and metabolic problems and to prevent introducing variables into the research.

Food can be a source of environmental enrichment, particularly in terms of how it is presented (e.g., scattered food for foraging; puzzle feeders; whole fruits, vegetables or seeds; or live invertebrates for fish). All use of supplemental food for environmental enrichment must be discussed with, and agreed upon by, the investigator and veterinarian. Any food provided as environmental enrichment should be managed to ensure animals consume the necessary amount of their primary diet to meet their nutritional requirements.

If an animal becomes sick and reduces its food or water intake, whether as the result of an experimental procedure or not, the animal must be monitored and action must be taken to provide for the nutritional needs of that animal (e.g., by providing soft, palatable food, making food and/or water more accessible to the animal, or conducting subcutaneous rehydration). Any potential food-related problems should be investigated and documented.

The amount of food provided at a given time will depend on the method of delivery (e.g., when a hopper is used, enough feed must be added so that the feed flows freely and is easily available to the animals). Any leftover food that could spoil should be removed within a timeframe that ensures the animals are offered only appetizing food (i.e., not discoloured, moldy or soiled) that has the proper nutrient content.

Light, temperature, humidity and food turnover at the cage/pen level must also be controlled to prevent degeneration of micro- and macro-molecules of the food over time. Moreover, any particular requirements for special diets should be noted (see manufacturer's instructions); for example, some medicated foods should not be exposed to light or some high fat diets need to be changed twice daily. Feeders should be checked regularly for wet, moldy, or encrusted feed.

Food dispensers should reliably provide food in a secure manner. They should be reasonably vermin-proof and able to shed occasional water-droplets and other airborne debris without affecting food contents.

When there is a change in the food delivery system, each animal must be closely monitored to ensure they are able to access the food. For automated feeders, a back-up strategy for providing food to the animals should be in place in the event of failure in the system.

Food containers, such as bowls, must be cleaned regularly to prevent bacteria, mold or insect colonization. This is particularly important for moist food. There should be regular verification of the cleaning process.

5 WATER

Guideline 5:

Fresh, clean drinking water should be supplied in a manner that makes it available to all animals at all times.

Where water restriction is part of a scientific study, it must be described and justified in an ACC-approved protocol. The duration of water restriction must be suited to the species and always be as short as possible.

5.1 WATER QUALITY

Water quality monitoring is important to ensure the chemical composition of the water remains within specified limits and that any potential sources of contamination do not affect the health of animals or the research. In general, water quality should be tested regularly and should meet *Canadian Drinking Water Guidelines*. However, the specific water quality parameters to be measured and acceptable ranges for these will depend on the species and the research.

Water quality should be monitored at least annually; however, more frequent monitoring may be required, depending on the water dispensing system and the type of water being delivered, i.e., treated water (reverse osmosis, UV, acid, autoclave), municipal water or well water. The quality of municipal and well water supplies is very site specific. The quality of municipal water can be confirmed by municipal procedures; however, water from a well needs to be analyzed regularly.

The type of water treatment should be selected with consideration of the effects on the animals and the research, as some methods can affect the health and physiology of the animals (Fidler, 1977; Hall et al., 1980; Hermann et al., 1982). Acidification of water is a common practice to control the growth of micro-organisms; however, it may cause leaching of heavy metals from the water dispensing system (Nunamaker et al., 2013). Monitoring the quality of drinking water should therefore occur at the point where water is available to the animals (Nunamaker et al., 2013).

For aquatic animals, additional precautions for ensuring appropriate water quality are warranted (CCAC, 2005).

5.2 WATER DISPENSING SYSTEMS

Selection of the water dispensing system should be based on the ability to: 1) provide sufficient quantities of water to all animals at all times; and 2) minimize the potential for contamination of the water supply, spread of disease, and flooding of the environment. Regardless of the type of system, it should be checked and maintained frequently, following manufacturer's recommendations, to ensure these criteria continue to be met.

Where animals are housed outdoors, precautions must be taken to prevent freezing of the water supply, for example by providing heated water bowls or circulating water. Snow is not a sufficient water source for animals in science.

5.2.1 Water Containers

Water containers (e.g., bottles and bags) should be suited to the species and type of husbandry.

Water bottles for regular drinking water should be transparent to permit easy observation of cleanliness and water level, and be designed to facilitate cleaning and sterilization. Facilities should be aware of the possibility of leaching from plastic water bottles.

Care must be taken to cycle dispensed water and keep records of when bottles are replaced. Water bottles should be replaced with clean, freshly filled bottles, rather than refilling them when empty, and sippers should be changed when bottles are replaced. Bottles containing regular drinking water should be checked daily to ensure water continues to be available and there is no leakage. They should be replaced at cage change or when levels are low, or more often if necessary, depending on the species, research and animal density in the cage.

For medicated water, the bottles must be identified and the drinking solution must be changed as recommended by the manufacturer. The use of medicated water may require shading of water bottles to maintain the efficacy of a particular compound. It is important to regularly resuspend medication, if not completely soluble. The residual volume that is not consumed by the animal must be noted to ensure the animal receives the correct amount. Disposal of the residual volume must be done according to regulations to avoid environmental contamination.

Water should be dispensed into bottles as close to the time of use as possible. When water is dispensed and not used promptly, it should be tested for bacterial levels and the maximum time appropriate for water storage in that facility and bottle should be identified. The shelf-life is longer for sterilized water.

5.2.2 Water Delivery Systems

When animals are introduced to a new water delivery system, each individual animal must be observed until there is assurance that they are appropriately adapted to the new system. The flow rate in water delivery systems should be suited to the needs of the individual animals and checked regularly according to manufacturer's recommendations. Practices to facilitate access to the water should be implemented, if necessary.

Water delivery systems should be checked, maintained and sanitized (including the cleaning and changing of valves) according to the manufacturer's recommendations. At the cage or pen level, daily observations are necessary in order to identify possible floods or problems with accessibility of water for the animals (e.g., valve blockage). Where possible, structures should be provided within cages/pens for animals to escape flooding should it occur. A back-up system for providing water to the animals should be in place in the event of failure of the water delivery system.

If a problem with the water delivery system occurs, water quality must be verified once the issue is resolved to ensure clean water is delivered to the animals.

BEDDING, NESTING MATERIAL AND SHELTER

Guideline 6:

Species-appropriate bedding materials should be provided.

Bedding appropriate to the species, strain, size and number of animals, as well as the housing system, should be provided (see the CCAC guidelines developed for specific types of animals). Bedding can provide the following benefits, depending on the species:

- assist in maintaining a healthy environment – by absorbing moisture, minimizing growth of microorganisms, reducing contact with feces and urine, and reducing ammonia accumulation;
- provide animal comfort – by providing a comfortable substrate for resting and a means to manage the microenvironment (e.g., nest building to avoid cold stress (Gaskill et al., 2013)); and
- allow expression of species-specific behaviours – by providing opportunity for foraging, digging, rooting and burrowing.

Where bedding is not provided to a species that typically has bedding, it must be justified to the ACC.

Bedding must be safe for the animals and suited to the requirements of the research. For example, cedar shavings emit aromatic hydrocarbons that induce hepatic microsomal enzymes and cytotoxicity (Torronen et al., 1989; Weichbrod et al., 1988). Other types of bedding may produce significant levels of dust that may cause health problems for some animals. Moreover, unsterilized bedding materials are a possible source of disease introduction. Bedding materials should also be unpalatable to the animals, so as not to affect their diet or the research.

Bedding material should always be considered in experimental design, and efforts should be taken to maintain the same type of bedding throughout a study, as a change could impact the results of the research.

Nesting material and shelter should be provided where they are important to the welfare of the animals; such that they allow the expression of species-specific behaviours and/or provide comfort (see the CCAC guidelines developed for specific types of animals).

6.1 PURCHASE OF MATERIALS

As with feed, bedding and nesting materials must be purchased from a supplier whose practices ensure the quality of the material and minimize the potential for exposure to vermin and contaminants during production, storage and transport.

If an institution is contemplating the acquisition of new bedding or nesting material, the applicability and safety of that material should be tested before it is used in routine husbandry practices.

6.2 STORAGE OF MATERIALS

Bedding and nesting material should be stored according to manufacturer's recommendations, if available. Bags should be placed off the floor on easily cleaned pallets, preferably plastic or metal, with bags rotated so that the oldest is used first. Bedding for livestock (e.g., straw or wood shavings) should be stored in a manner that prevents excessive moisture build up.

If bedding is autoclaved, it can absorb moisture, so appropriate drying time and storage conditions should be implemented to ensure its quality and effectiveness are maintained and reliable (NRC, 2011).

Institutions should have SOPs in place to maintain the cleanliness of the storage area, including a program for vermin control.

6.3 BEDDING CHANGES

Bedding should be changed as appropriate to keep animals clean, dry, and relatively odour free, and to ensure that the level of ammonia does not irritate the animals. SOPs should be in place for bedding changes, suitable to the particular animals and housing conditions. For animals that rely on odours for communication (e.g., mice), it may be important to transfer a portion of the nesting material when enclosures are cleaned (Van Loo et al., 2003). Cage changes may be a source of stress for some species (e.g., mice and rats), and this should be a consideration in determining the appropriate frequency (Gerdin et al., 2012; Rasmussen et al., 2011; Meller et al., 2011). Bedding should be cleaned out between animals or groups, as appropriate for the species (see the CCAC guidelines developed for specific types of animals).

7 ENVIRONMENTAL ENRICHMENT

Guideline 7:

Measures should be sought to improve the welfare of the animals by enriching their environment in a manner that further addresses their species-specific and individual physical and behavioural needs.

7.1 DEFINITION AND OBJECTIVES OF ENVIRONMENTAL ENRICHMENT

Environmental enrichment is a term used to refer to provision of stimulating and responsive environments (Shepherdson, 1998) above the basic conditions that meet the animal's physical, physiological and psychological needs (see the CCAC guidelines developed for specific types of animals for details about basic conditions and enrichment). Implementation of environmental enrichment should focus on improving the quality of the animal's environment by expanding the choice of activities conducive to good welfare and increasing the animal's ability to control its environment (Newberry, 1995 and Stauffacher, 1995, as cited in Baumans, 2005). Bayne and Würbel (2014) categorize enrichment into three groups: social enrichment, structural enrichment, and physical and cognitive activity. They further note that environmental enrichment should be considered part of a broader behavioural management program and be tailored to the particular animals involved. In addition, because animals in enriched environments have shown less anxiety-related behaviour in experimental situations (Würbel, 2007), there may be less variation between results, which may ultimately reduce the number of animals required.

Modifications to an animal's environment should focus first on the fundamental 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). Morton (2013) refers to the low-ranking needs of animals as "wants" and states, "For animals to be contented and free from states of mental distress, such as boredom and frustration, it is important that some of their wants are met as well as their physiological needs, such as food, water and clean air." This approach should result in the induction of positive affective states, while reducing negative affective states, recognizing that sometimes what animals want may not always be good for them (such as eating more of the environmental enrichment food than their normal diet).

It is imperative to recognize and maintain standard conditions (i.e., the basic conditions that must be met for animals); however, constant refinement of environmental enrichment, and of the environment in general, should be provided when possible. Any request for withdrawal or reduction of enrichment must be justified and approved by the ACC.

7.2 CONSIDERATIONS FOR ENVIRONMENTAL ENRICHMENT

7.2.1 Effects on the Animals

Effective environmental enrichment considers the species-specific needs and “wants”, and any particular requirements for the strain and individual animals. Some strains may have unique characteristics; for example, some transgenic animals exhibit a greater stress response in particular situations (Brown and Murray, 2006). Additionally, individual animals will show variation in response to environmental enrichment due to previous housing conditions and experiences.

The impact of any environmental enrichment should be evaluated in relation to the physical or behavioural need/want that it is intended to address (Baumans and Van Loo, 2013) and whether it is safe for the animal. This could be accomplished through observation of the animals and how they respond to the change in their environment, as well as through the use of preference tests (Baumans and Van Loo, 2013; Dawkins, 2003). Consideration should also be given to the way in which environmental enrichment is presented, or the amount of enrichment presented, such that it does not lead to competition, fighting, hoarding, etc.

Possible means of enriching an animal’s environment are provided in the CCAC guidelines developed for specific types of animals.

7.2.2 Effects on Research

In terms of scientific outcomes, attending to the well-being of research animals is important to ensure that avoidable stress is minimized, thus limiting the introduction of variables into the research model (Poole, 1997). Animals with compromised welfare may be physiologically and immunologically abnormal, which can confound research results.

Würbel (2001) reviewed a number of studies on rodents that examined the impact of environment, and suggested that housing conditions can negatively impact the usefulness of rodents in research, particularly in behavioural neuroscience. For example, the brains of rats housed in enriched environments are structurally different from those in impoverished environments, resulting in a difference in behaviour and neurochemistry (Würbel, 2001).

Because changes to the environment can result in modified behaviours, it is recognized that environmental enrichment must be implemented in line with experimental objectives. As always, in developing and reviewing an experimental protocol, consideration should be given to any potential impacts on the scientific validity of the animal model, statistical power, relevance of comparisons to historical data, and reproducibility of the study (Baumans and Van Loo, 2013). However, as noted by Baumans and Van Loo (2013), “For the scientific validity of results, it is most important that the environmental refinement program leaves the animal model intact, and that even if results differ from historical data, valid conclusions can still be drawn.”

It is important for environmental enrichments to be reproducible. All environmental characteristics, including enrichment, should be reported in the literature alongside the research (Kilkenny et al., 2010). Where possible, a described standard of enrichment should be in place for the facility or study, and where this is not done, justification and a description of the enrichments implemented should be provided.

7.2.3 Effects on Husbandry Personnel

It has been recognized that providing environmental enrichment to animals has a positive effect on personnel involved in experimental protocols (FELASA, 2006). However, all personnel involved with animals in a study should be informed where it is necessary to withhold specific forms of environmental enrichment as justified by the nature of the study.

7.2.4 Potential Costs

Cost should not be the sole consideration in determining whether a particular aspect of the well-being of the animals should be addressed through environmental enrichment; however, it may be a factor in deciding how best to implement suitable enrichments (such as a particular condition of husbandry). Potential costs include financial and human resources (workload for personnel and investigators), as well as potential adverse effects on personnel or the operation of the facility. For example, providing hoses for pigs to manipulate can result in broken pieces of hose clogging the drainage system or becoming a choking or obstruction risk to the animals.

Decisions to implement enrichment strategies should not be based on convenience where the result is to the detriment of animal welfare.

8 PHYSICAL ACTIVITY

Guideline 8:

Animals should have opportunities to perform physical activities that allow the expression of species-specific behaviours.

Opportunities to perform physical activities suited to the species, breed, age, temperament and physical condition of the animal, and the experimental protocol, should be provided by the housing design and furnishings and/or other means (e.g., dog walking programs). Consideration should also be given to the available space, the environment, the conditions under which the animals have previously been kept, and the length of time the animals will be confined. For some animals, opportunity for exploration addresses species-specific behaviours and provides mental stimulation.

Opportunities for additional activity and how they can be accommodated within any space limitations must be discussed among the veterinarian, investigator and ACC. The welfare of animals housed in systems that allow limited movement, such as cattle in tie-stalls, can be improved significantly when the animals are provided with access to areas with greater space for physical activity (i.e., paddocks or pasture in the case of cattle) (Popescu et al., 2013).

Additional physical activity as an environmental enrichment aims to provide animals with sensory and motor stimulation that promotes psychological and physical well-being. For example, physical activity has been shown to reduce stress and improve the welfare of dogs (Menor-Campos et al., 2011), have a positive impact on the speed of wound healing in mice (Pence and Woods, 2014), and improve neurological outcomes (Würbel, 2001; Patten et al., 2015).

HUMAN CONTACT, HANDLING AND RESTRAINT

Guideline 9:

Any contact, handling or restraint must take into account species and individual animal differences, and be conducted in a manner that minimizes stress for the animals and risks to the animals and personnel.

9.1 HUMAN CONTACT

Many experimental animals are social and benefit from interactions with humans. Positive human contact can often reduce stress for animals during handling and interventions (e.g., cattle (Lensink et al., 2000), nonhuman primates (Hosey and Melfi, 2012; Waite et al., 2002) and dogs (Rehn et al., 2014)). However, for contact to be “positive”, it must be positive for the animal, regardless of the apparent positive experience from a person’s perspective. Some species or individuals, especially those originally from the wild, may not benefit from, and may be harmed by, excessive human contact. Depending on the species, it may be challenging to determine the experience of the animal and care should be taken to ensure that the relationship that develops is of benefit to the animals, first and foremost, as well as to the personnel and the research (Shyan-Norwalt, 2009).

Human interactions must also be appropriate to the type of animal and the research. For example, large animals that lose a flight zone response to humans (i.e., they no longer move voluntarily in a directed manner in response to human presence) are very difficult to move and require special consideration, as handling can become dangerous (Grandin, 2015). Human interactions should not compromise the consistency of research data.

Predictability and routine timing of interactions, with a calm, gentle and consistent approach, can reduce stress for the animals and enhance their well-being. Where conditions permit interaction of personnel with the animals, talking, handling, training and grooming, as appropriate, should be encouraged.

Some people are more readily accepted by animals than others and awareness of this feature should be used to maximize benefits for the animals. There may be sex-based influences of handlers that have an impact on the animals and experimental results (Sorge et al., 2014). It is important to be aware of this when there is a change in personnel. Research has shown that perfumes/scents affect animals (Dhanjal, 1991, cited in Howard et al., 2011), and scent-free zones may be useful to consider in animal facilities. This may be particularly important for rodent breeding facilities or behaviour studies.

For animals that become accustomed to humans, consideration should be given to preventing the animals from experiencing distress when circumstances change, such as when the animal is returned to standard husbandry after a study or a particular caretaker is no longer available.

9.2 HANDLING

Competent, confident and consistent handling is important in reducing stress in animals. Routine laboratory procedures, such as handling, can cause stress (Gouveia and Hurst, 2013). Positive or negative handling experiences that occur at a young age can affect responses to stress later in life (Sternberg and Ridgway, 2003). Tailoring handling methods to the particular species, strain and individual animal can significantly reduce stress and make future handling easier.

Handling can be positive or negative from a human viewpoint, and very difficult to interpret or measure from the animal's perspective. Successful handling requires the ability to recognize the animal's state of mind, which may include aversion, confusion, fear and discomfort or pain.

Training animals to cooperate with handling procedures through positive reward should be encouraged, as this can reduce stress. Familiarizing animals to handling during routine husbandry and procedures is important in reducing stress, both to animals (Swennes et al., 2011; Verwer et al., 2009) and personnel. An exception to this is fish, which would not benefit from repeated handling prior to an experimental procedure. Familiarizing animals to humans and procedures may also refine the animal model and reduce variation, leading to a reduction in the numbers of animals required (Verwer et al., 2009). Inconsistent and incompetent handling can cause undue or additional stress and harm, and have negative implications for factors such as reproduction (Hemsworth, 1987; Hemsworth et al., 1986).

In general, light latex, nitrile or other thin gloves should always be worn in the laboratory when handling animals, as part of regular personal protective equipment (PPE) to reduce exposure to contact allergens and to protect the animals. Gloves should be worn when coming into contact with most species, but they may be less critical for some species (see the CCAC guidelines developed for specific types of animals).

9.3 RESTRAINT

The decision to use physical or chemical restraint must be undertaken through consultation with knowledgeable individuals, such as the veterinarian, investigator or other experts. This should be based upon the length of the procedure, the invasiveness of the procedure, the need for analgesia, the degree of stress involved in the capture and restraint of a particular species, and the safety of personnel.

Physical restraint causes stress to the animals and alternatives should be used where possible. Restraint stress is well described in the literature as a profound source of stress, and is used in creating repeatable stress models in rodents (Campos et al., 2013). When physical restraint is required and justified to the ACC, details for monitoring the animals and the impact of restraint should be described in the protocol. The restraint should involve the minimum amount of force for the least amount of time necessary to accomplish the objectives.

Any equipment that is used should be appropriate for individual animals, and should minimize pain, distress and discomfort and ensure the safety of the animals and personnel involved. Animals should be trained gradually to the procedure using positive reinforcement. Even the best approach results in stress for the animal, but this can often be reduced through habituation and pre-conditioning.

While pre-conditioning or training of animals takes time and effort, and may require additional personnel time and training, non-aversive training techniques for restraint procedures can improve animal welfare and enhance the value of using animal models (McMillan et al., 2014). The amount of time and training

required will depend on the species and the individual animals, as well as the type and duration of restraint to be applied.

For protocols involving the use of metabolic cages, investigators should outline the procedures that will be used to help animals adapt to the metabolic cages.

It may be useful, and in some cases necessary, to administer calming or relaxing (tranquilizing) drugs before physical restraint for the safety of the animals and/or handlers (e.g., for large animals), or to assist with training animals to adapt to restraint equipment and personnel. The use of drugs may be necessary where there is insufficient time to train the animals for physical restraint.

When the use of chemical restraint is being considered, a veterinarian with knowledge of the species must be consulted and chemical restraint applied only by trained personnel. Where food animals that may enter the human food supply are involved, withdrawal time must be in accordance with relevant laws and regulations.

The principal goals of chemical restraint are: 1) to render the animal unconscious or deeply sedated, with a minimum amount of stress and no injury to the animal, in such a way that facilitates handling; and 2) to ensure safe and rapid recovery. Personnel involved in chemical restraint must be competent in the procedures being undertaken, including being able to recognize and address potential complications. Chemical restraint protocols for any animal must include details for the close monitoring of cardiovascular, respiratory and thermoregulatory systems and behaviour.

9.4 TRAINING AND PROTECTION OF PERSONNEL

As noted in the *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015), training programs must: foster a philosophy of compassion and respect for animals in science; and provide personnel with the necessary knowledge and technical skills to carry out required procedures, including handling and restraint, with the opportunity to update their knowledge and skills as needed. The development of positive relationships between personnel and animals can be enriching for both, and may promote a greater commitment to the care of those animals (Bayne, 2002). It is clear that the attitudes, behaviour and personal characteristics of people can affect the behaviour of the animals (Hemsworth et al., 2000; Waiblinger et al., 2002).

Training of personnel is important for the well-being and safety of the animals and for the safety of people, as appropriate interactions can result in animals being less frightened and/or aggressive and more manageable. The “creation of positive human-animal relationships” and “ensuring adequate skill and knowledge among animal handlers” are among the World Organisation for Animal Health’s 10 General Principles for the Welfare of Animals in Livestock Production Systems (Fraser et al., 2013). Personnel handling animals must be properly trained, and should be closely supervised when undergoing such training.

In the context of human-animal interactions, good personal hygiene, adherence to requirements for PPE and an adequate health and safety program, suited to the particular risks of the work environment, are also important for ensuring safety of personnel.

Investigators and facility managers need to be aware of the emotional cost of caring for and working with research animals. Individuals who are continually subjected to emotionally difficult tasks should be monitored

for potentially negative impacts. These individuals may become desensitized as a coping mechanism, which could affect their ability to properly care for and handle the animals. Individuals experiencing emotional difficulty should be offered assistance from their supervisors, and in some cases, tasks/responsibilities should be altered in order to protect people, animals and the quality of research.

10

ANIMAL MONITORING

10.1 HEALTH AND BEHAVIOURAL MONITORING

Guideline 10:

Health and behavioural monitoring must be in place to ensure that sick or injured animals are identified quickly and treated, as appropriate, and that the health status of the animals is maintained.

Health and behavioural monitoring should be based on the source and species of animal, husbandry practices, the nature of the research, and any direct or indirect contact of personnel with other animals. Monitoring should be commensurate with the size of the facility and the type and length of studies the animals are involved in. There should be detailed SOPs for the following:

- routine assessment of physical health;
- monitoring for obvious external signs in the animals (e.g., coat and skin condition, the appearance of the eyes, gait/swim pattern) and behaviour (e.g., stereotypic behaviour);
- procedures to be followed if a change in the health or behaviour of the animals is detected;
- pathogen surveillance, as appropriate to the species and research; and
- requirements for the introduction of new animals.

Health monitoring, which could include the use of sentinel animals, serology and other laboratory techniques, as appropriate for the species, should consider the Three Rs and adopt methods that reduce the level of invasiveness and the number of sentinel animals needed. Where quarantine or barrier systems are required, rigorous monitoring for viral, bacterial or parasitic infection is necessary to maintain and verify the health status of animals.

Investigators should consult veterinary personnel about regular monitoring of animal health status in order to verify the microbiological standing for reporting in the publication of experimental results, and to minimize cross-contamination between areas in a facility (Baker et al., 1979; FELASA, 2014).

In situations where it is difficult to maintain appropriate environmental parameters (such as a sufficient level of relative humidity at certain times of the year), animals should be closely monitored for negative effects (e.g., excessively flaky skin in birds and mammals, difficulties with ecdysis (molting) in reptiles, desiccation stress in semi-aquatic amphibians, development of ringtail in rodents, and avascular necrosis of ear extremities in mammals).

For aquatic animals, water conditions should allow adequate visualization of animals to facilitate monitoring of their health.

10.2 MONITORING RESPONSES TO CHANGES IN HOUSING AND HUSBANDRY

Any change to an animal's housing situation should be carefully monitored. The introduction or re-introduction of animals to established groups should be supervised by trained personnel to avoid problems of incompatibility and disrupted social relationships; procedures should be in place for managing and minimizing aggressive interaction (Council of Europe, 2006).

It is important to monitor the impact of any modification to an animal's environment to ensure the intended objective of improving the physical and/or psychological well-being of the animal is achieved (see Section 7, "Environmental Enrichment"). While well intended, enrichment that is not properly designed can have negative welfare implications, such as triggering biting behaviours where none previously existed.

10.3 ANIMAL CARE MONITORING IN RELATION TO RESEARCH, SURGERY AND ANESTHESIA

Investigators are responsible for identifying on a protocol any particular requirements for animal monitoring that relate to the specific nature of the research, and for communicating this information to animal care personnel involved. In some cases, increased monitoring may be needed (e.g., when highly novel variables are introduced), or there may be specific husbandry needs for a particular animal strain/model (e.g., fragile genetically modified mice). Cage/pen cards and monitoring records (see Section 12.4.2, "Experimental Records") are useful communication devices, particularly with smaller animals.

New lines of genetically modified animals have the potential for unanticipated phenotypes; consequently, these new lines must be carefully monitored and any information on special husbandry requirements should be recorded and updated as new information becomes available.

Where available, information on the welfare and husbandry requirements of a new line must accompany animals transferred to another investigator. Ideally, any particular information that is essential to the welfare of those animals should be relayed to the recipient in advance of receiving the animals. See *Animal passports: an example of documenting animal information* (CCAC, 2014) for recommended practices.

For animals undergoing invasive procedures, such as surgery, investigators need to ensure that the animal care personnel are aware of the procedures (both planned and completed) and any associated requirements for monitoring, care and pain control. Invasive studies with severely compromised animals may require continuous monitoring, including overnight and on weekends. Animals under anesthesia should not be left unattended and those that undergo invasive procedures should be continuously monitored until completely recovered.

For animals on protocols that involve regulation of their food, fluid or nutrient intake, the level of intake must be monitored, and the animals must be closely monitored for weight loss and signs of dehydration, stress, and deterioration in health. Communication among animal care personnel and investigators should be facilitated by the maintenance of good monitoring records in the location/site where animals are housed, which are readily available to both.

A process must be in place to ensure that animal care personnel and all research team members listed on the protocol (for example, including undergraduate students doing health checks during a critical post-

operative period) are aware of the animal monitoring requirements and endpoints that have been set in the specific approved protocol (see the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing*, 1998). Facility managers must ensure that animal care personnel are provided access to, and time to review, all protocols and related documents pertaining to the animals they care for.

Animal care personnel are responsible for informing the investigator and/or veterinarian if they notice animals experiencing any signs of pain and distress or clinical signs that may require mitigation. It is also important that animal care personnel monitor the length of time the animals are kept for scientific purposes, including teaching (see Section 12.2, “Animal Records”), and that endpoints are established for geriatric animals.

11 SANITATION

11.1 SANITATION PROGRAM

The objective of a sanitation program is to reduce microbial contamination to a level that promotes the health of the animals, reduces the possibility of introducing variables in the research, and protects the health and safety of personnel. Cleaning and sanitation practices should be based on the species and phenotype of the animals, type of housing, density of animals, and the ventilation system. SOPs should be developed and records kept.

An animal's housing must be cleaned and sanitized at a frequency that maintains good health for the animal. The specific requirements will depend on factors such as the species and type of animal (e.g., diabetic), density of animals, type and size of housing, bedding, and research requirements. The relevant CCAC guidelines developed for specific types of animals should be consulted.

Settings on equipment washers and washing times should be in accordance with the manufacturer's specifications, and the efficacy of the system should be monitored regularly and recorded. Problems of cross-contamination may arise if cages are not properly sanitized/sterilized.

A vermin control program must be in place and humane methods of control should be used.

11.2 SOURCES OF CROSS-CONTAMINATION

In addition to the animals themselves, other possible sources for the transfer of contaminants include:

- pressure spraying and dumping bedding, which can aerosolize microorganisms, allergens, and animal wastes;
- opening doors or failures in the ventilation system that alter the airflow;
- moving equipment and personnel (especially clothing and footwear) between areas and not establishing and maintaining room entry order from clean to dirty;
- using procedure rooms for animals from different sources;
- use of shared equipment (e.g., imagers, biosafety cabinets, anesthetic or surgical equipment);
- transfer of cell lines, biologicals and transplantable tumors;
- uncontrolled discard of water or pressure-washing mist in wet-lab facilities; and
- storing unprotected cages in common areas.

For more detailed information, see Scientific Institute of Public Health, Biosafety and Biotechnology Unit (2011) and CCAC (2003b).

Procedures must be in place to minimize the potential for cross-contamination, such as using anterooms and unidirectional air flow and air pressure differentials with heating, ventilation and air conditioning (HVAC) monitoring, using dedicated equipment, and having clearly posted SOPs for disinfection of surfaces and equipment before and after each use.

11.3 CARCASS AND WASTE DISPOSAL

The institution is responsible for ensuring suitable carcass disposal methods and services are available and that they are provided for all carcasses generated through ACC-approved protocols.

If a dead animal is discovered, it should be removed from the enclosure, identified, and placed in a suitable container or tagged, as appropriate for the species and age of the animal. The veterinarian or designate should be notified, and the body kept for post-mortem examination or disposal in accordance with the investigator's and/or veterinarian's instructions and local and provincial laws and regulations. Animal tissues, excreta, bedding and unused food should also be disposed of in accordance with institutional SOPs and local and provincial laws and regulations.

Biohazardous, radioactive and toxic waste must be disposed of in accordance with institutional and government guidelines. Hazardous waste must never be placed in the regular garbage waste stream.

Infectious waste should be incinerated or chemically inactivated on-site. If it needs to leave the facility, it should be sterilized (autoclaved) or decontaminated according to the *Canadian Biosafety Standards* (PHAC and CFIA, 2015) before removal, unless local and provincial regulations allow off-site disposal of the particular infectious agents by a certified company.

Cold-storage areas or freezers for the temporary storage of waste that needs to be collected for a time prior to a scheduled disposal time must be vermin-free, easily cleaned and disinfected, and physically separated from other storage facilities.

12

RECORD KEEPING

Records appropriate to the species and animal-based procedures must document the proper care of the animals and identify factors affecting scientific studies.

12.1 ACCESS TO RECORDS AND RECORD RETENTION

Guideline 11:

Records appropriate to the species and animal-based procedures must be accessible.

Animal records, husbandry records and other records relevant to husbandry activities should be comprehensive and readily accessible to investigators and their research technicians, veterinary personnel, animal care personnel, ACCs, auditors and inspectors.

Medical and experimental records must be kept onsite for the duration of a study and in a format that is readily accessible to veterinary personnel.

Retention of records is important for research accountability, and requires collaboration between investigators and facility personnel regarding the type of records and length of time they need to be retained. Health records and records for food, water and bedding should be retained for a period of time suited to the type of research and institutional requirements; a minimum of one year is required, or as long as necessary to meet the requirements of the government, relevant professional associations and the research. Investigators should also be aware of any additional requirements by publishers or granting agencies funding the research regarding record retention, publication and availability.

Responsibilities for maintaining and retaining records are as follows:

- Animal records – animal care personnel² in consultation with investigators and the veterinarian.
- Husbandry records – animal care personnel in consultation with investigators and the veterinarian; facility manager for records of feed and bedding purchase and storage.
- Medical records, including use of drugs – veterinary personnel or investigator under supervision of the veterinarian.

² While some animal care may be entrusted to well-trained students or other members of research/testing teams with the approval of the ACC, the work by these persons must always be overseen by animal health professionals (*CCAC policy statement for: senior administrators responsible for animal care and use programs* (CCAC, 2008)).

- Experimental records – investigator, in consultation with the veterinarian and/or facility personnel as necessary.
- Water quality and environmental monitoring records – animal facility personnel.

It is important that each institution establish good record-keeping practices suited to their particular facilities. The following sections outline the various types of records indicated above.

12.2 ANIMAL RECORDS

Records for rodents, rabbits, fish and other small animals may be at the cage/tank level; for farm animals, records may be at the herd level. However, individual records should be kept for any animal where there is a health or welfare concern.

Records should specify the investigator's name and contact information, and the protocol number. Written communication between investigators and facility personnel regarding plans for the animals is important to ensure animals are not kept beyond their intended purpose. Specifying an "end date" in the animal records can be useful in monitoring the length of time the animal should stay in the facility. This date should be revised as necessary, according to any modification in the research schedule, and reviewed during the annual protocol renewal.

The level of detail required in the records will depend on the species and procedures being done, and the requirements of provincial/territorial regulations. General information that should normally be recorded includes: arrival date and origin of the animals, species and breed/strain/stock, sex, date of birth or age estimate, colour, markings, and physical abnormalities or other identifying features. Clinical history, prior experimental procedures, previous housing and care, breeding experience, and behavioural observations are all important to include. For genetically modified animals, it is important to record the genotype using standardized nomenclature for the species where such standards exist and to ensure the use of abbreviations is consistent and unambiguous. Previous experience with husbandry of the particular strain (if known) should also be recorded. Any change in practices such as handling of the animal should be recorded if it could affect the research. All entries should be signed or initialed.

Animal records must contain enough information for institutions to accurately complete the *CCAC Animal Use Data Form (AUDF)* (consult the *Instructions for Completion of the CCAC Animal Use Data Form (AUDF)*) for the most up-to-date animal data reporting requirements. Animal records should contain enough information for investigators to be in compliance with funders, publishers and regulatory requirements.

12.3 HUSBANDRY RECORDS

12.3.1 Records of Daily Animal Observations

Recording observations of animals, initialed by the observer, is part of an institutional quality assurance program. Any specific problems or concerns should be noted. Even if no concerns are found, a daily record should be kept of the time when the animals were observed to monitor their health and welfare. Daily checklists with an option for comments may be useful to maintain consistency in observations and to monitor any concerns.

12.3.2 Records for Food, Water and Bedding

Good record-keeping practices for food, water and bedding should be established for each facility. Feeding regimes vary greatly among animals, and records should include the type and amount of food provided (which could be *ad lib*) and any other details pertinent to the type of animals and the research (e.g., acceptance of food). Special food or water requirements should be noted on the cage/pen/tank cards and in the feeding records to ensure animal care personnel are aware of these requirements.

Records of food supplies for the animal facility must be maintained to ensure adequate quantities of appropriately fresh and highly nutritious food are available for all animals. These records must include the expiry date and be used to help maintain an appropriate rotation of supplies. Records of feed purchases should also include the source/supplier, lot number, milling date, date of acquisition, nutrient content and quantity received.

Records maintained at the room level should include brief details on the type of food used, the expiry date (if applicable), and when the food packaging is opened.

Records should be kept of when water is dispensed, suited to the particular system in place (see Section 5.2, “Water Dispensing Systems”).

Records should be kept for the acquisition (delivery, type and amount) and use of bedding.

12.3.3 Cleaning and Sanitation Records

As part of an institutional quality assurance program, cleaning and sanitation procedures should follow institutional SOPs that have been developed for the particular species, type of housing, density of animals, ventilation system, biosecurity concerns, etc. Records of cleaning and sanitation activities should be kept, and the efficacy of equipment used for these purposes should be recorded regularly (e.g., for cage washers, the temperature, cycle duration, etc. should be recorded when the cage washer is used).

12.3.4 Breeding Records

Good record keeping is essential for proper management of breeding, whether for large animals, breeding colonies or broodstock. Records are critical for aligning the breeding program with the requirements of the research and the availability of resources to address the needs of any animals that are produced within the program. Large facilities with complex breeding programs involving numerous strains may benefit from having a system for recording this information in a reliable, searchable database that is regularly backed-up and securely stored to prevent loss during institutional systems renewal.

12.4 OTHER RECORDS RELEVANT TO HUSBANDRY ACTIVITIES

12.4.1 Medical Records

Medical records must be as complete as possible and at a minimum kept in accordance with the latest *CALAM Standards of Veterinary Care* (CALAM, 2007). Any veterinary care must be coordinated with animal care personnel and the principal investigator to allow for adjustments in husbandry and procedures, if necessary, and to minimize the impact on the animal. See Field et al. (2007) for a discussion of the types of medical records.

12.4.2 Experimental Records

The more invasive the protocol, the more detailed the monitoring and experimental records should be.

For surgical procedures, records must be kept for anesthesia, surgery, and postoperative care. The type, dosage, site and route of anesthetics, analgesics or sedatives must be recorded, and the animal must be monitored and records kept before, during and after surgery for depth of anesthesia, vital signs, and general condition, as deemed appropriate for the study and as approved by the ACC. Administration of antibiotics or other compounds and any procedures, including deviations from SOPs, must also be noted.

Records must be complete and in a format that is easily accessible for the veterinarian, veterinary or animal care personnel, the ACC, or any other applicable responsible party to review. A brief description of any experimental procedures should also be written on the cage/pen/tank card to keep animal care personnel informed and alert them to any changes in care.

The frequency of recording during monitoring and endpoints should be predetermined (see the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (CCAC, 1998)). Recording requirements could be subject to change during the course of a protocol, if for example, increased frequency of monitoring and additional supportive care are required to maintain animal well-being.

12.4.3 Records of Potable Water Quality Monitoring and Water Treatment

Records of water quality monitoring and water treatment should be maintained by the facility manager and be available to veterinary and animal care personnel as needed. Water quality monitoring should be performed and recorded at least annually, depending on the type of facility, site and water source, and more frequently if a problem has been detected.

12.4.4 Environmental Monitoring Records

Records of environmental parameters for animal rooms and other enclosures (e.g., barns), and for cages/pens/tanks should be maintained by the facility manager and be available to veterinary and animal care personnel and investigators, as needed. It would be useful to record any major facility management issues (e.g., noise, disruption, etc.) as they could be the source of husbandry problems that may arise. Disruption in normal parameters, such as inadvertent changes in photoperiod or significant changes in room temperature/humidity, may result in future animal welfare problems (e.g., poor production in mouse breeding colonies, or poor growth or productivity in livestock). Records of environmental parameters should contain enough information for investigators to be in compliance with funders, publishers and regulatory requirements.

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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.

Aromatic hydrocarbons – compounds which have a cyclical structure and delocalized electrons; these compounds are often toxic to animals and personnel.

Barrier systems – areas of an animal facility which are separated, thus reducing or minimizing cross-contamination; barriers are commonly used to separate animals of different or unknown disease statuses, such as dogs, cats, mice, specific pathogen-free (SPF) animals and genetically modified animals.

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 etc., appropriate for the species).

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

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.

Corticosteroids – hormones produced by the adrenal cortex that influence many body processes, including anti-inflammatory actions, breakdown of protein and fat, activation of the nervous system, salt and water balance, and regulation of blood pressure. Synthetic corticosteroids are prescribed by veterinarians for use in many procedures.

Cytotoxicity – the ability of an agent to cause damage to cells, either in vitro or in vivo; cytotoxicity may extend to cellular death.

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.

Ecdysis – the process of shedding the old skin (in reptiles) or casting off the outer cuticle (in insects and other arthropods).

Enclosure – the primary containment for an animal, which can be quite open (e.g., a fenced pasture used to confine cattle) or very contained (e.g., a ventilated cage housing mice). Enclosures include tanks for fish, pens or whole rooms for larger animals, aviaries for birds, and cages for smaller animals.

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

Endpoint – the point at which an animal's pain and/or distress is terminated, minimized or reduced by taking actions such as humanely killing the animal, terminating a painful procedure, or giving treatment to relieve pain and/or distress.

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

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

Five Freedoms – a framework for the assessment of the implications of husbandry practices that originated in a UK government report in 1965 and was then refined by the UK Farm Animal Welfare Council. It states that an animal's primary welfare needs can be met by safeguarding the following five freedoms:

1. **Freedom from Hunger and Thirst** – by ready access to fresh water and a diet to maintain full health and vigour.
2. **Freedom from Discomfort** – by providing an appropriate environment including shelter and a comfortable resting area.
3. **Freedom from Pain, Injury or Disease** – by prevention or rapid diagnosis and treatment.
4. **Freedom to Express Normal Behaviour** – by providing sufficient space, proper facilities and company of the animal's own kind.
5. **Freedom from Fear and Distress** – by ensuring conditions and treatment which avoid mental suffering.

Flight zone – the minimum distance an animal will try to maintain between itself and any perceived threat.

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

Genetically modified animals – animals in which there has been a deliberate modification of the genome (the material responsible for inherited characteristics).

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

HVAC – an acronym for heating, ventilation and air conditioning, which refers to the complete air handling system in an animal facility.

Hepatic microsomal enzymes – liver enzymes (cytochrome P450 system) that catalyze oxidation, which generally makes substrates more water-soluble and more readily excreted by the kidneys.

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

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

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

Mouth swab – a procedure usually carried out by wiping the inside of the buccal cavity with a cotton swab; the buccal cavity cells are used for genotyping.

Pain – an unpleasant sensory and emotional experience associated with actual or potential damage or described in terms of such damage.

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

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

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 of time to allow for evaluation.

Quality of life – the welfare of the animal throughout its entire life-span.

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

Ringtail – a condition in young suckling rats and mice believed to be caused in part by low relative humidity (less than about 30%); annular constrictions appear on the tail that may progress to necrosis and sloughing of the tail tip.

Sedative – drug which is used to produce a state where the animal is not entirely unconscious, but its awareness of the surroundings is severely altered and it does not have control of its muscles; sedatives may be appropriate for some situations where pain and distress are anticipated.

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

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

Specified Pathogen Free (SPF) – a designation used to describe the health status of animals for which a specific list of potentially infectious organisms have been tested for and not found.

Standard Operating Procedure (SOP) – written documents that describe 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*.

Water restriction – specialized protocols which limit an animal's access to water, in order to be able to use sips of fluid as a reward for performing a particular behavioural or cognitive task.

Water quality – water characteristics, assessed and maintained to ensure that the water is potable, or in the case of fish, will sustain life.

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