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Canadian Council on Animal Care
Conseil canadien de protection des animaux

CCAC guidelines: Wildlife

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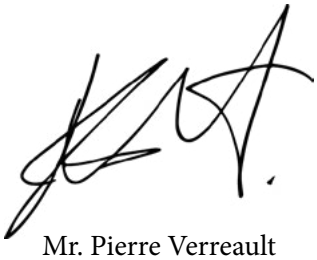

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NENA DAK'ANUTA – TAKING CARE OF ANIMALS

"Who does the term 'Wildlife' refer to? Many First Nations people say, 'All My Relations'. My grandchildren are seven generations from first contact. Since then, how have we changed how we see animals? We have now given ourselves authority over animals. By stating these kinds of guidelines, we are assuming authority over animals." (Gùdia- Mary Jane Johnson, Lhu'ààn Mân Ku Dań Elder, Whitehorse, Yukon, December 2021).

The definition of 'Wildlife' used in this document is colonial. While the CCAC guidelines were developed to minimize suffering and negative effects on wildlife imposed by research and researchers, the guidelines are limited to the perspectives and contexts represented by those who were and are authorized to create, review, and enact them. They are limited to western, colonial contexts and perspectives.

The deep, holistic knowledge of wildlife held by First Nations, Inuit, and Métis peoples is not reflected in this version of animal care guidelines. Future versions must go far beyond and instead be built on an entirely new foundation that includes First Nation, Inuit, and Métis Knowledges. We must consider many questions, including:

"How would the guidelines and the actions of researchers be different if we always thought of effects seven generations ahead? How would the guidelines be different if we thought of 'wildlife' as one of our Earth relatives? How do we want our actions to reflect respect of other beings, our relations? How did we get here, where animals are seen as experimental units in a scientific study, and where animals can be collared and tattooed without even talking to people who see those animals as relations, as food, and as beings who deserve gratitude and respect? How do we want to move forward from here?" (Gùdia – Mary Jane Johnson, Lhu'ààn Mân Ku Dań Elder, Whitehorse, Yukon, December 2021).

At first we were wary of writing an introduction to this version of the wildlife care guidelines because we did not want to appear as if we endorsed the guidelines as currently written. However, we welcomed the opportunity to stress the importance of including First Nation, Inuit, and Métis worldviews. In particular, we emphasize the need for individual researchers to assume responsibility for their actions, and in taking actions of Reconciliation with First Nations, Inuit, and Métis peoples in their research. Protocols for handling and studying animals that respect Indigenous Knowledges will vary greatly among communities. It is the responsibility of the researcher to reach out and learn these protocols, understand them, and respect them. We thank CCAC staff and Board of Directors for inviting us to write these introductory words as a start towards Reconciliation with Indigenous peoples in this aspect of science.

Elder Gùdia – Mary Jane Johnson
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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 of ethical care and use of animals in science throughout Canada.

The *CCAC guidelines: Wildlife* provides information for investigators, study directors, instructors, animal care committees, and veterinarians to help facilitate improvement in both the care given to wildlife and how procedures are carried out. Where conditions required by scientific activities differ from the guidelines, they must be justified to, and approved by, the animal care committee.

The individual guideline statements in the document have been developed based on expert peer advice and current interpretation of scientific evidence.

CCAC guidelines are intended to provide a framework for implementing Russell and Burch's Three Rs: replacement, reduction, and refinement (Russell and Burch, 1959), primarily the principle of refinement. These practices are constantly evolving, and refinements should result in continual improvement in animal welfare.

For studies outside of Canada, investigators based at CCAC-certified institutions are subject to these guidelines and the relevant legislation and regulations pertaining to ethical animal care and use in the country where the scientific activity is conducted.

LIST OF GUIDELINE STATEMENTS IN THIS DOCUMENT

The following list of guideline statements serves as an executive summary covering the most important aspects of the care and use of wildlife. These guideline statements are included within this document alongside details and references that provide support and context for their implementation. Throughout this document, the term 'should' is used to indicate an obligation for which any exceptions must be justified to and approved by an animal care committee. The term 'must' is used for mandatory requirements.

1. INTRODUCTION

Guideline 1

Scientific activities that do not interfere with the animal in any way (e.g., observations from a distance that are unlikely to affect normal behaviour) should be prioritized over more intrusive approaches.

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Guideline 2

Scientific activities conducted in the field must be designed with the welfare of the animals in mind and to minimize disturbance of the animals and their habitat.

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Guideline 3

If conducting a scientific activity in the field is likely to have lasting negative effects on a local animal population or to affect the persistence of an animal population or ecosystem, the investigator must demonstrate that the scientific activity is necessary. When such impacts are likely, the scientific activity should not be undertaken except under extraordinary circumstances, as approved by the animal care committee.

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Guideline 4

Consultation and involvement of veterinarians with wildlife experience should be sought on projects involving potential animal health and welfare concerns and, in some cases, must be sought where procedures can be performed or drugs prescribed (e.g., medically important antimicrobials), only by a veterinarian.

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Guideline 5

When morbidity is observed during or following handling or manipulation, the situation should be investigated and documented, and refinements of protocols should be addressed in protocol updates or

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Before initiating field projects involving capture, investigators should be familiar with the sensitivity and tolerance of the target and non-target species to methods of capture and restraint. Every effort must be made to minimize stress to avoid distress and ensure the welfare of the captured animal. The post-handled animal should be comparable to non-handled animals in terms of such factors as reproductive success, behaviour, and survival.

Section 4.1 General Considerations, p.24

Guideline 9

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Guideline 12

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Section 4.3 Chemical Capture, p.27

Guideline 13

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Section 4.3.1 Muscle Relaxants, p.28

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Section 8.1.1 Blood and Tissue Samples, p.46

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Guideline 35

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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.

1.1 ENGAGEMENT WITH INDIGENOUS COMMUNITIES AND ORGANIZATIONS

This revision of the *CCAC guidelines on: the care and use of wildlife* (CCAC, 2005) is intended to provide updated guidance on practices commonly used in scientific activities involving wildlife, in line with advances in animal welfare. The CCAC recognizes the need for engagement with Indigenous communities and organizations in further revision of this document. Consultation with Indigenous communities and organizations has not yet taken place; however, the CCAC acknowledges the importance of incorporating and respecting Indigenous worldviews and protocols as outlined in *Towards Reconciliation: 10 Calls to Action to Natural Scientists Working in Canada*.

As a first step to meaningfully contribute to reconciliation, enable and improve wildlife research, and help avert potential misunderstandings with Indigenous communities, investigators and animal care committees are encouraged to learn and be respectful of Indigenous protocols within the communities associated with proposed wildlife studies.

1.2 DEFINITION OF WILDLIFE AND SCOPE OF THE GUIDELINES

For the purposes of this document, wildlife refers to free-ranging and captive wild vertebrates and cephalopods, including amphibians, reptiles, birds, and mammals, while excluding fish (see the *CCAC guidelines on: the care and use of fish in research, teaching and testing* (CCAC, 2005)). This includes all introduced and native species and domestic animals that have become feral. It does not include domestic animals that have been transported to a wildlife setting for the purpose of in situ research (this situation is addressed in other CCAC guidelines for particular types of animals). The present guidelines focus on free-ranging wildlife and wild-caught animals that have not been habituated to captivity. The *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017) must be consulted where wild animals are to be maintained in animal facilities over a period of time that is likely to significantly impact successful reintroduction back into their native environment.

The CCAC requires animal care committee approval of protocols for scientific activities involving animals in accordance with the *Requirement for submitting an animal protocol: Addendum to the CCAC policy statement on terms of reference for animal care committees* (CCAC, 2020).

For animal-based activities that fall within the CCAC's mandate but do not require animals be included in a protocol, such as observational activities in which there is no expected impact on the animals or those around them, investigators must still inform the animal care committee for confirmation that the activity does not require an animal protocol (CCAC, 2020). Guidance on the conduct of observational activities in the field is provided in this document (Section 3, "Observational Field Activities") because it is difficult to account for inadvertent disturbances that may occur from the presence of an observer or remotely operated equipment, and a protocol may be required.

1.3 THE THREE RS

These guidelines are necessarily broad and are limited to basic principles that will assist investigators and animal care committees in the development and review of protocols and standard operating procedures (SOPs) specific to scientific activities involving wildlife (see the *CCAC policy statement on: terms of reference for animal care committees* (CCAC, 2006) for information to be included in a protocol form). As with any animal-based scientific activity, the scientific validity of a protocol involving wildlife must be established carefully, and the Three Rs (replacement, reduction and refinement) (Russell and Burch, 1959) must guide decisions concerning the design of the scientific activity and the care of wildlife when conducting procedures.

Animals may be used only if the researcher's best efforts to find a non-animal replacement or a method that does not interfere with the animal (e.g., a non-invasive method such as camera traps, scat collection, or use of tracks) to obtain the required information have failed. Where the use of animals is necessary, consideration must be given to the substitution of non-cephalopod invertebrates for vertebrates or cephalopods. It is recognized that where the aim of a scientific activity in the field is to understand the ecology, ecophysiology, or behaviour of wildlife, replacement by a non-animal-based method, or even replacement of one species with another, may not be an option (Griffin and Gauthier, 2004). However, there may be opportunities to minimize contact with animals to obtain the data required for the scientific activity (e.g., environmental DNA (eDNA) (Thomsen and Willerslev, 2015; Neice and McRae, 2021), genetic non-invasive sampling (gNIS) (Schultz et al., 2021), hair traps, remote imaging, and data banks). In addition, research involving endangered or threatened species may be necessary to support the species' conservation. While replacement of a rare or threatened species with a more common species or a species of lesser conservation concern (Curzer et al., 2013) may be more desirable in terms of conservation impacts, it does not address the Three Rs principle of replacement.

To address the Three Rs principle of reduction in scientific activities that require live animals, the appropriate number of animals to provide valid information and the statistical power to support conclusions should be used, and these numbers should be supported by an appropriate power study or prior research. Good design of the scientific activity is the primary means of reducing the number of animals required to demonstrate outcomes in the field. Prior statistical evaluation of sample size is necessary, even when sources of variation only can be roughly estimated. Familiarity with the literature on similar scientific activities regarding sample size and design is important. Reducing animal numbers can also be attained by cooperation between investigators in obtaining animal samples for their animal care committee-approved scientific activities, better sharing of data through data repositories, and publication of results in generally accessible formats.

The primary focus of this guidelines document is on refinement, with the aim of minimizing disturbance, stress, pain, and distress to wildlife while undertaking approved scientific activities. The animal's physical and psychological well-being should always take precedence over considerations of cost and convenience,

while also taking into consideration human safety. In addition, refinement efforts should aim to use techniques that have the least potential to impede normal behaviours (e.g., using hair or fur traps that do not involve capture, using low-invasiveness tagging, minimizing handling time, reducing frequency of visits to nesting sites, or ensuring family groups are released together). When distressed, animals may behave abnormally and possibly be placed at greater risk of injury or predation. Excessive stress, whether acute and severe or prolonged and chronic, can reduce health, performance, immune function, and reproduction. Protocols must be developed to treat wildlife involved in scientific activities humanely, not only for ethical and legal reasons, but also for the quality of scientific results (Animal Behavior Society (ABS) and the Association for the Study of Animal Behaviour (ASAB), 1997)). In general, acceptable procedures should minimize interference to individual animals, populations, and habitats, and thereby increase the validity of the data and results. Additionally, minimizing disturbance to the environment will reduce the number of other animals affected by the scientific activity.

Guideline 1

Scientific activities that do not interfere with the animal in any way (e.g., observations from a distance that are unlikely to affect normal behaviour) should be prioritized over more intrusive approaches.

Investigators should keep up to date with new techniques and implement them when they can reduce or eliminate the need for direct contact with animals. Examples include:

- using remote imagery to estimate seabird (Barber-Meyer et al., 2007) and polar bear (Stapleton et al., 2014) populations;
- using artificial intelligence and facial recognition software to identify individual bears without the need to capture and tag (Anderson et al., 2007; Clapham et al., 2020);
- identifying individual amphibians (Loafman, 1991; Eitam and Blaustein, 2002) and cetaceans (Hauser et al., 2006; Bergler et al., 2021) by scanning or photographing patterns; and
- understanding bird movement and migration through databases supported by citizen science observations (Sullivan et al., 2009; Walker and Taylor, 2017).

Scientific activities involving wildlife in the field and in captivity include a wide range of techniques that must be refined to accommodate the target species and their response to the presence of humans. The tremendous variation in size, physiology, and behaviours of animals needs to be taken into account to determine the most effective means of capture, restraint, and handling. During the development of protocols and SOPs, investigators should consult those who have experience with the species and expertise in the procedures to maintain good animal welfare practices. Investigators and project personnel should be competent in the procedures they will be undertaking prior to the start of the scientific activity.

1.4 CONSIDERATIONS FOR ANIMAL CARE COMMITTEES

Animal care committees that regularly deal with field-based projects should have two or more wildlife professionals with expertise in captive or free-ranging wildlife on the committee. Animal care committees that rarely review wildlife studies and do not have such expertise must consult wildlife professionals with relevant expertise when reviewing protocols involving wildlife. Given the wide range of species and methodologies

employed in scientific activities involving wildlife, even animal care committees experienced with reviewing protocols involving wildlife may need to periodically consult additional wildlife professionals.

When evaluating protocols for scientific activities that are to take place in the natural habitat of wild animals, animal care committees should recognize that conditions are likely to require different approaches, sample sizes, and procedures than those dictated by a laboratory environment or those used for domestic or semi-domesticated animals. Scientific activities in the field often require larger samples than in the laboratory to overcome natural attrition and environmental and individual variation that cannot be controlled in the field, and some scientific activities involving wildlife require marking and following complete populations, rather than a sample of those populations. Animal care committees should also be aware that protocols for testing devices or techniques that evaluate the most humane and effective methods for subsequent use may involve some unpredictability and flexibility. For these protocols, pilot studies may be important.

It is important that the veterinarian on the animal care committee evaluates the need for veterinary involvement (i.e., direct involvement in procedures, oversight, training, and communication) in protocols, in line with provincial and territorial regulations and the welfare of the animals.

Where field sites cannot be visited easily and procedures cannot be assessed or validated by members of the animal care committee in person as part of their post-approval monitoring (PAM) program, investigators should be prepared to photograph and record procedures and share these materials with the animal care committee for information and approval. Approved materials can be helpful in training other investigators and animal care committee members and in developing SOPs.

2 SCIENTIFIC ACTIVITIES CONDUCTED IN THE FIELD

2.1 GENERAL CONSIDERATIONS

Investigators must obtain all applicable permits prior to initiation of scientific activities and understand and comply with all regulations relevant to the jurisdiction and species involved. When working outside of Canada, Canadian investigators may be subject to additional regulations in the country where the scientific activity is conducted. Appendix 1 provides a starting point for consulting relevant regulations and authorities. Investigators should contact the appropriate authority for the most up-to-date permit requirements, as regulations and procedures may change over time.

Investigators must ensure their use of live animals is in accordance with applicable permit terms, conditions, and guidelines. When a permit is required, the permit number under which an animal was collected should be retained by the investigator throughout the scientific activity to demonstrate that the sample was legally obtained. Publishers may also require permit numbers to be reported in the submission of journal articles.

Investigators should take into account traditional or local knowledge and community values and, where appropriate, share knowledge gained through their scientific activities with the local community. The benefits of establishing a mutual exchange of information between investigators and stakeholders, including those with traditional knowledge, is well recognized by the International Council for Science (ICSU) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) (ICSU, 2002). In particular, investigators should be aware that traditional knowledge may be considered intellectual property, and in such cases guidelines on intellectual property would apply (The First Nations Information Governance Centre, 2014). Collaborating with local people and organizations (e.g., guiding services, trappers) that obtained wildlife for other purposes (e.g., roadkill, hunted, trapped, culled) where the investigator is not involved in the collection process can provide access to wildlife and samples from wildlife for use in scientific activities as secondary data.

Guideline 2

Scientific activities conducted in the field must be designed with the welfare of the animals in mind and to minimize disturbance of the animals and their habitat.

The use of wildlife in science raises ethical concerns. Adequate review of protocols by animal care committees is critical to ensure that proposed field procedures and techniques minimize pain and distress, distortion of animal behaviour, changes to habitat, and other risks. Although the acquisition of scientific knowledge and understanding may justify a scientific activity involving wildlife, the effects of field procedures on animals or their habitats cannot always be predicted. Consideration must be given to the potential risks to animals and their populations and environment, and these risks must be weighed against the potential benefits of the scientific activity.

Many scientific activities involving wildlife are based on observation or other methods that do not require contact with the animals; however, some scientific questions can be answered only by manipulating animals to some degree, either in the field or in captivity (ABS and ASAB, 1997). Ensuring manipulations are carried out in a humane manner that minimizes discomfort for the animal is an ethical requirement. Understanding how procedures impact animals is also important to the interpretation and integrity of scientific results. Therefore, procedures that disrupt normal animal activities should be of short duration or they should be mitigated and refined to minimize disruption.

To understand and potentially reduce the impact of observational and experimental procedures, investigators should conduct appropriate pilot studies whenever possible, particularly to evaluate a new technique or a proven technique being used in a novel manner or on a novel species. For example, there is evidence that some methods of capture and handling negatively impact animal welfare for a longer time and to a greater degree than previously recognized (Cattet et al., 2008), and some tracking devices, such as implants, have negative welfare impacts on some species that extend well beyond the timeframe of most scientific activities (Arnemo et al., 2018).

The Three Rs principles, described in Section 1.3, "The Three Rs", should always guide scientific activities involving wildlife. Where possible, scientific activities should be designed so that the information or samples collected can be used for multiple purposes or so that the scientific activity can be combined with information from additional field seasons and sites to maximize the value of the animals used. For example, the collection of biological and genetic samples for archiving can reduce or eliminate the need to capture additional animals to obtain these same samples at a later date. Additionally, investigators should collect information in a manner that is conducive to evaluating techniques and use opportunities to publish refinement techniques to improve welfare outcomes for animals. Formal reporting of results from scientific activities involving wildlife is expected (e.g., formal report, scientific paper, accessible database) and open access is encouraged. Dissemination of information on negative results (e.g., failed procedures, harmful techniques) is valuable for demonstrating the need to improve techniques for future scientific activities.

Investigators must take responsibility for the welfare of the animals involved in their scientific activity. Investigators must be prepared to respond appropriately to accidental injury of any animals that occurs during protocol procedures, and to record all instances such that they can be reported to the animal care committee at or prior to protocol renewal. While pain and distress can be difficult to determine, when animals are believed to be experiencing unrelievable pain or distress, immediate veterinary assistance should be sought, and where appropriate the animal should be euthanized. At the end of a scientific activity, removal of any devices must be given consideration in light of the long-term welfare of the animal.

When animals are temporarily held for short duration and released back into the wild for purposes other than translocation, they should be released at, or as close as possible to, the original capture site so that the animals can rejoin their social group or occupy an area with which they are familiar. Animals that are released into unfamiliar areas may be driven away by territorial animals, preyed upon, die from lack of shelter or starvation, or spread disease. Even when released at the original capture site, temporary absence may result in some of these problems.

If animals are to be held in captivity for more than a temporary period while conducting a procedure, they must be provided with housing and husbandry that addresses their needs (e.g., isolation, rest) based on the duration they will be held (see Section 10, "Housing and Husbandry").

For animals that are to be released back to the wild after a prolonged holding period, investigators must attempt to maximize each individual's ability to quickly resume normal behaviour and to minimize effects on existing populations (see the International Union for Conservation of Nature and Natural Resources (IUCN), 2013, and Section 12, "Release of Animals", for more details). Investigators should provide animal care committees with assurance, based on transparent reasoning that released animals do not represent an unacceptable risk to themselves, the public, other animals, or the environment.

For studies that require animals to be killed, the methods used must be in accordance with Section 6, "Killed Specimens", and measures must be implemented to minimize impacts on non-target animals.

Investigators should plan ahead for animals that cannot be released at the conclusion of the scientific activity. Any plans to relocate live animals to a sanctuary or educational facility must be evaluated on a case-by-case basis, based on applicable regulations, the welfare of each individual animal during transport and for their lifetime in captivity (taking into account the animal's age, health, temperament, and previous experiences), and the conditions of the potential rehoming situation. For animals that are to be euthanized at the end of the scientific activity, efforts should be made to maximize the information obtained from the animal (e.g., donate carcasses to museums, to investigators studying contaminants in the area or to other suitable scientific activities or tissue banks) in accordance with any permit restrictions.

Guideline 3

If conducting a scientific activity in the field is likely to have lasting negative effects on a local animal population or to affect the persistence of an animal population or ecosystem, the investigator must demonstrate that the scientific activity is necessary. When such impacts are likely, the scientific activity should not be undertaken except under extraordinary circumstances, as approved by the animal care committee.

Investigators must research the population status of the species involved in the scientific activity. If there is concern, consideration must be given to focusing on a species that is less sensitive to population effects where possible. However, some types of scientific activity may involve studies of population control or extirpation. For example, studies for rare birds may involve elimination or control of introduced or overabundant predators (e.g., rats on islands) or eradication of a disease agent (e.g., depopulation for the study of rabies control).

Investigators should review the current literature on the procedures they are planning to use to understand their impacts on the local population and ecosystem (See Appendix 2 for guidelines published by other organizations, which can be used as a starting point). Investigators should also consider the cumulative effects of any other procedures or scientific activities that have been conducted (e.g., previous disturbance of the habitat).

Scientific activities should not have any long-term detrimental impact on populations, and where this may result, mitigation measures should be taken (e.g., modification of the procedures, timing, number of animals involved), without compromising the validity of the scientific activity.

Unless a scientific activity entails manipulation of local populations (e.g., scientific activities designed to lower density or alter sex ratio), permanent removal of animals from an area for scientific purposes should only be done if there are no lasting negative effects on the local population. Practical alternative methods

of achieving the objectives must be considered to avoid long-term impacts (e.g., obtaining specimens from animals collected for other purposes, such as hunting).

To minimize detrimental impacts, investigators should take into account the seasonal social structure and behaviour of the animals involved during the scientific activity. For example, removal of adults should be avoided when young are dependent on parental care. For species with complex social organization, the removal of critical members of the group could impair the well-being of the remaining group members. These precautions may also apply to the temporary removal of animals.

Guideline 4

Consultation and involvement of veterinarians with wildlife experience should be sought on projects involving potential animal health and welfare concerns and, in some cases, must be sought where procedures can be performed or drugs prescribed (e.g., medically important antimicrobials) only by a veterinarian.

The advice and involvement of veterinarians with wildlife experience are particularly important in projects involving procedures with potential animal health and welfare concerns, such as translocation of animals, medical or surgical procedures, prescription of medically important antimicrobials, and immobilization activities. Combining the expertise and experience of wildlife biologists and other wildlife professionals with that of experienced veterinarians maximizes the likelihood of safe, humane, and efficient application of procedures on wild animals. See Cattet (2013) for further discussion.

Investigators should consult other biologists, veterinarians and Indigenous people who have experience with, or are knowledgeable about, the target species, procedures to be used, and the logistics of conducting scientific activities in the field. Investigators should be aware that in some jurisdictions only veterinarians are allowed to perform specific tasks (e.g., surgery). All medically important antimicrobials require veterinary oversight and a veterinary prescription due to national and international concerns regarding the development of antimicrobial resistance (Government of Canada, 2018). An important requirement that must be met by the registered veterinarian in order to appropriately prescribe medically important antimicrobials includes having a valid Veterinarian Client Patient Relationship. The definition of a valid Veterinarian Client Patient Relationship varies by province and territory, and applicable regulations must be consulted to ensure compliance. A valid Veterinarian Client Patient Relationship means that a veterinarian must be an integral part of scientific activities where they prescribe medically important antimicrobials or immobilization drugs for the use of investigators. Drugs used for wildlife immobilization are also available directly to trained wildlife professionals under special authorization from Health Canada (CAZWV, 2019).

Major, unanticipated disruptions that threaten the ability of investigators to continue their work on projects that have already been initiated should be addressed through the institution's crisis management plans (see CCAC, 2006). Such disruptions include pandemics, warfare, and loss of funding. During these situations, investigators have a continued responsibility for the welfare of animals in scientific activities underway, but there may be limitations placed on their ability to act. Welfare concerns from shutting down scientific activities are related to the animals involved in the studies (e.g., collars not dropping off as intended) and to the inability to retrieve data. If data are not retrieved, animals were unnecessarily used without accomplishing the intended purpose. Additionally, if the data is required, more animals will need to be used to gather that

data in the future. The institution, animal care committee, and investigators should work together to ensure scientific activities are modified or terminated in a manner that addresses the welfare of the animals.

2.2 PROTOCOLS INVOLVING WILDLIFE

The basic requirements for protocols involving wildlife are outlined in the *CCAC guidelines on: animal use protocol review* (CCAC, 1997), the *CCAC policy statement on: terms of reference for animal care committees* (CCAC, 2006) and the addendum to the policy, *Requirement for submitting an animal protocol: Addendum to the CCAC policy statement on terms of reference for animal care committees* (CCAC, 2020). Due to unpredictable conditions in the field, animal care committees should be aware that some of the procedures described within a protocol may have to be adapted depending on the prevailing conditions.

Investigators are responsible for obtaining animal care committee approval of their protocols before any work involving animals begins, including pilot studies. When a scientific activity will be conducted outside the jurisdiction of their institution (including in another country) or in collaboration with investigators from other institutions, the approval process described in the *CCAC policy on: animal-based projects involving two or more institutions* (CCAC, 2003) applies. This policy highlights the need for communication among animal care committees of all institutions involved to ensure protocols are properly and efficiently reviewed, and that each animal care committee is aware of all projects that involve investigators from their institution.

In addition to procedures to be used, investigators should identify and describe potential risks of morbidity or mortality for wildlife in their protocols and how those risks will be minimized. Prior studies or pilot studies may be helpful in determining expected risk and estimating levels that may be acceptable. Where information is limited (including out-of-date methods), this should be indicated by the investigator. Stated levels of risk provide useful benchmarks at the time of protocol renewal when investigators must report morbidity and mortality of animals. Investigators must keep records on morbidity and mortality, and if these numbers exceed expectations as defined in their protocols, investigators must inform the animal care committee and put in place actions to lower the risk to an acceptable level (CCAC, 2016). This may include protocol refinement, additional training for field personnel, predator control or management, or evaluation of equipment.

Investigators must establish scientific endpoints and humane intervention points for their scientific activity, which must be approved by the animal care committee (see the *CCAC guidelines: identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)).

When designing research and teaching protocols that involve wildlife, investigators must make an effort to:

- prevent, or at least minimize the intensity and duration of, any pain or distress experienced by animals;
- prevent demographic and other population effects unless part of the design of the scientific activity; and
- minimize the cumulative effects of procedures of concurrent or consecutive scientific activities.

Investigators must also consider any necessary measures to:

- minimize effects on the behaviour and physiology of all animals directly and indirectly affected by their activities; and
- minimize the effects on the ecosystem in which the research is conducted.

In the protocol, investigators must:

- articulate the goal of the scientific activity, put the work in a broad context, explain how the scientific activity contributes to the general state of knowledge or desired outcomes, and justify the significance of the anticipated results in light of any potential pain, distress, injury, or death of animals;
- ascertain the conservation status of the animal to be studied and ensure that the animals chosen are best suited to provide the information sought;
- ensure protocols and SOPs are up to date, are comprehensive in describing aspects of animal-based procedures accurately, follow recognized good practices that adhere to the Three Rs principles and the ethical treatment of animals, and minimize effects on the environment (e.g., *Decontamination Protocol for Field Work with Amphibians and Reptiles in Canada* (Canadian Herpetofauna Health Working Group, 2017)). Where similar procedures will be used in several protocols (e.g., capture or marking techniques), writing procedures as SOPs maintains efficiency. All SOPs require approval of the animal care committee and regular review (CCAC, 2006);
- include contingency plans, where appropriate, that describe actions to be taken in the event of any adverse reaction or an animal emergency, including for non-target species. If animals could become incapacitated, a plan must be provided for protecting them, such as protection from conspecifics, predators, environmental conditions, or other disturbances when recovering from immobilization procedures; and
- if capture is involved, outline precautions to avoid capturing non-target animals and contingency plans in case they are caught (e.g., release, rehabilitation, or euthanasia, as appropriate and in line with the Three Rs).

Investigators must inform the animal care committee in writing of changes in the protocol (via an amendment or new protocol) for their review and approval, particularly when changes have implications for animal welfare or ecosystems. Animal care committees must be alerted to, and approve of, potential major changes (e.g., increase in numbers of animals affected, invasiveness of scientific activity, severity of pain or distress, or duration of pain or distress, unexpected injury, or death), unless under situations where factors such as time or field conditions do not allow for immediate review and approval by the animal care committee. In such situations, the animal care committee must be contacted and made aware of the changes as soon as possible.

Outcomes of non-target animals must be recorded and reported to the animal care committee. Any anticipated procedures (e.g., blood sampling or application of a mark) on captured non-target animals must be included in the protocol and be in line with the Three Rs.

Basic animal first aid and appropriate treatment options must be available during all procedures relating to the handling of wildlife, and this information should be incorporated into an SOP. An individual with appropriate training and direct experience with the species of interest or related species must be on site for all animal first aid, physical and chemical restraint, and anesthesia. Investigators should consult with a veterinarian with expertise in wildlife during the planning phase of the project and ensure they can contact the veterinarian during the field season for additional advice, and to seek veterinary medical attention in the case of adverse events such as fractures, wounds, or other injuries and illnesses. Experts in wildlife rehabilitation can also be a useful resource.

If the investigator is anticipating medicating animals for any pre-existing health problems or prophylactically, a veterinarian experienced with the species of interest or related species should be consulted during the planning phase of the project, and administration must be done in accordance with regulations and animal care committee approval.

2.3 MORBIDITY AND MORTALITY IN THE FIELD

Guideline 5

When morbidity is observed during or following handling or manipulation, the situation should be investigated and documented, and refinements of protocols should be addressed in protocol updates or amendments. Mortalities should receive a postmortem to determine the cause of death, with the results included in the annual report to the animal care committee at the time of protocol renewal.

Prior to beginning field work, investigators should make arrangements for postmortems to be carried out in a veterinary pathology facility and consult with a pathologist on best practices for handling and preserving carcasses and tissues. Proper sample collection and preservation techniques are key to maximizing the identification of disease agents and causes of death and for archiving (e.g., genetics, stable isotopes). Ideally, postmortems should be conducted by experienced wildlife veterinary pathologists.

When it is not possible for carcasses to be retrieved from the field, a postmortem should be conducted by someone on the research team who is competent in postmortem techniques or a person in direct consultation with the animal disease specialists who will receive the specimens for further evaluation. In remote situations, a gross postmortem coupled with photographs of lesions and other conditions that may have contributed to the animal's death and the collection of a standardized set of samples (tissues, blood, feces, hair, swabs, etc.) can facilitate a diagnosis of cause of death and any underlying health issues. Field postmortem techniques are well described for some species (e.g., *Pathology of Wildlife and Zoo Animals* (Terio et al., 2018), *Field Manual of Wildlife Diseases—General Field Procedures and Diseases of Birds* (Friend and Franson, 1999), *Wildlife Disease Investigation Manual* (Canadian Cooperative Wildlife Health Centre and Office International des Épizooties, 2007), and *Necropsy Procedures for Wild Animals* (Munson, 1999).

Where the remains of an animal are not available or are insufficient to conduct a postmortem (e.g., predation), this should be noted and, where possible, photographed for inclusion in the annual report to the animal care committee at the time of protocol renewal.

Documentation of wildlife mortalities associated with the project that are considered not to be natural mortalities, including deaths that occur during or following handling, restraint, or manipulation, should be used to inform refinements in capture and handling protocols (e.g., Kreeger and Arnemo, 2018).

2.4 PERSONNEL INVOLVED IN FIELD ACTIVITIES

The principal investigator is responsible for all project activities in the field and is responsible for ensuring field personnel are competent in their tasks. Supervised field experience is required before leading a team in the field.

Investigators are responsible for their own conduct and for the conduct of all other personnel involved in the investigators' studies. In particular, investigators should ensure:

- all individuals involved with capture, handling, sampling, marking, monitoring, or euthanasia of animals are competent or under the direct supervision of someone who is competent in undertaking the

activities that they will be participating in (see the *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015));

- all personnel involved in any aspect of their study, whether students, volunteers, institution personnel, or contractors, receive a copy of, and comply with, the procedures specified in the approved protocol, with oversight for compliance and remediation; and
- all personnel involved in the project take appropriate precautions to reduce the risk of transmitting diseases or injuries to animals or humans.

Proper training of personnel is a major component of animal welfare. It is important that all personnel involved in working with wildlife have demonstrated training or work directly under a competent individual. The investigator must ensure personnel participating in a scientific activity are assessed by qualified persons as being competent in the procedures listed in the protocol that they will be undertaking before conducting any procedure. For information on assessing competency, see the *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015). As noted in Guideline 7: "All animal users must have the theoretical knowledge and practical skills necessary to be competent to perform their required tasks. When practical skills need to be acquired, the training should be timed in relation to when performance of the skills is required."

Documentation of training must be presented to the animal care committee to decide if it is sufficient to conduct the procedures without supervision or if supervision is required. Where a technique has not been performed by the investigator in the field, they initially may need to be accompanied by an individual who is proficient in executing the procedure in question.

Welfare assessments during scientific activities provide opportunities to learn and consider refinements, including where additional training is needed. These assessments should be conducted at the time of encounter with an animal whenever possible. See Section 11, "Welfare Assessment", and the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021).

3 OBSERVATIONAL FIELD ACTIVITIES

3.1 GENERAL CONSIDERATIONS

Animal care committees are responsible for determining the category of welfare impact of each scientific activity (CCAC guidelines on categories of welfare impact, in prep.). Investigators must inform animal care committees of their scientific activities and provide justification to their animal care committee regarding their choice of field techniques, even if activities are only observational in nature. Activities that are considered only observational must be justified as such in reference to the most recent literature and expert advice for the species, so that the animal care committee can determine whether a protocol is required (CCAC, 2020). Consideration must be given to the impact of observer presence because it can lead to disruption of normal target and non-target animal activities whether part of, or incidental to, the scientific activity.

Guideline 6

Investigators should ensure observational activities minimize disturbance to the animals and their habitat.

Access to or through sensitive areas (e.g., breeding sites or colonies) can result in negative impacts such as nest or young abandonment (Blackmer et al., 2004), increased vulnerability to predation (Götmark, 1992), injuries or mortality resulting from an escape response, increased energy use (Price, 2008), disruption of daily activities and social structure (Maldonado-Chaparro et al., 2018), spread of disease, or long-term harm of fragile habitats.

When designing observational activities, investigators must aim to minimize the potential impact of the observers. This can be done by determining the appropriate number, duration, and timing of visits, and identifying mitigation measures to minimize the intensity, persistence, and broadness of any disturbance. In particular, consideration should be given to how the timing and location of observation impacts dependent offspring, pair bonds, and breeding behaviour or increases exposure of individuals to the risk of injury or mortality. Disturbance during the breeding season should be justified and minimized. Potential negative impacts from observational activities should be evaluated.

Aerial, ground, or marine surveys should be conducted in a manner that minimizes disturbance to animals. This includes the use of directly and remotely operated motorized vehicles, as the animals' reactions may be extreme, depending on the characteristics of the vehicle, the particular animal, season, terrain, and other factors. Unmanned aerial vehicles (also known as drones) are increasingly being used for survey purposes. If used cautiously, they can have less impact on wildlife than traditional survey methods; however, their presence can still have significant observer effects (Mulero-Pázmány et al., 2017).

3.2 OBSERVATIONAL ACTIVITIES INVOLVING MANIPULATION OF WILDLIFE ENVIRONMENTS

Guideline 7

When manipulation of the animal's environment is necessary for a scientific activity, the investigator must make every effort to select the most appropriate method of observation for the species that will minimize stress to avoid distress and ensure the survival of the animals.

Examples of observational activities in the field involving manipulation of wildlife environments include the use of attractants (e.g., artificial models, supplemental food, scent, or sound) or deterrents (e.g., distasteful baits, odours, or animal models). Because experimental manipulation of the environment may expose target and non-target animals to stress, physical injury, predation, or social disruption (Waas et al., 2005; Linhart et al., 2012; Rivera-Gutierrez et al., 2015; Mennill et al., 2003), investigators should use the least invasive practical procedures required to achieve the objectives of the scientific activity, considering the biology and behaviour of the species of interest.

Whenever possible, negative effects should be anticipated and mitigated in the design of the scientific activity by defining clear scientific endpoints and humane intervention points (e.g., limits to the duration and frequency of manipulations) (see the *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)), providing protective barriers and escape routes, or using decoys or models to replace live encounters.

Well-defined scientific endpoints and humane intervention points for manipulations related to target and non-target animals must be approved by the animal care committee prior to commencement of a scientific activity (see the *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)). Investigators must monitor the animals and document their situation during manipulations, as specified in the protocol or SOP, with the intent to stop any manipulations at predetermined points (ABS and ASAB, 1997).

4 CAPTURE

4.1 GENERAL CONSIDERATIONS

Guideline 8

Before initiating field projects involving capture, investigators should be familiar with the sensitivity and tolerance of the target and non-target species to methods of capture and restraint. Every effort must be made to minimize stress to avoid distress and ensure the welfare of the captured animal. The post-handled animal should be comparable to non-handled animals in terms of such factors as reproductive success, behaviour, and survival.

Capture includes physical and chemical capture methods. When the capture and handling of wild animals is necessary, reducing the impact on the individual target and non-target animals that may also be captured (bycatch) and maximizing the information obtained while minimizing the handling time is an ethical imperative (Karesh, 1996). It is important to be aware of the sensitivity and tolerance that may apply to the species, and when such information is not available, the investigator must exercise caution and design procedures in full consideration of the knowledge available.

Distress occurs when an animal has to devote substantial effort or resources to respond to challenges emanating from a stressful situation and is unable to cope. There is considerable variability among species regarding their ability to compensate for stressful conditions, and it is imperative that investigators be aware of this ability in the species of interest and any non-target species that are likely to be caught to minimize stress.

Minimizing stress and ensuring the welfare of captured animals requires selection of the most appropriate methods of capture and handling for the species and the application of mitigation measures that limit disturbance and ensure integration of released animals back into the wild. Considerations for target animals (e.g., minimizing stress) also apply to non-target animals (as noted in Section 2, "Scientific Activities Conducted in the Field").

Investigators must be familiar with the advantages and drawbacks of available methods of live capture, particularly those that have been used with the target species. Protocols involving capture should draw from the scientific literature, consultation with experts, personal experience, and local and traditional knowledge. Scientific endpoints, humane intervention points, handling times, frequency of checks, and when to abort the capture attempt must all be defined in the protocol or an SOP. Capture often entails considerable risks for the animals and personnel, and personnel must be competent in performing the chosen method on the species of interest or work under the direct supervision of a competent individual.

Whereas many capture procedures can cause stress for the animals, any procedures with the potential to cause distress must be avoided or well controlled to limit the effects on the animal. A major cause of distress in capture and handling situations is excessive exertion, such as running or struggling, which may lead to

negative physical or physiological changes that could have fatal consequences immediately or at a later time (Jenkins and Kruger, 1973). Examples of negative physical or physiological changes include hyperthermia, hypothermia, acute stress, capture myopathy, shock, abortion in pregnant animals, and injury. Maximum pursuit time, as defined by the SOP, and signs of animal distress must be approved by the animal care committee prior to the initiation of capture.

Physiologic stress caused by temperature (hot or cold) should be prevented or minimized by selecting daily and seasonal times best suited for captures, post-capture monitoring, and using mitigation methods as needed. Distress during captures may also be induced by lack of water or nutrition and capture-induced disturbance such as unfamiliar sights and sound. Frequent monitoring should occur. As appropriate, animals should be provided with water and appropriate, high-quality food, particularly when held in captivity during capture (see Section 10.2, "Nutrition"). See Section 11, "Welfare Assessment", for more information on assessing the welfare of wild animals.

Animals compromised by pre-existing conditions are poor candidates for capture and handling and their capture should be avoided unless necessitated by the objectives of the scientific activity. Pre-existing conditions for an animal (e.g., pregnancy, lactation, social stress, inadequate supply of food or water, disease, exposure to temperature extremes) may decrease an animal's ability to deal with the intense and sometimes prolonged stress of being captured.

Capture and handling of animals with dependent young must be carried out with particular attention given to the care and welfare of the young so as not to detrimentally disrupt parental care (Dudeck et al., 2017) or cause abandonment. The removal of animals with dependent young from the wild should be avoided unless specifically approved as part of a protocol. Because the timing of hatch or births can be variable, local expertise (e.g., biologists, trappers, wildlife rehabilitators, and knowledgeable people within the community) should be consulted regarding local conditions, and their advice should be documented and incorporated in the protocol.

Guideline 9

Investigators conducting scientific activities in the field that involve capture must be prepared to handle reasonably anticipated conditions that may cause undue stress or injury to the animal.

Investigators must be prepared to stop activities when they are considered to cause distress or likely to cause injury, for example during extremes of temperature or weather, the presence of predators, longer than anticipated handling time, or difficulty collecting a sample. These should be outlined as scientific endpoints and humane intervention points in protocols and SOPs (see *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)).

A sufficient complement of competent personnel must be on hand to deal with all reasonable eventualities (e.g., larger than expected number of animals in nets and traps, non-target species). If insufficient personnel are available, excess animals captured must be released as soon as possible and the operation reduced in scale or stopped.

Guideline 10

Investigators must be prepared to recognize and respond to animals injured as a result of their actions and euthanize those suffering unrelievable pain or distress if they are not expected to survive when released and cannot be rehabilitated.

Investigators must develop contingency plans in advance for responding to injuries and euthanizing animals, and this includes obtaining appropriate permits to conduct such interventions in advance. Investigators must ensure that any proposed medical or euthanasia interventions on wild animals are conducted in accordance with relevant regulations and permit conditions or permission from the relevant authorities. Contingency plans should also cover unanticipated emergency humane killing of injured animals where permits are not in place, or when a delay in seeking permission would cause critical distress. For considerations and methods of euthanasia, see Section 13, "Euthanasia".

4.2 PHYSICAL CAPTURE

Investigators must review the various traps, nets, and associated techniques to ensure the equipment used is legal, effective, and suited to the species and situation, and that it will minimize animal stress and injury and the capture of non-target species (e.g., Powel and Proulx, 2003; Proulx et al., 2012; Sikes et al., 2011). Capture methods must also be selected based on the climatic conditions and efficacy of handling. Restraining (live holding) traps must comply with federal, provincial and territorial regulations and other legal requirements. Investigators must also consult authorities (e.g., federal, provincial, territorial, municipal, Indigenous) regarding any local restrictions or requirements for the use of certain devices and their placement, and the type of public notification and signage at a site.

Investigators must be competent in the correct use of the selected method or technique and should be able to ensure the safe release of any non-target animal that may be accidentally captured. For some larger animals such as grizzly bears and cougars, immobilization may be required (see Section 4.3, "Chemical Capture").

Investigators must plan capture events, including timing and monitoring frequency, to keep captive animals alive, uninjured, and exposed to minimal stress. Investigators must also make every effort to avoid trap deaths from factors such as exposure to extreme environments, shock, capture myopathy, and predation or disturbance from other species. Investigators must be aware of the specific behaviours, physical sensitivities, and bodily requirements of the species they are capturing and make provisions to accommodate these prior to processing and release (where appropriate, provide food, water, insulation, shelter, shade). If there is a lack of information about the species or potential non-target species, investigators should make these assessments based on similar or related species. Where possible, capture must be avoided when weather conditions threaten the welfare and survival of animals unless steps can be taken to mitigate these risks.

When traps and nets are not in use, they must be closed, deactivated or removed. Equipment used in trapping must be inspected regularly and maintained in good working order.

Investigators must justify the use of animal models or decoys, audio lures, or live lure animals to capture animals, as they may have short- or long-term effects on target and non-target species and individuals.

If live lure animals are to be used, investigators must provide justification to the animal care committee and must minimize the level of stress to lure animals (McCloskey and Dewey, 1999). Investigators are responsible

for the welfare of any lure animals, and scientific endpoints and humane intervention points for their use must be approved by the animal care committee. Lure animals must be reported to the animal care committee for inclusion in the *CCAC Animal Data Report*.

When bait or chemical lures are used alone or in combination with another lure to attract animals into a trap, precautions should be taken to minimize detrimental effects of conditioning or habituation.

Investigators must monitor the effects of capture on both target animals and non-target species and record both the results and numbers of animals as part of the annual report to the animal care committee at the time of the protocol renewal. This also applies to the protection of live lure animals used to attract predator species.

4.2.1 Monitoring Frequency

Guideline 11

Investigators must specify monitoring requirements for traps and nets in the protocol, and these must be appropriate to the capture method and target species to minimize stress to avoid distress in the captured animals, and to avoid injury or death.

Monitoring frequency of traps and nets must be described in the protocol or SOP and should be justified by references or consultation with experts. The frequency will depend not only on the species and trap or net type, but also on the objectives of the scientific activity, weather, location, environment, remote monitoring capabilities (real-time cameras), and applicable regulations. Monitoring frequency must also consider the potential capture of non-target species. Frequent trap checking is key for the well-being of all animals and especially for those that are particularly sensitive to exposure to heat, cold, dehydration, and energy deprivation. Dehydration can happen quickly under certain environmental conditions. Long periods spent in traps or nets during the reproductive season should be avoided to reduce impacts on breeding, behaviour, and dependent young.

To reduce disturbance at the trap site, investigators should consider devices such as telemetry signals and remote cameras that alert investigators when traps close. However, these should not replace on-site monitoring as specified in the protocol. When remote devices are used for this purpose, the time taken to respond should be addressed in the protocol to ensure the response time is appropriate for the circumstances.

4.3 CHEMICAL CAPTURE

Guideline 12

Investigators must make an effort to minimize the risks associated with drugs used for and during capture. As always, the animal's welfare must be the primary consideration, taking into account human safety.

Working with capture drugs requires veterinary oversight and specific training. Investigators must consult recently published literature and veterinarians with current knowledge of chemical capture of the species

involved to ensure they are using the most suitable drugs for the species and situation. The use of these drugs is regulated by Health Canada and investigators should be aware of the specific regulations on the drugs they intend to use.

Chemical capture of free-ranging wildlife can be particularly challenging. Prior to administering drugs, many factors must be considered with regard to their potential risk to animals in the field. These include the animal's age, sex, weight, condition, health status, and metabolic state; the possibility of gravid females or accompanying dependent young; terrain, ambient temperature, weather, and visibility; and the proximity of predators.

Investigators must anticipate potential hazards to the animal during the induction period of anesthesia or sedation when animals are not fully aware or fully ambulatory (e.g., cliffs, water bodies, ice, steep slopes, roads, fences, falling out of trees) and should avoid captures where these pose imminent danger. Investigators must fully describe scientific endpoints and humane intervention points in the protocol, such as pursuit times and unacceptable weather conditions for capture. Pursuit times must be minimized within the established scientific endpoints and humane intervention points.

The drug dose should be calculated to deliver an adequate volume in a single dose to ensure effective, rapid capture within the maximum safe dosage margins for the drug being used. Induction of anesthesia or sedation is a particularly hazardous time for wildlife and capture personnel. If prolonged, there may be increased risk of injury to the animal and capture personnel, as well as increased risk of losing the animal if it is free ranging and cannot be readily tracked.

The capture crew must be proficient with the capture equipment through regular practice that is specific to the field conditions, use of aids to reduce errors in judgment (e.g., a rangefinder to determine darting distance), and regular maintenance of the equipment.

Anesthetized or sedated animals may remain under the influence of the drugs for several hours or days following capture, and they must be monitored continuously at a safe distance that minimizes disturbance until they can be released to reduce the risk of injury and death from predators or conspecifics (see Section 5.3.3, "Recovery, Monitoring and Supportive Care"). To reduce these risks, investigators should use drugs or drug combinations that can be reversed or partially reversed, and are short acting, if available. They must also take steps to protect the animals until purposeful movement and the ability to defend themselves from conspecifics or predators has returned.

4.3.1 Muscle Relaxants

Guideline 13

Depolarizing muscle relaxants (e.g., succinylcholine chloride) produce paralysis without anesthesia and must be used with an anesthetic agent.

In the past, muscle relaxants were used as the sole agent for wildlife capture; however, when given on their own, they produce a situation of extreme distress for the animal because they are not anesthetic and the animal is fully aware of their surroundings. Muscle relaxants are titrated to produce paralysis of the limbs, but are not selective for the muscles of locomotion, and thus produce varying degrees of paralysis of the respiratory muscles, resulting in depression of the respiration system, potential suffocation, and often death

(Delvaux et al., 1999; Jolicoeur and Beaumont, 1986; Kreeger and Arnemo, 2018). Therefore, muscle relaxants must be used only when there is sufficient scientific justification that is approved by the animal care committee, and they must be used with an anesthetic agent and respiratory support.

4.3.2 Drug Delivery

Guideline 14

Investigators must ensure the remote drug delivery systems for administering anesthetic agents to free-ranging wildlife are appropriate for the size of the animal and for the volume of drug to be administered and are used based on established protocols.

Many equipment systems are available for the remote injection of drugs, including high velocity dart rifles, low velocity systems, pole syringes, and blow pipes. Investigators should choose a system that will deliver the required volume of drug with the least amount of physical trauma and stress to the animal (Bush, 1992; Kreeger and Arnemo, 2018; West et al., 2014).

Hitting the proper injection site is critical when remotely injecting immobilizing drugs in wildlife, and therefore, certified training[1], regular practice with delivery systems, and knowledge of animal anatomy are necessary to be able to consistently hit the appropriate anatomical site. Investigators should describe the major target areas in their protocols. A large, superficial skeletal muscle mass is usually the most desirable target to achieve an intramuscular injection. However, factors such as season, age, body condition, and behaviour of the animal at the time of immobilization should all be considered, as they can radically alter the target site and the dose required. Additionally, an appropriate needle length and gauge and dart size should be chosen for the size, age, and body condition of the target animal to allow effective injection and reduce the risk of laceration and trauma.

Only experienced personnel should use high velocity dart rifles because they are capable of killing most mammalian species and generally are much less accurate than traditional firearms. Accidental animal deaths involving dart rifles can result from using excessive velocity to propel the dart or missing the target area, causing penetration of a vital organ or body cavity or broken bones.

Low velocity systems, including CO_2 powered pistols, low velocity dart guns, and blow pipes, cause less trauma than high velocity dart guns because the projectile travels at a much lower speed. However, these systems require a shorter distance for hitting a specified target.

Slow-injection darts, pressurized with air or gas, cause less tissue damage when injecting than rapid-injection darts, which contain an explosive charge. Low velocity systems with slow injection darts should be used when feasible. If a dart powered by an explosive charge is used, the system should be designed to minimize tissue trauma from drug injection.

Pole syringes and blow pipes are useful for trapped or restrained animals. They create less trauma than high-velocity systems due to the slow speed of impact and injection. However, pole syringes can result in

1 Provided by the Canadian Association of Zoo and Wildlife Veterinarians or equivalent

lacerations or needles can break off, especially when needles are too long or of a higher gauge, or when administering a large volume of drugs. Placement of the syringe needs to be accurate, as an incorrectly placed pole syringe or dart from a blow pipe may harm the animal.

5 HANDLING AND RESTRAINT

The decision to handle an animal or use physical or chemical restraint should be undertaken through consultation with knowledgeable individuals. Considerations before handling and restraining an animal include the length and invasiveness of the procedure, the need for analgesia, the potential degree of stress involved for the animal, and the safety of personnel. Predictable chemical restraint protocols with good analgesia and antagonists exist for some species but not others. For some species, effective handling or physical restraint can be accomplished more quickly and with fewer complications than chemical restraint.

Investigators must be competent in the techniques to be used and must not allow unsupervised or inexperienced personnel to handle any animal until they have received training and been determined to be competent in the planned handling and restraint and release of the animals (CCAC, 2015). The investigator must consult the current literature and experienced professionals before handling an unfamiliar species. When a new technique or approach is to be used, it should be tested through a pilot study.

Improper handling or restraint, especially of distressed animals, may lead to major and potentially fatal physiological disturbances or physical trauma. In addition, handling or restraint of some animals may alter their behaviour or predispose them to predation. As noted in Section 4.2, "Physical Capture", when baiting is used for handling and restraint, precautions should be taken to minimize detrimental effects of food conditioning or habituation.

Personnel should keep the amount of talking and noise to a minimum. Helicopters and other motorized vehicles or equipment should be turned off or moved away from the handling area. If tracking dogs are used, they should be moved far enough away from the animal when it is practical to minimize disturbance.

Chemical or physical restraint of wildlife may cause various forms of social and behavioural disruption (e.g., interfere with territorial defence or breeding), and this must be considered in planning the scientific activity. Social disruption is more detrimental at certain times of the year, such as during breeding, pregnancy, lactation, or parental care. Restraint of gravid, lactating, or incubating animals should be avoided when possible unless specifically required. This is particularly important for mammals during the last trimester of pregnancy and birds with developing eggs. If pregnant animals are to be captured, the methods used should minimize any potential adverse effects to the female and fetus (e.g., reversible or short-acting drug combinations, supplemental oxygen), and be the quickest and most suitable method for the procedure. Generally, chemical restraint of adult animals with dependent young should be avoided due to increased risk of abandonment (Côté et al., 1998). If handling young animals is necessary, handling time must be minimized, and precautions taken to reduce the risk of abandonment.

All procedures involving physical or chemical restraint must have a wildlife immobilization record completed for each animal. Data to be recorded should include:

- personnel involved;
- site-specific data such as the date, time of day, location (GPS coordinates), and climatic conditions;
- equipment and method used (e.g., type of dart rifle and darts, net guns);

- hazing (i.e., use of deterrents to move an animal) and pursuit duration if applicable;
- drugs used, including dose, route of administration, and induction and recovery times if applicable;
- biological data (e.g., age, sex, reproductive status, weight, condition, physical exam findings);
- procedures completed (e.g., ear tag, collar);
- codes, numbers, colour, and placement of identification marks;
- samples collected (e.g., blood, feces, hair);
- monitoring times and physiological data, including respiratory rate, pulse rate, oxygen levels, and temperature; and
- complications for wildlife and human safety experienced (e.g., accidental drug exposure, potential exposure to adverse events).

Pertinent information from these records should be reported to the animal care committee as part of the annual report at the time of the protocol renewal.

Investigators should monitor animals following handling and restraint, which can include tracking individuals and postmortems of any deaths that occur after release. Care should be taken so that post-release monitoring does not impair the success of released animals. For specific guidance on monitoring animals following chemical restraint, see Section 5.3.3, "Monitoring, Supportive Care, and Recovery".

5.1 HANDLING

Guideline 15

Personnel handling animals must be competent in the proposed procedures, alternative methods of restraint that may be required, and the use of sedatives, or they must work under the direct supervision of competent personnel.

Expectations in relation to competency of personnel are described in Section 2.4, "Personnel Involved in Field Activities", and in the *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015).

5.2 PHYSICAL RESTRAINT

Guideline 16

Within the limits of human safety, effective methods of physical restraint that minimize the possibility of physical injury and physiological and psychological stress must be used, and restraint should be for the shortest possible time necessary for the procedures being undertaken to be completed.

Physical restraint should be performed only by individuals who are familiar with the normal behaviour of the species and the equipment associated with the restraint procedure. If there is a lack of information about the species, investigators should make assessments based on similar or related species.

Investigators should minimize the length of procedures because restraint is stressful and the risk of significant health effects, including injury and death, increase with the duration of restraint.

Investigators must minimize sensory stimuli by handling animals quietly without sudden movement and with a minimum of the necessary personnel. When appropriate for the species, investigators should place blindfolds or hoods on animals and deploy earplugs or work in darkened environments to reduce stress. Hoods or masks should be designed to allow monitoring of eye reflexes (e.g., have flaps) or be flexible to allow temporary removal for monitoring.

Supplemental chemical restraint should be considered to prevent injury to an animal or personnel or to reduce stress for the animal (see Section 5.3, "Chemical Restraint"). Additionally, restraining devices (e.g., hobbles, nets, cones, or bags) should be used when appropriate to avoid injury to the animal or personnel. Investigators should be familiar with the appropriateness of such devices for the target species and be trained in their use. Devices should be well maintained, cleaned, and in good working order.

Investigators should be aware that seasonal changes in behaviour might influence the ease and safety of restraining animals. Planning for animal restraint must include contingency plans for inclement weather and other environmental conditions (e.g., having access to a temporary shelter such as a tarp in case of rain) and species-specific factors that will influence the outcome of the restraint procedures. Plans should identify scientific endpoints and humane intervention points that indicate when to stop attempting to restrain an animal.

5.3 CHEMICAL RESTRAINT

The principal goals of chemical restraint are to render the animal sedated or unconscious with a minimum amount of stress and no injury, and to ensure a safe and rapid recovery. When painful procedures are performed during chemical restraint, appropriate peri- and post-procedure analgesia must be considered. Drugs used for chemical restraint of wildlife have the potential to produce adverse effects in the animal and increase risks for personnel. The overall risk of significant morbidity and mortality increases if the methods are not suitable, used incorrectly, or applied without prior practice or regular use. Every possible effort should be made to decrease the risks of morbidity and minimize the mortality rate. The following sections outline guidelines to decrease stress and morbidity or mortality during chemical restraint and anesthesia. Additional useful information is found in the CAZWV course manual for the *Chemical Immobilization of Wildlife*, 4th edition (CAZWV, 2019), the *Handbook of Wildlife Chemical Immobilization*, 5th edition (Kreeger and Arnemo, 2018), and the textbook *Zoo Animal and Wildlife Immobilization and Anesthesia* (West et al., 2014). For guidelines on the using analgesics, see Section 8.2, "Use of Analgesics".

Guideline 17

When morbidity is observed in an animal during or following chemical restraint, it must be addressed. Following completion of the procedure, morbidities must be investigated and documented to refine and improve protocols. Any mortality during or following chemical restraint should receive a postmortem to determine the cause of death.

During chemical restraint, any observed morbidity (e.g., hypoxia) must be addressed as a priority; however, it is also important to determine if there is an issue that needs to be addressed in the protocol, particularly in the case of persistent morbidities.

Reporting information related to refinement and improvement of techniques to the animal care committee (*CCAC policy statement on: terms of reference for animal care committees* (CCAC, 2006)) and disseminating this information to the broader research community (as noted in Section 2, "Scientific Activities Conducted in the Field") will greatly assist in implementation of the Three Rs. For information on postmortems, see Section 2.3, "Morbidity and Mortality in the Field".

5.3.1 Training

Guideline 18

Personnel performing or supervising chemical restraint on wildlife must be competent and must use techniques and drugs that are appropriate for the target species.

Chemical restraint protocols and complications vary considerably among species. Therefore, personnel performing chemical restraint must have appropriate training and experience, must be familiar with the best techniques available for wildlife, and should be familiar with the target species.

At a minimum, personnel involved in chemical restraint of wildlife must have successfully completed training in chemical restraint that is relevant to the species of interest within the past three to five years, or an acceptable combination of initial training, refresher training, continuing education, regular hands-on practice, and participation in wildlife chemical restraint. Personnel should gain experience with the chosen method and target species through observation, mentoring, and direct supervision before attempting the procedure on their own. Current literature must be reviewed and personnel (e.g., wildlife biologists, wildlife management personnel, veterinarians with wildlife experience) who are familiar with the target species (or similar species where such expertise is not available) and the techniques to be used must be consulted.

5.3.2 Pharmacological Considerations

Guideline 19

Drugs used for the immobilization of wildlife should have the following properties: be stable in solution, be effective in small volumes, produce minimal deleterious physiological or toxicological effects, result in rapid onset, and be reversible. When painful procedures are to be undertaken, anesthesia and analgesia must be provided.

The drugs and dosages used in chemical restraint will depend on many factors, including species, length and invasiveness of procedure, drug safety, ability to reverse the drugs, and potential adverse effects. Depending on the scientific activity and the invasiveness of the procedures, the drugs should produce reliable sedation or anesthesia (unconscious) to allow for safe animal handling and render the animals unaware of the invasive procedures that are being performed.

Drugs used for wildlife immobilization should be potent and allow for the use of small volumes. Small drug volumes allow for smaller darts to be used, which facilitates accurate remote delivery of drugs and minimizes tissue trauma. Drugs should have a high therapeutic index and minimal toxic side effects to decrease

the risk of morbidity or mortality. Drugs should be used before the expiry date, and any outdated drugs must be properly disposed to prevent environmental contamination.

All drugs must be stored securely, accounted for (e.g., through a drug log book with expiry dates documented), and transported in the field appropriately (e.g., protected from theft, under aseptic conditions, and at the correct temperature, humidity and light requirements). An extreme range of ambient temperatures may be encountered in the field, and therefore the drugs should be stable over a large range of temperatures to promote precise delivery of a given drug dosage. Many of the commonly used drugs are water-based solutions and are subject to freezing in extreme cold temperatures.

Administration of a reversal agent should be considered to decrease recovery time and enable the animals to recover and defend themselves or escape predators more readily. Reversal drugs also antagonize the side effects of anesthetic agents and facilitate rapid recovery in an emergency. However, if opioids or alpha-2 antagonists are used, they will also antagonize analgesia, and additional drugs will be required to control pain if painful procedures have been performed. As noted in Section 4.3, "Chemical Capture", animals must be protected and observed from a safe distance until purposeful movement and the ability to defend themselves from conspecifics or predators has returned.

5.3.3 Monitoring, Supportive Care, and Recovery

Guideline 20

Appropriate supportive care and close monitoring must be provided during chemical immobilization to minimize the risk of morbidity or mortality, and ensure animals can recover and be safely released.

Consideration should be given to addressing potential complications and mitigating adverse effects of recovery (e.g., recover animals away from water, roads, or cliffs when possible). Personnel working with sedated or anesthetized animals must be competent in recognizing potential complications and dealing with them. Potential complications can vary based on the combination of drugs (i.e., the chemical immobilization protocol), the particular animal (e.g., factors related to the species, sex, developmental stage), and the season. The type of supportive care required varies considerably among species, and personnel must be aware of the appropriate care for the target species.

Sedation and general anesthesia subject the animal to potentially life-threatening complications. Animals subjected to chemical restraint require close monitoring of their cardiovascular, respiratory, and thermoregulatory systems. Ideally, these systems should be monitored continuously; if this is not feasible, close monitoring should be performed every 5 to 10 minutes. The depth of sedation or general anesthesia should also be closely monitored to detect sudden changes that may indicate distress or premature recovery of the animal or a hazard for personnel (e.g., rapid recovery). It is advisable to have one person whose sole job is to monitor the physiological status of the immobilized animal throughout the handling procedure and record data on an animal immobilization form.

Dependable, lightweight, highly portable equipment to assist in monitoring is available (e.g., pulse oximeters and digital thermometers). Personnel conducting field sedation and anesthesia must become proficient in the use of the appropriate aids and have access to this equipment in the field. Personnel should be prepared

to administer oxygen in the field, and animals showing signs of hypoxia should be treated with supplementary oxygen. Compressed medical grade oxygen is easily transported in D-size cylinders under most field conditions (Read et al., 2001) with appropriate safety precautions taken. Portable self-inflating resuscitation apparatus (e.g., bag-valve-mask ventilation bags) can be useful in smaller species, as they are very portable and provide ventilatory support. Portable oxygen concentrators can also be used in field situations to deliver constant or intermittent oxygen to wildlife (Fahlman, 2014).

Anesthetized mammals that have lost the reflex to swallow require correct head and neck positioning to prevent complications associated with regurgitation and potential aspiration. The head and neck should be extended, with the tongue pulled out to open the airway and allow drainage of saliva. Monogastrics should be positioned in lateral or sternal recumbency on a soft, even surface with the head and neck extended; ruminants should be positioned in sternal recumbency on a soft, even surface with the head held slightly above the ground, neck extended, and the chin directed downward (nose pointing down) to allow for drainage of saliva and reduce the likelihood of regurgitation and aspiration. If, for any reason, sternal recumbency cannot be maintained with ruminants, the animals may be maintained in lateral recumbency (right side down is preferable) if carefully monitored for ruminal tympany and regurgitation. Large, heavy-bodied animals should not be placed on one side for extended periods of time due to lack of circulation, which could potentially lead to myopathy. Handling personnel must be competent in recognizing and treating airway obstruction, and equipment should be available to treat severe ruminal tympany (e.g., a rumen tube).

Dissociative anesthetics are frequently used for wildlife anesthesia and may eliminate the palpebral or blink reflex and reduce tear production. Eye lubrication is required to decrease the risk of corneal ulceration or trauma. The eyes and eyelids should also be protected by blindfolding, with access to the eyes via flaps in the blindfold for monitoring eye reflexes.

Drugs used for wildlife immobilization often impair thermoregulation, making the animals susceptible to hypothermia or hyperthermia. This risk is further increased by the field conditions under which wildlife immobilization frequently is performed. Personnel using immobilizing drugs must be able to recognize these complications (Ozeki et al., 2015). Because there is a high degree of variability among species, personnel should know the range of normal body temperature for the target species and must monitor the animal's temperature frequently throughout anesthesia, taking preventative and corrective actions as needed. These actions should be described in the protocol or a relevant SOP.

5.3.4 Drug Residues

Guideline 21

Investigators must take all possible steps to ensure that drugs used in procedures on wildlife do not enter the food web.

Drugs used for wildlife, including antibiotics and analgesics, have the potential for adverse effects on humans and other animals if the target animal is consumed soon after administration. Animals that may be consumed by people should be clearly tagged in a manner that indicates they have received a drug, with contact information on the tag. Personnel administering these drugs should be aware of approximate withdrawal times for the target species. The Veterinary Drugs Directorate at Health Canada, provincial, territorial and federal wildlife agencies, or drug manufacturers and distributors may be able to provide

information for some drugs regarding the length of time during which an animal is not to be consumed after receiving the drug, and government agencies may have specific requirements for animals administered drugs under their authority. However, many of the drugs used in wildlife are "off label", in which case the drug residues and long-term effects have not been determined for these species. Information on withdrawal times for some drugs commonly used for wildlife is given by Craigmill et al. (1997) and the Western Wildlife Health Committee (2000), and is available to veterinarians through the Canadian gFARAD (global food animal residue avoidance databank). These withdrawal times should be used at the discretion of veterinary authorities in each region.

Chemical restraint should not be performed close to the hunting season for game species unless allowed in the permit and any required notification has been given. Local consumers of wild meat (e.g., Indigenous communities, regional hunter groups, trappers' associations) should be consulted whenever possible prior to chemical restraint of wildlife and fully informed about any potential risks from consumption of the meat.

6 KILLED SPECIMENS

When dead specimens are required, investigators should attempt to obtain them from other sources (e.g., specimen banks, museums, road kills, hunter kills, wildlife rehabilitators, other permit holders) whenever possible. When this is not possible, other opportunities for reduction should be considered, for example, sampling eggs.

Federal and provincial and territorial regulations concerning lethal methods for collection of wildlife must be consulted for local requirements.

Guideline 22

Lethal methods for collection of wildlife must be species-specific and humane. Personnel involved with administering lethal termination must be competent in the proposed method(s) or under the direct supervision of personnel competent in the method(s) to ensure effective, humane death.

The killing of animals for studies requires scientific and ethical justification to the animal care committee. The date, time, species, location of the animal collection, and other pertinent information must be recorded and reported to the animal care committee during protocol renewal.

All reasonable efforts must be made to maximize the use of each animal, thus increasing the scientific value of those animals killed and potentially contributing to an overall reduction in animal use. For example, consideration should be given to their potential use as voucher specimens (see Animal Research Review Panel (2020)).

Wildlife should not be killed for the sole purpose of dissection or mounting. Whenever possible, these activities should be done with animals that have been found dead (e.g., window strike birds) or euthanized for other purposes, or replacement tools, such as educational videos and simulations, should be used.

The collection method chosen will depend upon the circumstances (e.g., species, season, purpose for collecting the specimen); however, the most efficient and humane method that will serve the objectives of the scientific activity must be used. The method should not compromise the quality of the biological samples. For some species and studies, live traps (see Section 4, "Capture") followed by an acceptable method of euthanasia (see Section 13, "Euthanasia") may be more appropriate for minimizing pain and distress, ensuring death occurs rapidly, and avoiding physically harming or killing non-target animals (American Veterinary Medical Association (AVMA), 2020). In other cases, lethal traps may be more appropriate to ensure rapid death, minimize pain and distress for the animals, or reduce dangerous situations for the animals or personnel (AVMA, 2020).

Lethal traps should be the most humane and effective available, based on the published literature (Proulx et al., 2012; Sikes et al., 2011; Powel and Proulx, 2003) and persons experienced in using lethal traps. Only traps that are legally permitted for the intended species may be used unless special authorization has been

given by the provincial or territorial authorities. Traps for some species, particularly furbearers, may also be subject to additional requirements (e.g., certified as having met the standards specified in the Agreement on International Humane Trapping Standards (AIHTS), European Community, Government of Canada, and Government of the Russian Federation, 1997).

All lethal traps should be deployed and operated according to the manufacturer's instructions, monitored for performance, and adjusted to ensure effective and humane deaths. Investigators must describe the set-up in their protocols, along with the minimum frequency with which the traps must be checked to prevent specimen loss due to scavengers or spoilage. Ideally, there should be electronic signalling to inform the investigator when an animal has entered the trap.

Shooting with firearms may be the most efficient or only practical means of killing some species. In such cases, a competent marksperson who is legally permitted to use a firearm must be used, and they must be familiar with the targeted anatomical structures for the most efficient killing. Investigators must also adhere to any restrictions on where firearms can be discharged or animals may be legally taken, such as in ecological reserves or within defined distances from human settlements and houses.

The firearm and ammunition that will produce a quick death in the species should be used. Whenever possible, non-lead shot should be used (Canadian Veterinary Medical Association (CVMA), 2021; Thomas, 2019). If lead shot is used, the carcass or portions of the carcass contaminated with the lead shot should be removed to prevent inadvertent lead poisoning in scavengers or contamination of the environment (Arnemo et al., 2016; Pain et al., 2019). Situations that may lead to a high risk of losing the carcass (e.g., aquatic or marine mammals in open water, land mammals on steep terrain) should be avoided. When possible, an attempt should be made to shoot the animal while minimizing stress to other animals.

Shooting an animal from a moving platform (e.g., helicopter) increases the welfare risks to the animal and must be justified as necessary. Shooting must be conducted by a competent marksperson with experience in shooting animals from that platform. The capability of the pilot must also be considered when planning the procedure. Alternatives to shooting an animal from a moving platform must be considered (e.g., capture using a net gun, followed by restraint and euthanasia); however, capture and restraint may not lessen the negative welfare impacts for some species and situations. A record should be kept of the type of platform, firearms, and ammunition used, pursuit times, platform velocity and movement patterns, environmental conditions (e.g., lighting and weather), daily success rates, and the timing of any wounded animals. These data should be used to improve success rates, including assessment of whether personnel fatigue was a potential factor (Hampton et al., 2017; Bengsen et al., 2021).

The investigator or marksperson must be able to confirm death after euthanasia of the target species and be prepared to retrieve and quickly euthanize any target animals they have wounded (see Section 13, "Euthanasia").

7 MARKING OF ANIMALS

Marking of animals includes the application of identifying marks on the skin, fur, scales, or feathers, and the attachment or insertion of tags, microchips, telemetry devices and others.

7.1 GENERAL CONSIDERATIONS

When possible, investigators should use unique natural features to identify individuals (e.g., frogs' skin patterns, turtle plastron patterns, whale tail fluke patterns) or technologies that avoid the need for capture and marking animals (e.g., artificial intelligence, facial recognition (Clapham et al., 2020)).

When marking animals is necessary and appropriate, investigators should use marks that:

- are appropriate for the species;
- are the least invasive, with minimal pain or discomfort;
- do not require recapture for identification or removal;
- allow for growth of juveniles and seasonal changes in adults;
- remain effective for the duration of the scientific activity to minimize the need for replacement marks; and
- do not interfere with the animal's activities during or after the scientific activity.

The marking process should be quick and easy, minimize animal handling, and limit the number of animals marked to that which is appropriate to meet the goals of the scientific activity.

Accurate records must be kept for all marked animals, including replacement marks.

All methods of marking animals must comply with relevant government regulations and guidelines.

Guideline 23

Investigators must aim to minimize all short-term and long-term adverse effects of the marking procedures and the marks on the animals.

Minimizing the impacts of marking procedures and marks on animal welfare requires consideration of the least invasive approach and means of reducing the potential for animals to experience pain, discomfort, stress, changes in health (e.g., infection and susceptibility to disease), and altered behaviour and physiology. Investigators also must consider the nature and duration of any restraint required in deploying marks. If possible, marking procedures should be conducted at the same time as other required procedures (e.g., taking blood or morphometric measurements) to avoid cumulative stress. Justification must be given for any tissue removal or damage and whether anesthesia or analgesia is required for invasive marking. The permanence of the mark should be weighed against the subsequent need for recapture. When non-permanent marks are used, the procedures should involve removal without recapture when possible (e.g., collar drop-offs).

Investigators must consult the literature and seek expert advice to determine any potential problems with marks and identify new developments in marks and marking approaches for the species of interest. Investigators should provide justification for the selected procedure.

When previous research is not available on mark impacts and marking procedures, investigators should conduct a pilot or controlled study to assess their effects on animal welfare. Effects of marks may be unknown, partly because of the lack of adequate controls. When at all possible, investigators should compare the impact of different types of marks in controlled situations. In particular, new marking procedures and marks should be evaluated on captive animals or in a pilot study where feasible. Investigators are encouraged to publish results of studies evaluating the effectiveness of the mark type or design, including any negative impacts, particularly for novel or experimental marking procedures. Such studies can help other investigators.

If a marked animal from another scientific activity is captured and is not part of a collaborative project, investigators should not interfere with the previous scientific activity, such as by removing marks without prior permission, unless there is evidence of the mark causing injury to the animal. If marks are removed, efforts should be made to notify the investigator and return any non-permanent marks.

7.2 VISIBLE IDENTIFICATION MARKS

Guideline 24

When considering the use of visible marks, investigators must weigh the requirements of the scientific activity against the potential risks of morbidity, mortality, or altered behaviour or reproduction of the animals, and minimize these risks.

The risks associated with visible marks depend on the species, the type of mark, and the location of attachment. The size, shape, and placement of marks should allow normal behaviour of the animals. Marks that project from the body (including collars) may impair physical activities or cause entanglement in undergrowth or aquatic cover. In addition, projecting marks may be torn as a result of the animal's movements. An analysis of avian wing marks shows that a variety of wing marks can have negative impacts on survival and nest success (Trefry et al., 2013; Saraux et al., 2011). In addition, brightly coloured marks may compromise an animal's camouflage or possibly act as a predator attractant. The use of marks that pose such risks must be justified and the risks minimized.

Application of some marks can be painful, for example, ear tags that displace substantial tissue and tags that are sutured. These applications must be justified to the animal care committee or their use avoided. Analgesics should be used if pain during application of the mark is anticipated.

In some cases, means of applying marks can be improved, for example, use of a small biopsy punch to create a clean incision hole, rather than a standing tagging punch or gun, may reduce tissue trauma. For marks requiring an incision, the area surrounding where a mark will be placed should be cleaned and disinfected prior to any attachment. Analgesia must be considered.

7.3 TELEMETRY DEVICES

Telemetry devices, such as very high frequency (VHF) and satellite transmitters, data loggers, video cameras, and maximum depth gauges, are commonly used for remotely monitoring animal physiology, movement, behaviour, habitat use, survival, and reproduction. The effects of these devices on energetics, survival, reproductive success, and behaviour of the animals should be considered in protocol development and justified to the animal care committee. This is especially important for flying and swimming animals, where additional drag or weight and buoyancy can impair locomotion (Bodey et al., 2018).

Guideline 25

Telemetry devices and their attachment materials should be as light weight and as streamlined as possible for the species on which they are deployed. Investigators should use devices that minimize discomfort and hindrance of normal behaviour, health, or other aspects of the animal's welfare.

Investigators must justify the use of all telemetry devices and their mass, attachment location, and attachment methods in terms of meeting the research needs while minimizing negative impacts on the animal. For external telemetry devices, investigators are encouraged to use breakaway or drop-off devices that allow device removal without recapture at the end of their useful life. The health and survival of the tracked animal must be a priority.

The appropriate mass of the transmitter will depend on the species, where and how the device is attached to the animal, and the length of time it will remain attached. Investigators must review the literature and seek expert advice on device weight, shape, positioning, and attachment method (e.g., collars, harnesses, ear tags) for the species they are working with or similar species if the information does not exist.

The lightest devices possible are encouraged; however, the mass of the transmitter will be a balance between minimizing the total mass of the device and the inclusion of drop-off devices and features necessary for the scientific activity (e.g., battery life, mortality sensors, GPS capability). Consideration of the mass of the transmitter must also include any attachment materials and other marks that will be applied (e.g., additional visual marks).

A percent body mass approach is likely to have different effects for different taxa and species (Bodey et al., 2018; Weiser et al., 2016; Elliott, 2016; Barron et al., 2010; Casper, 2009; Sikes et al., 2011). Recommendations for the mass of the transmitter and attachment materials in the literature range widely across taxa and species, from less than 2% to less than 5% of the body mass of the animal. For some flying and swimming animals, an upper transmitter mass limit of 3% of the body mass of the animal is considered appropriate (Phillips et al., 2003). However, a comprehensive approach must be taken for decisions regarding the devices and attachment methods to be used. A device and attachment method with a mass over 5% of the body mass of the animal must be justified based on the requirements of the scientific activity and the welfare of the animals.

Consideration must also be given to the design, fit, and materials of the device to ensure there is no unacceptable hair or feather loss, skin damage, choking, impairment of movement (e.g., flight and diving for birds) or thermoregulation, or other welfare effects for the animal. The fit of the device and attachment method must account for animal growth, including seasonal changes, which can be significant. Other con-

siderations should include limiting the visibility of the device to prevent or reduce disruption of the natural camouflage of the animal unless justified in the protocol. If predators can potentially ingest the devices, consideration must be given to non-toxic materials that do not cause blockage in the predator's digestive system.

For birds and aquatic animals, investigators should consider placing tags as far back on the animals as possible to reduce drag, while taking into account any change in the animal's balance that may be caused by placing tags away from their centre of gravity (Elliott, 2016; Bodey et al., 2018). Consideration should also be given to internal implants such as Radio Frequency Identification (RFID) (Bandivadekar et al., 2018) to reduce drag; however, some require invasive surgical techniques (White et al., 2013).

Transmitters implanted into the abdominal or coelomic cavity require invasive surgery and a second surgery if the device is to be removed. These devices can lead to complications related to animal welfare, and the devices cannot be easily monitored for these complications (e.g., Arnemo et al., 2018). Therefore, justification must be provided for why less invasive alternatives cannot be used. If devices are surgically implanted, the procedures must use recognized veterinary techniques (see Section 8.4, "Surgery"), with signs of complications (e.g., adhesions, inflammation, and infection) documented and reported to the animal care committee as part of the annual report at the time of protocol renewal.

Investigators are encouraged to use external devices that fall off once the scientific activity is completed or completely drop off when remotely triggered. Because trigger mechanisms may fail, two self-removal mechanisms (e.g., a rot of fabric and a programmed drop-off) should be considered, especially when capture and removal of the collar may adversely influence the welfare of the animal. Investigators should indicate when the device is due to drop off or rot off in their protocol. Any signs of complications with the removal of devices should be documented and reported to the animal care committee as part of the annual report at the time of protocol renewal. If devices are retained past the completion of the scientific activity (e.g., from collar drop-off failure) and are causing or are likely to cause animal welfare concerns that outweigh the effects of additional handling, effort must be made to remove the device, document any issues, and investigate alternatives or refinements for future use.

Decisions of whether or not to remove an implanted device or an external device that has failed to fall off at the end of the scientific activity must be based on consideration of the welfare costs of removal and of leaving the device in place.

A permit or license for the use of specific frequencies for radio transmitters may be required from Innovation, Science and Economic Development Canada. The appropriate spectrum office of the Ministry of Innovation, Science and Economic Development should be contacted regarding the licensing process (see Radiocom Information Circular 66 (RIC-66) for district offices).

7.4 TISSUE MARKING (INVASIVE)

Researchers should ensure that the tissue marking process does not cause unnecessary tissue damage, pain, or severe blood loss. Adequate pain control is necessary when undertaking marking procedures that can have such consequences. Certain techniques can lead to infection if not carried out under aseptic conditions.

Tattooing has been used on many animals, including mammals, birds, amphibians, and reptiles (e.g., Williams et al., 1997; Nietfeld et al., 1996). One limitation of tattoos is that they might be misread due to loss of legibility, and therefore other primary marking methods should be considered.

Guideline 26

Marking techniques that cause significant tissue injury, such as branding and toe and tail clipping, must not be used unless based on evidence that the procedure is necessary and alternative methods cannot achieve the required results.

The use of branding in scientific activities involving wildlife is not encouraged. Branding causes pain and serious tissue damage and is considered to be a form of mutilation. Therefore, it must be used only in exceptional circumstances. If branding is the only option, cryo-branding must be considered first, before hot-branding. Cryo-branding must be performed with sedation and analgesia, and hot iron branding must be performed under anesthesia and with post-operative analgesia. In either case, the operation must be conducted by competent personnel and every effort must be made to minimize the animal's pain and discomfort. Anesthesia is particularly risky for cetaceans; however, hot iron branding without anesthesia causes severe pain and distress (category of welfare impact E) and alternative marking procedures should be used.

Removal or damage to tissue by toe, ear, or tail clipping must be used only when no alternative marking methods are available. Technologies such as RFID should be considered over these methods, except when tissue samples are also required. When used, tissue removal should not impair normal activities or survival of the target animal. Toe clipping must not be used for animals that burrow, climb, or otherwise use toes with specialized functions. If no alternative methods to toe clipping are available, justification must be provided to the animal care committee and only the most distal phalanx should be cut.

8 BIOLOGICAL SAMPLING AND SURGERY

Procedures used in wildlife studies include minimally invasive techniques for the collection of biological samples and more invasive techniques such as the removal of blood and tissue, administration of substances, and surgery. While investigators may be the most knowledgeable persons regarding the handling of the animals for minimally invasive procedures, the advice and direct assistance of a veterinarian should be sought during the planning stage of a scientific activity involving the administration of antibiotics or other drugs and invasive procedures, particularly with a species or procedures new to the investigator. Appropriate veterinary expertise should also be sought for training or to perform the procedure. Depending on the jurisdiction, laws or regulations may require that some procedures be undertaken only by a veterinarian (see Section 2, "Scientific Activities Conducted in the Field").

Invasive procedures include tissue sampling, physical measurements, ageing techniques (for some species), and surgery. Some procedures require chemical restraint for the safety of the animal and personnel (see Section 5, "Handling and Restraint") or complete general anesthesia (i.e., for painful or invasive procedures). Investigators must minimize handling time, stress, and pain and ensure that any effects do not last beyond the normal recovery period of the animal.

Protocols should include plans for problems that could be encountered during procedures, which should include consultation with a veterinarian with wildlife experience when feasible.

The development of SOPs can promote best practices and make the process of writing and reviewing protocols more efficient; however, they must be specific to the species involved and allow flexibility for field conditions. For some scientific activities involving wildlife, it is often more efficient to describe the procedures in a protocol, rather than develop and refer to SOPs.

8.1 COLLECTION OF BIOLOGICAL SAMPLES

Guideline 27

Investigators should use the least invasive method of collecting biological samples from the animal that is suited to the goals of the scientific activity.

Where possible, biological samples should be collected from the environment (e.g., eDNA, shed feathers, fur, feces, pellets, regurgitate). Where studies require that samples be taken directly from the animals (e.g., blood, claw clips, breath, saliva, mucosal swabs), consideration should be given to obtaining these samples when animals are captured or killed for other purposes. However, opportunistic, nonessential sampling activity should be balanced against any additional restraint time required.

The use of biopsy darts to remotely sample animals must be performed by personnel who are competent in the procedure on the species of interest to ensure that sampling is done safely and with as little stress to the animals as possible.

8.1.1 Blood and Tissue Samples

Guideline 28

Sampling of blood and other tissue, including tooth extraction, must be performed only by, or under the direct supervision of, personnel competent in the procedures, and must avoid or minimize pain and distress.

The advice of a veterinarian can be helpful for developing and practising proper blood and other tissue sampling methods that minimize stress and pain to the animal and provide an adequate sample for analysis. Proper collection and specimen handling and preservation protocols should be followed to ensure valid field data. The need for an anesthetic and analgesic depends on the species, the method of restraint, the physical condition of the individual animal, and the volume (amount of blood) and tissue required.

The literature and experienced field personnel must be consulted to determine the appropriate volume of blood, intervals between blood collection, and sampling site for the particular animal and situation. As a general rule, the volume of blood collected for a single sample should be no more than 1% of the total body mass of the animal (or 8-10% of the total blood volume; blood volume is approximately 100 ml/kg body weight) and less (approximately half that volume) in sick or debilitated animals. Smaller volumes should be targeted based on the age, condition, and species of animal, or when repeated sampling is necessary. Consideration must also be given to the sampling site (Brown and Brown, 2009) and seasonal energy demands for the animal (Voss et al., 2010). Justification for blood sampling volumes and site selection should be provided to the animal care committee, especially when volumes are at the upper end of the acceptable range.

When appropriate, blood and other tissue samples should be collected during the handling of animals for other purposes (e.g., radio collar attachment, migration monitoring) to maximize data collection, reduce the need to recapture animals, and minimize the number of animals needing to be immobilized in future studies. The samples may be analyzed immediately or archived for later use.

Cardiac puncture is not appropriate for birds or mammals, except in terminal procedures, and it must be performed under general anesthesia. For some herptiles, it may be an option in particular situations where justification is provided to the animal care committee.

8.2 USE OF ANALGESICS

Guideline 29

Appropriate analgesics should be used for any procedure that may produce pain.

For invasive procedures, such as laparotomy, biopsy, tooth extraction, ear tagging, or surgery, measures must be taken to control pain during and after the procedure. Acute pain triggers catecholamine (epinephrine, norepinephrine) release, resulting in immediate multiple changes in physiology and organ function. Chronic pain can result in decreased healing, decreased resistance to disease, and malnutrition. For both acute and chronic pain, there are negative effects on the welfare of the animals, and the loss of function due to pain may make the animal more susceptible to predation.

Analgesics must be administered prior to performing a painful procedure to reduce the pain response, with additional analgesia provided as indicated. A variety of approaches may be available to provide analgesia for wildlife, including local anesthesia, narcotics, anti-inflammatory drugs, other prescription pain medications, or a combination of these. The choice of medication and route of administration will vary among species (Whiteside, 2014), as will the duration and mode of action. The current literature and experts must be consulted to make an appropriate choice.

Opiates such as morphine and opioids such as fentanyl, buprenorphine, and butorphanol, are classified as narcotics and are included in the *Controlled Drugs and Substances Act* (Health Canada, 1996). An individual wishing to use these drugs must have appropriate training and make a written request directly, or through a veterinarian, to the Drug Strategy and Controlled Substances Program at Health Canada for every project in which they plan to use these drugs. These drugs may cause side effects and should be administered only following careful consideration and consultation with a veterinarian having experience with wildlife.

Local anesthetics can provide effective analgesia of wildlife for some procedures. SOPs should be developed that detail the route of administration, duration of action, toxicity, and competency of personnel in their administration. Duration of action required for the procedure often dictates the choice of local anesthetic agent. For surgery, local infiltration of the surgical site is the simplest method; however, this approach is not effective for all procedures as infusion around a tooth may be of no use for tooth extraction and fracturing a tooth without full-root removal can lead to pain and infection. Personnel must be competent in the procedure. If administered incorrectly, injection of local anesthetics may penetrate too deep into the nerve bundle (e.g., for maxillary or mandibular freezing) and cause permanent damage. Those administering anesthetics should also be trained in drug properties and onset and have knowledge of the animal's anatomy.

8.3 PHYSIOLOGICAL MEASUREMENTS

Investigators should minimize stress and pain when capturing animals for the purpose of taking physiological measurements and when undertaking those measurements. See Section 5, "Handling and Restraint", for more information on handling procedures.

8.4 SURGERY

Guideline 30

Investigators must consult a veterinarian on projects involving surgical interventions, including laparotomies, transmitter implants, and other invasive procedures that expose the abdominal cavity or other deep tissues. In some cases, procedures can be performed only by a veterinarian according to relevant laws governing the practice of veterinary medicine.

As noted in Section 2, "Scientific Activities Conducted in the Field" (Guideline 4), investigators should consult with veterinarians who have experience with, or knowledge of, the species in question and the logistics of scientific activities in the field in their jurisdiction. During the planning phase, investigators must establish who will perform any surgical procedures on the animal, taking into consideration jurisdiction and regulations. Many jurisdictions limit surgery to veterinarians.

Recognized veterinary procedures must be used for all invasive procedures, including asepsis, anesthesia, analgesia (pre-, peri-, and post-operative), appropriate surgical techniques, and animal monitoring. The advice of a veterinarian must be sought and followed prior to the administration of antibiotics or other drugs used during invasive procedures.

Animals must be observed and cared for during recovery from anesthesia, and they should not be released from traps or enclosures until fully recovered (i.e., showing purposeful movement, not showing signs of ataxia, and able to defend themselves) unless this poses a safety risk for operators. Post-operative monitoring should be performed, either remotely or via direct observation, with follow-up treatment provided if appropriate.

9 TRANSPORTATION

All transport of wildlife, whether over a short distance (e.g., carrying an animal from the point of capture to a processing location) or a longer distance (e.g., by road or airline), requires planning and adherence to procedures that will ensure the animal's welfare and humane care for the duration of the journey. The transport process must be described in the protocol. Any necessary permits must be acquired, and the requirements listed on the permits must be followed.

Guideline 31

Investigators must ensure that the care, containment, and mode of transportation of wildlife are suitable for the species and the animal's condition, based on the best available information for that species or similar species, and that the animal will be transported in a manner that minimizes stress and injury.

The species involved, the method of transportation, appropriate temperature, the duration of the journey, frequency of monitoring, and environmental conditions (e.g., heat or cold) are important factors in determining the type of care and conditions of containment required to transport the animal in a safe and humane manner.

Personnel involved in transporting animals must be knowledgeable of the procedures for the particular type of container and must ensure food, water, and bedding are available as appropriate for the duration and method of travel. Veterinary assistance may be required to prescribe or administer short- or long-acting tranquilizers to an animal if the transportation process is anticipated to be stressful or if complications occur.

The transportation process should be as brief as possible. Transportation of some species requires periodic rest periods to allow the animals to feed and drink undisturbed, whereas other species may only be transported when they are normally inactive. Animals must be monitored during transport. Remote, continuous monitoring of animals via video surveillance during transport can be an option to minimize disturbance associated with direct observation and allow for prompt action if complications occur (Slater et al., 2021). In the absence of remote monitoring, scheduled welfare checks on the animals should be conducted during transportation at a predetermined frequency based on the risks to the animals.

9.1 TRANSPORTATION BY ROAD OR AIR

If planning to transport wild animals using an airline, investigators must consult the most recent edition of the International Air Transport Association (IATA) *Handbook on Live Animal Regulations*, for information on species-appropriate containers, care, and handling. The airline may also have more restrictive rules (number of animals per crate, destination, temperature maximums and minimums, etc.) and should be contacted for further advice. The IATA document is also a good resource when preparing for ground transport.

To avoid delays, anticipated relevant documents (e.g., permits, health certificates) must be available prior to transport. The trip should be scheduled to minimize transfers and delays and to ensure that a person competent to provide appropriate care is available to meet the animals upon arrival at the destination. Multi-day transport may require a qualified person to accompany the animals or appropriate alternative measures. Any required clearance of animals by animal health and customs inspectors should be arranged prior to transport to avoid unnecessary delays.

Contingency plans must be in place to deal with transport delays and emergencies such as breakdowns, collisions, or extreme weather. This may include carrying twice the amount of food and water that is needed for the anticipated length of the journey.

Animals that have received general anesthesia should be fully recovered prior to transport.

10 HOUSING AND HUSBANDRY

There is considerable variation in the biological needs of each wildlife species, the health and welfare of individual animals, and the nature of scientific activities involving wildlife. Therefore, only general guidelines are provided for the housing and care of wildlife in either short- or long-term captivity for scientific activities.

Guideline 32

Investigators must review the literature and seek expert advice to gain an understanding of the relevant requirements, habits, and behaviours of any wildlife species to be held captive.

An understanding of the normal ecology, morphology, physiology, biology, and behaviour for each species being held will assist in providing optimum care and housing. When this information is not available, literature and expertise in housing similar species should be consulted. It may be necessary, especially when dealing with unfamiliar species, to test and compare several methods of housing to find the one most appropriate for the needs of the animals and the objectives of the scientific activity.

Husbandry methods should be used that minimize or prevent detrimental behaviours (e.g., food conditioning, habituation, interaction with people) that are likely to result in harm to the animals or people during the holding period or after the animals are released. Consideration must be given to ensuring the housing provides protection for the animals from predators, other animals (whether of the same species or not), and human harassment.

10.1 HOUSING

Guideline 33

Animals held for up to 24 hours must be placed in appropriate containment that allows adequate ventilation, and be provided with suitable food, water, and furnishings (e.g., bedding for mammals, perches for birds and other species) based on the length of time they are being held.

Containers must be kept at a temperature appropriate for the species and have appropriate shelter and ventilation. The animals should be protected from direct sunlight, wind, and precipitation. Care should be taken to minimize psychological stress by shielding containers from excessive light, noise, and human activities. Containers should have an area where the animal can escape from view. Live trap-type containers with open visibility may be suitable for short-term holding if they provide adequate space and ventilation, but they are suitable only for up to a couple of hours.

The environment near the container should be selected to avoid stressors. Containers should be secured so that no predator can enter.

Short-acting tranquilizers can help reduce the level of stress and anxiety associated with temporary confinement and should be discussed as an option with a veterinarian with wildlife experience.

Animals should be monitored frequently with as little disturbance as possible. Remote video surveillance is recommended where appropriate.

Guideline 34

A captive environment for the long-term (>24 hours) confinement of wildlife must provide for any anticipated behavioural, physical, nutritional, and security needs, while providing enrichment opportunities for physical and psychological stimulation.

While in captivity, wild animals must be maintained under conditions that are anticipated to meet their needs for food, water, nesting, space, microclimate, and safety. Long-term containment should attempt to duplicate all aspects of the species' natural conditions, including social groups, or replace these with artificial elements or conditions of comparable value to ensure the survival and well-being of each individual. For enclosed housing, it is important that environmental humidity and temperature are maintained within the animal's thermoneutral zone to minimize energy demands. Animals that hibernate require special housing to maintain and monitor ambient temperatures and humidity at the optimum level for each species. Animals housed in open pens must be provided with natural or human-constructed shelter and sources of water.

Wild animals kept or raised in captivity for more than a few days will have additional requirements for enrichment to accommodate features of their ecology, morphology, physiology, biology, and behaviour, including social needs (see Miller (2012) as a starting point for information on keeping various groups of wild animals in captivity). These may include feeding strategies, visual barriers, refuges, natural materials, perches, dust and water baths, and opportunities and space for exercise and play. Animals that are not provided with these features may develop signs of acute and chronic stress, including poor health and abnormal behaviours.

In natural settings where animals are maintained in fenced pens, consideration must be given to predator-proofing the fencing (e.g., belowground fencing and double fencing) and taking precautions to modify or eliminate potential entry points, such as trees along the fence line.

Short or long-acting tranquilizers should be considered to facilitate reduced stress levels while the animals initially acclimatize to captive environments.

Ease of restraint and maintenance should not be the only determinants of housing conditions. The suitability of housing should be assessed by monitoring a number of biological indices over time, such as changes in general health, appetite, growth and weight, survival rates, breeding success, birthing, activity types and levels, general behaviour, and appearance of skin, pelage or plumage (see Section 11, "Welfare Assessment"; Kleiman et al., 2010; and Fowler and Miller, 2007).

10.2 NUTRITION

Guideline 35

Diet and feeding schedules should reflect the animal's normal foods and feeding behaviour.

Consultation with experienced nutritionists or those with experience meeting the nutritional requirements of the species or similar species held in captivity (e.g., wildlife rehabilitators and zoo keepers) should occur prior to housing a wild animal. In addition to attempting to duplicate critical aspects of a species' natural environment, it is also important for both normal health and behaviour to ensure that the animals' nutrient requirements are met and that the animals are kept in body condition appropriate for their age and sex and the season. Where possible and appropriate, it is advantageous to supplement standard dietary requirements with a variety of natural feeds. For wildlife species with specialized diets (e.g., sage grouse, moose), it may be necessary to consider adding supplements such as natural vegetation. Animals must have access to fresh water at all times; however, the appropriate water source and quality will depend on the species.

Dietary changes should be made gradually over a few weeks. Providing food *ad libitum* may be problematic for some species; overfeeding and consumption of less nutritionally balanced but preferred food items may be a concern.

10.3 SOCIAL INTERACTIONS

Guideline 36

Social relationships and social behaviour of captive wildlife must be taken into consideration when designing and maintaining captive facilities for wildlife populations.

Group housing is necessary for some species, but for others it increases stress and the risk of injuries. Similar concerns may arise for group housing individuals of different sexes. Consideration must therefore be given to the appropriateness of visual, auditory, olfactory, and tactile contact for the particular animals involved. Prey and predator animals should not be kept in close proximity where olfactory or visual cues can cause distress.

10.4 HEALTH

Wild animals that are maintained or raised in captivity should be provided with a preventive health care program. This program should assess the need for procedures such as:

- health monitoring (e.g., visual and physical exams, including assessment of hair or feather condition, behaviour or stereotypies, nutrition analysis, blood sampling and testing, bacterial and viral cultures, and serology or molecular diagnostics for diseases of concern);
- vaccinations;
- diagnosis, monitoring and treatment for internal and external parasites; and

- dental, skin, and foot (fins and flippers for marine mammals) care.

Section 11, "Welfare Assessment", provides information on health indicators.

10.5 HYGIENE

Guideline 37

Facility setup and cleaning routines should be designed to maintain adequate hygiene levels while minimizing disturbance of the animals.

The frequency of cleaning the animal's housing (e.g., cage, pen) should be a balance of the level of cleanliness necessary to prevent disease and the amount of stress on the animals imposed by disturbance, handling, and exposure to unfamiliar surroundings and bedding (ABS and ASAB, 1997). Particular attention should be paid to maintaining adequate hygiene in concentrated use areas, such as around feed bins. Cleaning procedures suited to the particular situation should be described in an SOP to maintain consistency.

11 WELFARE ASSESSMENT

When working with wildlife, it is important to consider the effects of the scientific activity on each individual animal, where possible, even when the scientific activity is designed at the level of the population. Assessing animal welfare and reporting potential animal welfare issues, along with any mitigation taken and their efficacy where appropriate, is important to minimizing stress and avoiding distress for the animals during the activity in question and to improving wildlife techniques and results for future scientific activities (Harrington et al., 2013).

General guiding principles for welfare assessment of all animals used for scientific purposes are described in the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021). Information in this section builds on the general guidelines by focusing on indicators for assessing the welfare of individual wild animals, keeping in mind that any indicator must be tailored to the species and age of the animal and the type of scientific activity.

Any welfare concerns must be investigated, documented, and reported to the animal care committee at protocol renewal. Responsibility for assessing the welfare of wild animals usually ends with termination of the research or teaching activity (CCAC, 2021). However, in designing studies, investigators must review the literature and seek expert advice to assess and minimize any potential long-term impacts of procedures on the welfare of the animals (see Section 2, "Scientific Activities Conducted in the Field").

Guideline 38

The welfare of all wild animals involved in scientific activities must be assessed according to a plan that is suited to the type of scientific activity and designed to optimize the collection of information without adding procedures that would cause stress for the animal.

The *CCAC guidelines: Animal welfare assessment* (CCAC, 2021) state that information can be obtained through a mixture of animal-based measures, resource-based measures, and data management-based measures. For wildlife, animal-based measures are observations and additional information on the health, behaviour, and physiology of individual animals obtained through the conduct of procedures that are part of the scientific activity. Where wild animals are to be held in captivity, an assessment of their captive environment (i.e., resource-based measures) can provide information to determine the source of any negative welfare observed in these animals. In addition, records of the scientific activity, mortality and morbidity records, husbandry records, etc. (i.e., data management-based measures) can be useful in identifying potential causes or sources of impacts on the welfare of animals used in scientific activities, particularly when viewed over time. Harvey et al. (2020) provide an example of a template for assessing the welfare of wild animals.

11.1 APPLICATION OF WELFARE ASSESSMENTS AND REPORTING

Welfare assessments can be used to identify potential risks to animals and inform decisions regarding their inclusion in a particular scientific activity, the level of monitoring required, and the need to implement

mitigation strategies to improve animal welfare. For example, animals that are pregnant, injured, or have a debilitation may not be suitable for capture and collaring based on risks to their welfare. The information should also be used to refine techniques and procedures, including the refinement of humane intervention points that are aligned with scientific endpoints (see the *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)).

As noted in the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021), information gathered in relation to welfare assessments should be recorded in a format accessible to those involved in the scientific activity, including the animal care committee. Welfare assessment records or a summary of the information should be reviewed by the animal care committee at least annually during protocol renewal. However, the animal care committee must be notified as soon as possible if animals experience unanticipated "severe" or "unacceptable" welfare states[2] in response to the scientific activity.

11.2 WELFARE INDICATORS

The following sections list potential indicators for consideration in designing a plan for assessing the welfare of wild animals. As mentioned, the indicators selected must be tailored to the particular animals involved (e.g., species, age, physiological state) and the type of scientific activity, and they should not impose stress for the animals beyond that expected from the procedures described in the protocol.

11.2.1 Health Indicators

Potential health indicators that could be noted only through observation:

- gait or flight pattern;
- movement patterns and rates;
- posture and attitude;
- presence of obvious health concerns (e.g., physical injuries, deformities, or pathologies);
- increased susceptibility to disease or severity of disease;
- general appearance of coat, feathers, scales, skin;
- overall behaviour (e.g., isolation from the herd or conspecifics);
- respiration rate and effort;
- food and water intake;
- general estimate of body condition score;
- appearance of feces (and urates in birds) and urine;

2 Defined in the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021) as follows:

Severe: welfare concerns have been identified that require extensive mitigation measures and close monitoring. Discussion with the animal care committee may be required to rectify the situation or terminate the protocol.

Unacceptable: overwhelming welfare concerns have been identified, providing justification for immediate euthanasia. Discussion with the animal care committee is required to rectify the situation or terminate the protocol.

- survival compared to control animals; and
- reproduction and appropriate parental care.

Potential health indicators that could be assessed during handling or holding of the animals, providing they do not cause additional distress for the animals:

- body weight;
- body temperature, pulse, heart rate, and respiration;
- coat, feather, scale, and skin condition;
- appearance of the eyes and teeth;
- possible physical injuries, deformities, pathologies, diseases, parasites;
- wound healing;
- more objective scoring of body condition (e.g., ultrasound);
- respiratory rate (and open-mouth breathing);
- appearance of feces and urine or urates;
- oxygen levels; and
- capture myopathy.

11.2.2 Behavioural Indicators

Investigators should be aware of species-typical behaviours; however, assessing behavioural indicators at the individual level can be difficult when there is no known history of the particular animal. For example, some individuals are inherently more aggressive than others of the same species. However, an attempt should be made to define the normal behaviours of these animals (e.g., the generally observed behaviour of captured animals). When an animal displays changes in the frequency, duration, or intensity of their usual behaviours or displays abnormal behaviours such as stereotypies or apathy (e.g., animals of social species that appear alone (see Brakes, 2019) or animals that are not eating), these should be investigated and recorded.

The behaviour of wild animals can be influenced by the presence of an observer and, where possible, remote observation for welfare assessment purposes should be used to determine the overall behaviour of the animal. Remote monitoring (e.g., via video cameras or biologgers) is recommended when possible to allow analysis of behaviours via ethograms and other objective methods.

11.2.3 Physiological Indicators

Measurement of physiological indicators should be obtained through non-invasive methods whenever possible, such as observation of respiratory rate and effort, collection of shed hair and feathers, and excreted urine and fecal samples. The use of infrared thermography to measure peripheral body temperature may also provide an indication of core temperature (Jerem et al., 2015). More invasive physiological measurements should be used only when they are a component of an approved protocol.

Potential physiological indicators may include:

- body temperature;
- immunological functions (rates of lymphocyte proliferation or suppression of their activity);
- blood pressure;
- blood analysis (hematology and biochemistry);
- respiratory and heart rate;
- energy expenditure;
- levels of various 'stress hormones' (e.g., cortisol, noradrenaline); and
- remote measurement of eye temperature using infrared thermography.

12 RELEASE OF ANIMALS

12.1 RELEASE OF ANIMALS FOLLOWING PROCEDURES

Captured animals should be released as soon as possible after the procedures at the site of the original capture, with consideration of escape pathways, unless they are involved in a translocation project. For studies that do not involve translocation, if release at the original site of capture is not possible (e.g., to avoid recapture), the animals should be released as close to the capture site as possible, preferably within their home range if known.

Food, water, and a period for stabilization prior to release should be provided as necessary and appropriate. Consideration must be given to how the time of release may impact the animal's probability of survival, weighed against the impact of continued holding. For example, releasing a Great Horned Owl in broad daylight risks exposing it to harassment from crows, which can be avoided by scheduling the release for the end of the day or at night.

When an animal is held in captivity for a prolonged period prior to release, consideration should be given to the potential impact of that time in captivity on a successful release. For example, the welfare of the animals may be affected by seasonal changes in diet that have occurred during captivity or there may be changes related to migration.

Consideration should be given to releasing family groups or cohorts, young with parents, and mated individuals together.

12.2 SCIENTIFIC ACTIVITIES INVOLVING TRANSLOCATION OF ANIMALS

Translocation is defined by the IUCN (2013) as "the human-mediated movement of living organisms from one area, with release in another." The IUCN definition includes the movement of animals from the wild or from a captive environment and includes release of those animals "either within or outside the species' indigenous range."

Guideline 39

Translocation must be justified for an individual, population, or species, taking into consideration the impacts on the source and recipient populations and ecosystems. The possible negative consequences of translocation on the individual animal, source and recipient populations, and the ecological conditions at the release site must be considered and mitigated or minimized within the constraints of the scientific activity. When translocation occurs, the health and disease status of the source and recipient populations and the individuals to be translocated must be evaluated prior to translocation. Capture, transportation, and release of translocated individuals must follow current best practices for animal welfare, and individuals should be released to maximize their ultimate survival and minimize impacts on other species and the environment.

Translocation of captive or free-ranging wildlife should be carefully planned to ensure animal welfare during translocation (see Section 9, "Transportation") and to enhance the probability of subsequent survival and eventual reproduction of released animals. A multidisciplinary approach to planning may be required, involving government and non-government agencies with expertise in resource management, wildlife biologists, veterinarians, personnel with husbandry and academic expertise, and persons with traditional and local knowledge.

Translocation of animals brings risks associated with diseases and genetic and ecological integrity. It has the potential to compromise the welfare of animals in the population into which individuals are being released and to negatively impact other components of the ecosystem, including human health. Prior feasibility studies and a formal risk analysis should be undertaken to show that the adequacy of available habitat, the level of existing competitors and predators at the site, and the genetics and condition of animals to be released will promote the welfare and success of translocated individuals. A decision tree for wildlife reintroductions developed by Harrington et al. (2013) provides an example of relevant welfare considerations at each stage of the translocation process. For additional useful information, see *The IUCN Position Statement on Translocation of Living Organisms: Introductions, Re-introductions and Re-stocking* (IUCN, 1987), the *Guidelines for Reintroductions and Other Conservation Translocations* (IUCN, 2013), the *Guidelines for Wildlife Disease Risk Analysis* (IUCN, 2014), and Leighton (2002).

Federal, provincial and territorial wildlife agencies must be contacted for consultation and authorization at the earliest stages of planning for a proposed release of any native or non-native wild animals, including those that have been captive-bred. All applicable local, provincial, territorial and federal legislation must be followed and necessary permits obtained before release.

If animals bred or held in captivity are to be released, they should be assessed for normal behaviour and their ability to survive and reproduce in the wild prior to release and post-release when possible. There should also be an assessment of the target environment to accommodate them. Investigators should thoroughly review research into 'hard' release versus 'soft' release to determine the method with the greatest potential for success.

12.2.1 Animal Welfare Considerations

Guideline 40

Appropriate measures must be taken to ensure the welfare and humane treatment of animals throughout all stages of translocation.

Prior to release, wild animals should be assessed for factors that can affect their survival or reproductive success, such as the presence of known infectious agents or pathological parasites, abnormal behaviours, and physical disabilities.

The principal welfare risks to be considered in translocation include:

- transport (see Section 9, "Transportation") and release;
- health of the animals being translocated;
- the availability of basic physical and nutritional requirements at the release site;

- potential encounters with unfamiliar mortality factors (e.g., novel predators) that prevent long-term survival and reproduction in the environment into which they have been translocated; and
- the impact of the animal on other species and the environment.

Before release, animals must undergo a quarantine and observation period if required by the permit. If the permit does not specify it, quarantine should be considered, and the period of quarantine should be based on current best practices to rule out many health concerns. The duration (generally 30 to 90 days) should reflect the incubation period of known diseases, particularly those for which no reliable screening test exists. During this quarantine period, appropriate tests should be carried out (e.g., serology, viral and bacterial cultures, external and internal parasite screens). Archiving of tissue and serological samples for possible future testing should be encouraged wherever possible. For more specific guidance, see Woodford (2000) and the current literature for the particular species.

Species-appropriate vaccinations and treatments to control parasites may be desirable prior to release to improve animal welfare if such treatments will not interfere with post-release health monitoring (e.g., serology) or are restricted by permit regulations. If used, treatments and vaccinations should be appropriate for the species and, in the case of vaccines, timed to allow appropriate immunity to develop before release.

Post-release monitoring is an important component of release programs and should be conducted. This monitoring can include tracking of individuals, postmortem investigation of natural post-release deaths, demographic studies of released stock, and disease monitoring through serology of recaptured individuals. Knowledge of the age-specific natural recruitment rates can help to assess the success of translocation efforts. Care should be taken so that post-release monitoring does not impair the success of translocated animals.

12.2.2 Behavioural Considerations for Release

The impact of individual animal behaviour on translocation success is a growing field, and investigators should consult the literature to determine if there is information on the particular species involved. As one example, there is some evidence that for certain species, bold individuals are better candidates for translocation (e.g., Baker et al., 2016; Germano et al., 2017; Merrick and Koprowski, 2017). Another study has shown a loss of anti-predator behaviour in Vancouver Island marmots after only five generations in captivity (Dixon-MacCallum et al., 2021).

12.2.3 Environmental and Population Considerations at the Release Site

Guideline 41

Investigators should be confident that the habitat at the proposed release site can provide for the species' requirements for survival and reproduction, and that no impairment to the ecological integrity of the site will occur as a result of the release.

The seasonal and local conditions at the release site and in the surrounding area where the translocated animals are expected to settle should be conducive to ensuring that the released animals can meet their requirements to survive at the time of and after release. For example, adequate natural food sources are available or food is supplemented and suitable thermal and safety cover is present. Release should not occur during extreme weather events. The physiology and behaviour of the species should be considered in the timing of

release, for example, water birds should not have their water proofing compromised, and nocturnal animals should be released just after dusk. Consideration should be given to normal seasonal times of dispersal or migration for the species and how that might influence establishment.

Factors to consider with regard to potential impacts on local populations and ecosystems include:

- the animals may carry new diseases or parasites into the recipient ecosystem that cause harm to the ecosystem, or the animals being moved may encounter new diseases in the recipient ecosystem and be harmed by them (Leighton, 2002);
- the animals may not represent the same race or subspecies as those at the release site, or they may introduce undesirable genetic traits if interbreeding occurs; there may also be unintended consequences to local ecology.

13 EUTHANASIA

Euthanasia refers to the humane killing of animals for the purposes of the scientific activity and as a component of contingency plans to address unexpected circumstances of compromised animal welfare, if necessary.

Guideline 42

A method of euthanasia that minimizes pain and distress should be chosen and be suited to the objectives of the scientific activity. Consideration should also be given to techniques that least interfere with postmortem analysis.

Information on techniques appropriate for the species of concern and the scientific activity must be researched and the necessary materials and equipment must be obtained, prepared, and in good working order.

Planning for field procedures on wildlife must include contingency plans for euthanasia. For field research projects where anticipated scientific endpoints and humane intervention points do not include euthanasia, contingency plans for euthanasia are still necessary to be prepared to address unexpected circumstances, such as injury where animals cannot be released back into the environment or rehabilitated.

General guiding principles for euthanasia of all animals in science are described in the *CCAC guidelines on: euthanasia of animals in science* (CCAC, 2010). As noted in these general guidelines, death must be confirmed in any animal that is euthanized.

The following recommendations for euthanasia of wildlife in the field are based on the *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* (AVMA, 2020), which should be reviewed to determine the most appropriate methods for a given species and situation.

Many recommended means of euthanasia for captive animals are not feasible in the field; however, the challenges presented by field conditions do not lessen the ethical obligation of the responsible individual to reduce pain and distress to the greatest extent possible during euthanasia. Euthanasia must be humane and performed to the current standards for that species. The method of euthanasia should result in rapid loss of consciousness, followed by respiratory and cardiac arrest and ultimate loss of all brain function (CCAC, 2010).

Personnel involved in euthanasia must be competent in using the chosen method on the species involved. They must check the animal's vital signs to confirm death and apply a secondary method when necessary. SOPs should be developed when practices are repeatedly conducted.

One of the most important criteria for accepting a euthanasia method as humane is that it has an initial depressive action on the central nervous system to ensure immediate insensitivity to pain. This must be followed by cardiac and respiratory arrest and the cessation of electrical activity in the brain. For this reason, pharmaceutical methods are often advised; however, the use of pharmaceuticals requires proper disposal of the contaminated carcass.

Other considerations in choosing a method of euthanasia include:

- minimizing stress, distress, and pain for the animal prior to euthanasia;
- the reliability, consistency, reproducibility and predictability of the method;
- safety of personnel;
- impact on the scientific activity and the environment; and
- the psychological impact on personnel and the public.

13.1 METHODS OF EUTHANASIA

Sections 13.1.1 to 13.1.3 provide guidance on various methods of euthanasia; however, the particular species and situation must be taken into consideration. Acceptable and conditionally acceptable methods are defined in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) as follows:

- **Acceptable**: methods that are simple to perform and consistently produce death with minimal pain and distress when used on conscious or sedated animals.
- **Conditionally acceptable**: methods that may be acceptable for use in certain circumstances where there is scientific justification and following review and approval by an animal care committee and assurance that trained personnel are available. These are not considered acceptable methods because there is greater potential for operator error or safety hazards, they might not consistently produce humane death, or they are not well documented in the scientific literature.

The *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010) also states that "other methods of euthanasia may be acceptable when used on anesthetized or unconscious animals. For conditionally acceptable methods, the detailed descriptions of conditions provided in the AVMA guidelines (AVMA, 2020) should also be reviewed.

13.1.1 Pharmaceutical Methods

Non-Inhalant Pharmaceutical Agents – these agents should be administered intravenously, with added sedation as needed to decrease fear and distress in the animal. Intraperitoneal injection of non-irritating solutions is acceptable if intravenous injection is impractical or impossible. Intracardiac injection is only acceptable in fully anesthetized or unconscious animals, such that there is no reaction to an applied noxious stimulus (e.g., a hard toe pinch). Alternate routes may be appropriate based on the species and health of the animal (e.g., intraosseous).

- **Barbiturates** – depress the central nervous system, starting with unconsciousness and progressing to apnea and cardiac arrest. This is an acceptable method. The effects are rapid and smooth, and the solution is inexpensive. Disadvantages include: a) intravenous injection is required for best results; b) it is a controlled substance and therefore must be carefully accounted for and used under the supervision of a veterinarian; and c) there are potentially fatal toxic effects to scavenging animals consuming carcasses.
- **T-61** – non-controlled mixture of three drugs. T-61 is conditionally acceptable. It must be used intravenously and must only be given to an anesthetized animal. It must only be given at carefully monitored rates of injection according to the manufacturer's recommendations because of the differential rates of absorption and onset of action of the active ingredients when administered by other routes. Anesthesia

must be administered prior to the use of T-61 to protect the animal from adverse effects as the drug causes paralysis of the respiratory musculature, which can cause distress for the animal and result in suffocation.

- **Potassium Chloride (KCl)** – a conditionally acceptable method and the animal must be anesthetized. It is an inexpensive, non-controlled drug that is readily available and does not cause toxicity if the animal is scavenged nor cause histologic changes that can impair accurate evaluation of tissues during postmortems. It is easily acquired and safe to transport into remote field conditions. KCl must only be administered through intravenous or intracardiac injection after the animal is at an adequate plane of anesthesia (unconscious and no withdrawal reflex). Disadvantages include: a) the animal must be anesthetized (either with injectable or volatile anesthetics) and at an adequate plane of anesthesia prior to administering KCl; b) intravenous or intracardiac injection is required; c) muscle movements often occur for several seconds after administering KCl; and d) highly concentrated solutions are required for larger animals.

For other non-inhalant pharmaceutical agents, such as MS-222 (also known as TMS) for amphibians, the *AVMA Guidelines for the Euthanasia of Animals: 2020 Edition* (AVMA, 2020) should be consulted.

Inhalant Pharmaceutical Agents – volatile anesthetics (e.g., sevoflurane, isoflurane) are considered acceptable for euthanasia of small species. While an overdose of inhalation anesthetics is an effective method of euthanasia for many species, the time to death can be quite lengthy and use of a second procedure to ensure death of the animal should be applied once the animal is unconscious (CCAC, 2010). After anesthesia, administration of euthanasia solution via intracardiac injection in smaller species is easier to perform than intravenous injections. Because the liquid state of most inhalant anesthetics is irritating, animals should only be exposed to vapours. However, exposure to inhalant anesthetics has been found to be aversive in rodents (Leach et al., 2004; Makowska and Weary, 2009) and may also be stressful for other species. Ether and nitrous oxide are combustible and explosive, and they have the potential for human toxicity and abuse. They are, therefore, not recommended as volatile agents for use in euthanizing wildlife. Volatile anesthetics may be unsuitable for animals that have the ability to hold their breath for long periods of time (e.g., reptiles, diving birds, loons, grebes, diving mammals). Personnel safety must be considered to avoid exposure to the vapours.

13.1.2 Inhalant Gases

Euthanasia with inhaled gases is slow due to the requirement for any gas being inhaled to reach a certain concentration in the lungs before taking effect. A closed chamber to hold the gas is needed and personnel safety must be considered to avoid exposure to the toxic gas.

- **Carbon Monoxide (CO)** is conditionally acceptable based on safety hazards. It can only be delivered reliably in concentrations high enough to be effective through CO gas cylinders. Vehicle exhaust is not an acceptable source. Under the effects of CO, animals do not appear in distress as CO induces unconsciousness without pain or discomfort. CO may be explosive at levels exceeding 10%. CO is dangerous for personnel and steps should be taken to ensure they are not exposed to it.
- **Carbon Dioxide (CO_2)** is conditionally acceptable when used at appropriate concentrations, using pressurized cylinders. It can cause pain and distress to the animal and should not be used where other methods are practical for the scientific activity and the species (CCAC, 2010). CO_2 can be unsuitable for animals that hold their breath (e.g., diving or burrowing birds and mammals) or do not breathe at a very high frequency (e.g., amphibians and reptiles) and for aquatic species. Furthermore, CO_2 administered at 100% can cause distress in animals. See the *CCAC guidelines on: euthanasia of animals used in science*

(CCAC, 2010), Section 5.1, "Carbon Dioxide", for guidance on the application of CO_2 when its use is approved by the animal care committee.

13.1.3 Physical Methods

These methods, when properly applied by highly competent personnel, kill rapidly and cause minimal stress. They may offer a practical solution for field euthanasia of various sized animals and prevent pharmaceuticals from entering the food web.

Gunshot – A shot to the brain by a skilled marks person is an acceptable method. It produces a quick and humane death (Longair et al., 1991) and is best attempted when the animal is immobilized by injury or physical or chemical restraint. In free-ranging situations, a successful shot to the brain may be difficult to achieve at distance and can result in accidental injury to the animal or people if the shot deflects off the skull. Under these conditions, gunshot is conditionally acceptable and a shot to the heart and lung area or neck may be more feasible (AVMA, 2020) and is recommended in hunter education programs conducted by provincial and territorial agencies. Although death from these shots is not as quick, it is much more certain under free-ranging conditions. In some cases, a gunshot to the brain may prevent proper postmortem analysis. This is particularly important for research on rabies or chronic wasting disease (CWD). The size of shot to be used must be determined based on the species, skull thickness, and distance. Larger animals or those with thick skulls (e.g., moose, bear) require ballistics such as shotgun slugs. Ideally, euthanasia via gunshot to the head should be performed at close range to minimize risks. If the animal is in an urban or high human use area, it may be better to anesthetize the animal first, move it from the area, and euthanize it by gunshot at a more secluded location. In addition, care should be taken for animals that live in groups in order to avoid death, injury, and stress to conspecifics or heterospecifics. Appropriate resources (see Appendix 3 of AVMA, 2020) should be consulted for proper shot placement. Whenever possible, non-lead shot should be used (see Section 6, "Killed Specimens").

Penetrating Captive Bolt – This is an acceptable method in appropriate species and requires the animal to be well restrained in order to properly place the captive bolt (AVMA, 2020).

Manually Applied Blunt Force Trauma (Rather Than Concussion) – This method is conditionally acceptable for small animals with thin craniums and many neonates (CCAC, 2010; AVMA, 2020). In situations where a crushing blow to the head is the most rapid and practical method available, it should be carried out in such a manner that the animal is rendered unconscious almost instantaneously (CCAC, 2010). The procedure should be performed by someone with experience and should be conducted in an area beyond the sensory range of other animals.

Unless specifically designed for certain species and ages, non-penetrating captive bolts are not recommended as a sole means of euthanasia as they may cause unconsciousness without killing the animal (AVMA, 2020). In those circumstances where blunt force trauma causes unconsciousness but not instantaneous death, it must be immediately followed by a secondary technique to ensure euthanasia.

Cervical Dislocation – This method is used for mice, rats and bats (<200 g), other selected small mammals (small rodents <200 g and lagomorphs <1 kg), and birds (<3 kg). Manual cervical dislocation can be used on small birds (<200 g), but a mechanical cervical dislocation device is required for larger birds (Environment and Climate Change Canada (ECCC), 2020)). The manual technique involves stretching the neck to cause separation of the cervical vertebrae from the skull and can only be used on small animals. For immature rabbits (<1 kg), the neck is stretched, hyperextended, and dorsally twisted to separate the first

cervical vertebra from the skull. As noted in the *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010), "It is essential to check that the neck is broken at the end of the procedure by palpation of the vertebrae. If adequate separation is not observed, a backup method, such as decapitation or exposure to high concentrations of CO_2, should be used immediately." The procedure should only be performed on a small number of animals and by an experienced and trained professional to prevent human error due to fatigue. The animals should be anesthetized prior to cervical dislocation (CCAC, 2010) unless justified according to the species or situation. For example, where cervical dislocation is used as a contingency measure for animals that are suffering, it may not be appropriate to provide anesthesia.

Decapitation – This technique is conditionally acceptable for very small species that are already anesthetized or unconscious, including birds. It requires appropriate, specialized equipment. In certain emergencies where euthanasia is recommended, blunt force trauma can be used to render the animal unconscious prior to decapitation.

Exsanguination – This is generally a secondary method of euthanasia but can be an acceptable method in the field when performed on an anesthetized animal and another method is not available. It can be achieved by severance of both axillary arteries or both carotid arteries (ECCC, 2020).

Thoracic Compression (Rapid Cardiac Compression) – This method is conditionally acceptable for very small species that are already anesthetized or unconscious, including birds. It has the potential to cause substantial pain and distress prior to the animal becoming unconscious and is therefore unacceptable unless the animal is deeply anesthetized or unconscious. Thoracic compression is not generally used in emergencies, but rather for collection purposes. Therefore, anesthetic is more appropriate than blunt force trauma unless the drugs will interfere with the research results (e.g., for tissue or blood sampling). See Engilis et al. (2018) and Paul-Murphy et al. (2017) for recent information on the use of this method on small birds.

Stunning and Pithing – These methods will produce rapid unconsciousness when properly carried out, but not death, and they should only be used in combination with other techniques such as exsanguination. These methods are conditionally acceptable.

13.2 UNACCEPTABLE METHODS OF EUTHANASIA

Drowning is unacceptable for all animals. Freezing is unacceptable for all animals except reptiles of certain species and under specified conditions (CCAC guidelines on reptiles, in prep.).

Unacceptable agents for euthanasia include caffeine, strychnine, any neuromuscular blocking agents, nicotine, alcohol, and magnesium salts. Injection of an air embolus is unacceptable.

Unacceptable sources of gas include the reaction of sodium formate and sulphuric acid or vehicle exhaust to produce CO, and fire extinguishers, dry ice, or chemical means (e.g., Alka Seltzer® tablets) to produce CO_2.

13.3 DISPOSAL OF EUTHANIZED ANIMALS

Guideline 43

The euthanasia method that has minimal impacts on other wildlife and the ecosystem should be chosen. Any animal euthanized in the field that may contain residues of toxic euthanasia chemicals or other drugs (e.g., potent opioids, antibiotics, anti-inflammatories) or substances (e.g., lead shot) known to have impacts on other wildlife and the ecosystem should be disposed of in such a manner that contaminated tissues do not enter the food web or water sources.

Acceptable disposal methods for contaminated animals include incineration or liming the carcass and burying it in a deep hole. Carcasses may be disposed of at landfills where animal disposal is accepted (ECCC, 2020). Prior to disposal of the carcass in the field, investigators should also determine the need for necropsy or the suitability of euthanized animals for preparation and use as specimens in scientific activities, with accompanying relevant information.

Investigators must follow all relevant laws and regulations regarding euthanasia and disposal of animals. For situations where it will not be possible to dispose of carcasses, the method of euthanasia must be carefully considered and local authorities consulted to determine acceptable procedures and reporting requirements, unless stated in the permit. If an animal must be left in situ without disposal, the relevant authorities must be informed.

14 HUMAN SAFETY CONSIDERATIONS

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

Those working with animals must follow institutional policies and SOPs outlining appropriate measures of prevention and protection, in addition to provincial and territorial legislation on the matter. They should seek professional knowledge on potential health risks (including zoonotic diseases), injuries, and other risks or hazards that may be associated with the scientific activity they are involved in, as well as preventive measure to minimize the risks.

The following references can provide a starting point for identifying potential risks to personnel working with wildlife:

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APPENDIX 1
REGULATORY AGENCIES AND RELEVANT LEGISLATION

Anyone proposing to conduct a scientific activity involving wildlife must be familiar with, and comply with, the relevant legislation. Prior to engaging in any activity concerning wildlife, investigators must make themselves aware of any relevant regulations. The following sections are intended to provide a starting point for consideration of existing regulations. The list is not exhaustive and the regulations may change over time. Where conflicts arise between this list and regulations, regulations take precedence.

In most cases, licenses or permits are required to import or export wildlife or parts thereof across political boundaries (i.e., provincial, territorial, and international borders), to capture or kill wildlife, to band or otherwise mark wildlife, and to hold in captivity or release wildlife. It is the investigator's responsibility to ensure that all licenses, permits and approvals are in place before proceeding with any wildlife project.

A scientific activity that involves Indigenous communities requires permission from that community. Depending on the community, region, or land claim area involved, there may be established protocols for appropriate consultation, project approval, and community participation. There may also be regional organizations that must be consulted, in addition to the local community.

1. INTERNATIONAL

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), in force since 1975, has 184 member countries, including Canada. Member countries ban commercial trade in endangered species and regulate and monitor trade in other species that might become endangered. The import or export of any animals on the CITES list requires a CITES permit from Environment and Climate Change Canada (ECCC). CITES not only deals with live animals, but also with "parts and parts thereof", which includes all types of biological samples (skin, hair, bones, blood, serum, etc.). Any relevant wildlife permits issued by the host country must also be obtained.

Other organizations and agreements that investigators should be aware of include: the Convention on Biological Diversity, the International Union for Conservation of Nature and Natural Resources (IUCN), the *Red List of Threatened Species* and the *Animal (mammal) traps*, Part 4 (ISO 10990-4: 1999) and Part 5 (ISO 10990-5:1999) of the International Organization for Standardization (ISO). The United States and Canada share many species and populations of species, and investigators working in the US should determine whether the species or population to be studied is on the US endangered species list (US Fish and Wildlife Service). Additionally, the International Air Transport Association (IATA) *Live Animal Regulations* set standards for appropriate containers, care and handling during transportation.

Trapping of certain mammal species in the fur industry are subject to the Agreement on International Humane Trapping Standards (AIHTS) (European Community, Government of Canada, and Government of the Russian Federation, 1997). These trade agreement requirements must be met or exceeded for trapping animals primarily for scientific purposes.

2. FEDERAL

Environment and Climate Change Canada (ECCC) promotes the conservation of Canadian and international wildlife and biological diversity by protecting and managing migratory birds and federally listed Species at Risk along with nationally significant habitat and protected areas, and by providing leadership on other issues, such as recovery of endangered species. Many birds migrate cross international borders, and hence their use in scientific activities and consequent influence on survival may be of interest to several countries. The ECCC regulates the hunting of migratory birds and also requires that special permits be obtained for the collection, banding, and holding of these birds. In addition, permits are required to carry out activities such as wildlife research in National Wildlife Areas and Migratory Bird Sanctuaries.

ECCC is responsible for implementing the *Migratory Birds Convention Act*, 1994 (MBCA). The *Migratory Birds Regulations* (MBR) provides the authority to issue a scientific permit to allow the otherwise prohibited activities of kill, take or capture, and band migratory birds for a scientific or educational purpose. The Bird Banding Office (BBO) co-manages the North American Bird Banding Program with the Bird Banding Laboratory of the United States Geological Survey (USGS). The BBO manages the federal bird band inventory and issues scientific permits to capture and band birds, attach devices to birds, and collect biological samples. The BBO also coordinates bird marker use locally, nationally, and internationally and manages banding, encounter, and tracking data (the BBO can be contacted at bbo@ec.gc.ca). Section 73 of the *Species At Risk Act* (SARA) sets out conditions to be met before issuing a permit affecting SARA-listed species, their critical habitat or residences. Information on permitting provisions and how to apply for a SARA permit, consult the Species at Risk Public Registry. Permits are required to carry out activities such as wildlife research in National Wildlife Areas and Migratory Bird Sanctuaries under the Canada Wildlife Act. ECCC also regulates the hunting of migratory birds (for more information, contact the CWS regional permit office).

ECCC oversees the following Acts and Regulations:

- *Canada Wildlife Act*;
- *Wildlife Area Regulations*;
- *Migratory Birds Convention Act*;
- *Migratory Birds Regulations*
- *Migratory Bird Sanctuary Regulations*;
- *Wild Animal and Plant Protection and Regulation of International and Inter-provincial Trade Act* (WAPPRIITA);
- *Wild Animal and Plant Trade Regulations*; and
- *Species at Risk Act*.

The *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES) aims to protect wild animal and plant species by regulating the international trade of living and dead specimens, whole or parts of specimens, and any product made from any specimens of these species. Canada meets its international obligations under CITES by implementing the *Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act* (WAPPRIITA), which prohibits:

- the importation or possession of any wild animal or plant species obtained illegally in Canada or exported illegally from another country;

- the import or export of species listed in CITES without the appropriate permits; and
- the sale or possession of most species listed in CITES whose commercial trade is prohibited.

The CITES Canada - Management Authority administers WAPPRIITA and issues the CITES permits and certificates required for the commercial trade, import and export of species protected under the Act.

Fisheries and Oceans Canada (DFO) bears responsibility for marine reptiles and mammals. The *Marine Mammal Regulations* are listed under the *Fisheries Act*.

The *Canada National Parks Act* provides for regulations for the protection of fauna, the taking of specimens of fauna for scientific or propagation purposes, and the destruction or removal of dangerous or superabundant fauna.

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) is an advisory body that develops and maintains a national listing of Canadian species at risk, based on the best scientific evidence available. COSEWIC consists of co-chairs for each of ten taxonomic groups plus Indigenous knowledge, representatives from the wildlife departments of all thirteen Canadian provincial and territorial governments; federal departments and corporations concerned with wildlife, including the Canadian Wildlife Service (which provides the secretariat), Parks Canada, DFO, and the Canadian Museum of Nature; four non-government scientist positions; and two early-career scientist positions. It is the responsibility of the respective provincial and territorial jurisdictions where the species occurs to take whatever actions are appropriate to address the threats and limiting factors placing a species at risk.

Permits from the Canadian Food Inspection Agency (CFIA) are required for the movement of cervids within Canada under the *Health of Animals Regulations* in order to prevent the spread of brucellosis, tuberculosis, or chronic wasting disease. If the presence of these diseases, highly pathogenic avian influenza rabies, anthrax, or foreign animal diseases are suspected in wildlife, CFIA should be contacted. Additionally, the Public Health Agency of Canada should be contacted if wildlife diseases transmissible to humans are suspected.

The RCMP should be consulted for information on the possession and use of firearms.

3. PROVINCIAL AND TERRITORIAL

All provinces and territories in Canada have legislation governing activities involving wildlife. Therefore, it is imperative that investigators consult with the appropriate provincial or territorial agency when planning a project involving wildlife. Licenses or permits are required for the killing, capture, holding, marking, transport, trade, import, export, and sometimes release of most wildlife. This includes wildlife held for scientific activities. Provincial regulations also exist for the types of traps allowed and for the use of firearms or other weapons in specific areas. Additionally, permits are required for the movement of wildlife, or parts thereof, across borders, and such movement may necessitate obtaining permits in more than one province or territory.

Provinces and territories may have endangered species legislation and listings and associated permit requirements. Such legislation and listings may also be applied to species for which the normal management responsibility lies with another agency (e.g., migratory birds, and marine reptiles or mammals). Most terrestrial species (mammals, amphibians, and reptiles) and several species of birds (raptors, corvids, and resident non-migratory species) fall solely within provincial and territorial jurisdiction.

Permits may also be needed to conduct scientific activities involving wildlife in provincial and territorial wildlife areas, refuges, game sanctuaries, ecological reserves, wilderness areas, parks, or other specially designated lands. Additionally, permits may be required for active habitat manipulation or other activities on any provincial and territorial land holding.

Alberta

Ministry of Environment and Protected Areas

Wildlife Act

British Columbia

Ministry of Forests, Lands and Natural Resource Operations, Fish and Wildlife Branch

Wildlife Act

Manitoba

Department of Natural Resources and Northern Development, Fish and Wildlife Branch

Wildlife Act

Endangered Species and Ecosystems Act

The Polar Bear Protection Act

New Brunswick

Department of Natural Resources and Energy Development, Fish and Wildlife

Fish and Wildlife Act

Species at Risk Act

Newfoundland and Labrador

Department of Fisheries, Forestry and Agriculture

Wild Life Act

Endangered Species Act

Wilderness and Ecological Reserves Act

Northwest Territories

Department of Environment and Natural Resources

Wildlife Act

Species at Risk (NWT) Act

Nova Scotia

Department of Natural Resources and Renewables, Wildlife and Biodiversity Division

Wildlife Act

Endangered Species Act

Conservation Easements Act

Nunavut

Department of Environment, Wildlife Management Division

Wildlife Act

Ontario

Ontario Ministry of Natural Resources and Forestry, Wildlife and Nature Branch

Fish and Wildlife Conservation Act

Endangered Species Act

Ontario Ministry of Agriculture, Food and Rural Affairs

Animals for Research Act

Prince Edward Island

Department of Environment, Energy and Climate Action

Wildlife Conservation Act

Québec

Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs Act respecting the conservation and development of wildlife

Act respecting threatened or vulnerable species

Act respecting hunting and fishing rights in the James Bay and New Québec territories

Parks Act

Ecological Reserves Act

Forest Act

Saskatchewan

Ministry of Environment

The Wildlife Act

The Wildlife Habitat Protection Act

Yukon

Department of Environment

Wildlife Act

4. MUNICIPAL

Investigators must consult the appropriate municipal by-laws. Many municipal governments have regulations governing activities involving wildlife within municipal boundaries, including the holding of wildlife. There are usually restrictions on the use of firearms and other weapons, and there may be regulations relating to the use of traps or other tools and vehicles.

5. PRIVATE PROPERTY AND INDIGENOUS LANDS

Although wildlife is a public resource, wild animals may occupy private and Indigenous lands and certain rights of access are extended to land holders. Therefore, permission should be obtained from the owner to access private property and Indigenous lands, regardless of the permits held. In some cases (e.g., undeveloped lands, remote areas), it may be difficult to locate the owners for permission. In these situations, local provincial and territorial wildlife personnel should be consulted for advice. It is also prudent to inform local residents or interest groups (e.g., local fish and game organizations) of any scientific activities being conducted, whether on private or public land. In addition, government agencies likely to receive calls from the public should be notified prior to the activity (e.g., provincial, territorial and local conservation officers, the Coast Guard, Harbor Commission, ECCC, RCMP, local police).

6. PROFESSIONAL ASSOCIATIONS

Many professional associations have produced guidelines for the capture, handling, and care of wildlife. In addition, some scientific journals have developed guidelines that must be followed in order to have work published. For Canadian investigators, many journals specifically ask for demonstration of animal care committee review and approval before reviewing a paper that involves animals.

APPENDIX 2
WILDLIFE GUIDELINES PUBLISHED BY OTHER ORGANIZATIONS

This CCAC guidelines document provides guidance for investigators and animal care committees in the development and review of protocols and the conduct of scientific activities in the field. It is expected that more specific guidelines and references will be consulted to provide documented evidence for particular techniques. The following list of guidelines can be used as a starting point for additional resources; however, the list is not complete, the guidelines listed need to be checked for updated versions, and there may be instances where they differ from CCAC guidelines. Justification must be provided to implement any deviations from CCAC guidelines.

American Society of Ichthyologists and Herpetologists (2004) *Guidelines for Live Amphibians and Reptiles in Field and Laboratory Research* (accessed on 2023-02-09).

American Society of Mammologists (2011) *Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research*. Prepared by Sikes R.S., Gannon W.L. and the Animal Care and Use Committee of the American Society of Mammalogists (accessed on 2023-02-09).

Animal Behavior Society & Association for the Study of Animal Behaviour (2001) *Guidelines for the Treatment of Animals in Behavioral Research and Teaching* (accessed on 2023-02-09).

British Columbia, Ministry of Environment, Lands and Parks, Resources Inventory Committee (1998) *Live Animal Capture and Handling Guidelines for Wild Mammals, Birds, Amphibians and Reptiles*. Standards for Components of British Columbia's Biodiversity No. 3, version 2.0. Electronic document (accessed on 2023-02-09).

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Ornithological Council (2010) *Guidelines to the Use of Wild Birds in Research*, 3rd ed (accessed on 2023-02-09).

North American Banding Council. *The North American Banders' Manual for Banding Hummingbirds* (2019); *The North American Banders' Manual for Banding Passerines and Near Passerines (Excluding Hummingbirds and Owls)* (2001); *The North American Banders' Manual for Raptor Banding Techniques* (2001); *The North American Bander's Manual for Shorebirds* (2018); *The North American Banders' Manual for Waterfowl* (2017) (accessed on 2023-02-09).

GLOSSARY

Acclimation – a persisting physiological, biochemical or morphological change within an individual animal during their life as a result of a prolonged exposure to an environmental condition such as a high or low temperature; generally, the changes are reversible.

Analgesia – decrease in response to noxious stimuli.

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

Apnea – absence of spontaneous breathing.

Aseptic – absence of living germs, free from septic and poisonous putrefactive products.

Biopsy – the surgical removal of a cell or sample of tissue for diagnostic purposes.

Capture myopathy – the muscle damage resulting from anaerobic muscle function; predisposition may be due to improper capture procedures.

Cardiac puncture – the penetration of the heart, usually for removal of a blood sample.

Catecholamine – a type of biogenic amine; includes epinephrine, norepinephrine and dopamine.

Competent – able to effectively perform a particular task in relation to the care, maintenance or use of the animals, while ensuring their welfare is protected as far as possible within the constraints of any approved studies that the animals are involved in. Focusing on competency rather than training acknowledges that there may be a variety of ways of acquiring the necessary knowledge and skills, and places emphasis on learning outcomes.

Conspecifics – animals belonging to the same species.

Cull – selective killing to reduce a population.

Depolarizing muscle relaxant – an agent (drug) that produces a depolarization (contraction) of the muscles before it produces muscle relaxation; succinylcholine is an example of a depolarizing agent.

Discomfort – a mild form of distress.

Dissociative anesthetic – a drug that produces a type of anesthesia characterized by a cataleptoid state in which the eyes remain open and purposeful or reflexive muscle movements may occur.

Distress – a state of excessive stress which will occur if an animal has to devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation, or if the animal is unable to make the necessary adaptations.

Ecological – relations among living organisms and between living organisms and their environment.

Ecosystem – a complex of the plant and animal communities within an area, along with the non-living components of the environment and the interactions among these.

Endpoint – predetermined criteria for intervening in a procedure to terminate, minimize or reduce an animal's pain and 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 animals' environment that go beyond meeting their basic species-specific needs and further improve overall quality of life.

Euthanasia – literally, a good death; rapid loss of consciousness and death, with no pain or distress accompanying the procedure.

Exsanguination – a procedure causing extensive loss of blood due to internal or external hemorrhage.

Extirpation – elimination of unwanted species.

Herptiles – amphibians and reptiles.

Humane – conditions which promote physical and behavioural well-being of animals; in the case of euthanasia, humane methods are those which are painless, minimize fear and anxiety, and are reliable, reproducible, irreversible, simple, safe and rapid.

Heterospecifics – animals belonging to different species.

Humane intervention points – the pre-established criteria (e.g., physical, physiological, psychological, behavioural) that indicate when to intervene (e.g., supportive care, analgesia, euthanasia) to reduce welfare impacts to a level that has been approved by the animal care committee.

Hyperthermia – higher than normal body temperature.

Hypothermia – lower than normal body temperature.

Hypoxia – reduced oxygen in air and blood and tissues.

Immobilization – a procedure causing loss of the ability to make coordinated, purposeful movements.

Laparotomy – abdominal incision to access the peritoneal cavity.

Lateral recumbency – lying down on the side.

Mitigation strategies – actions taken to rectify instances of poor welfare.

Monogastric – having a single stomach.

Morbidity – visible manifestation of a diseased state.

Mortality – loss of life; death.

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

Palpebral – pertaining to the eyelid.

Postmortem – an examination of the body made after the death of the animal; an autopsy.

Prophylactically – preventing a disease or the process leading to a disease.

Protocol – a written description of a scientific activity that includes details of the goals, the use of animals, the procedures that are to be followed and the personnel involved; the purpose of the protocol is to ensure the quality and integrity of the scientific activity.

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

Radio transmitter – a piece of telemetry equipment that emits a signal (usually a 'beep') on a particular radio frequency.

Regurgitation – passive return of food or fluid to the mouth from the stomach.

Reversal agent – a drug that will reverse the effects of another drug or a drug combination. Reversal agents may specifically antagonize the pharmacological effects of another agent.

Ruminal tympany – bloat; an abnormal collection of gas in the rumen.

Ruminants – polygastric animals having usually four digestive compartments.

Scientific activity – includes all aspects of any research, teaching, and testing activity.

Scientific endpoints – the earliest points at which the scientific aims of the activity can be achieved while also ensuring that the negative welfare impacts experienced by the animals are minimized.

Sedatives – drug used to produce a state where animals are not entirely unconscious, but their awareness of the surroundings is severely altered and they do not have control of their muscles; sedatives may be appropriate for some situations where pain and distress are anticipated.

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

Sternal recumbency – lying down on the chest.

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

Telemetry – the use of devices to transmit information via radio to a distant station where it is recorded; commonly used in wildlife studies to monitor animals in order to answer questions about their physiology, behaviour, habitat use, survival and movements.

Therapeutic index – the ratio of dosage which kills 50% of animals (LD 50) to dosage which is effective in 50% of the animals (ED50) used in qualitative comparison of drugs.

Thermoregulatory – able to regulate heat.

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

Translocation – the movement of animals from one site to another.

Welfare – the physical and mental state of an individual animal and how this animal is experiencing the conditions in which it lives.

Welfare assessment – quantification of animal welfare by inferring affective states based on validated changes in physiology and behaviour.

Wildlife professional – a veterinarian, veterinary technician, biologist, research technician, or wildlife rehabilitator who has extensive experience with wildlife.

Withdrawal time – the length of time between when an animal is given a drug and when that animal could be safely consumed by a human.

Zoonotic – relating to the transmission of a disease from a non-human species to humans.