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Canadian Council on Animal Care
Conseil canadien de protection des animaux


# CCAC Ethics Principles for Animal Use in Science

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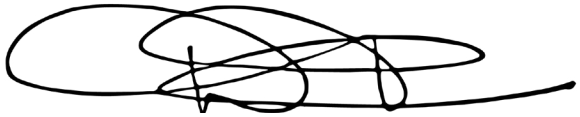

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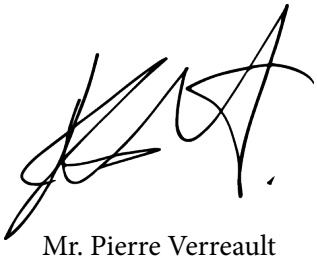

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# TABLE OF CONTENTS

# CCAC Ethics Principles for Animal Use in Science

## PREFACE

The Canadian Council on Animal Care (CCAC) is the national peer-review organization responsible for setting, maintaining, and overseeing the implementation of standards for ethical animal care and use in science throughout Canada. CCAC standards are based on professional expertise and current interpretation of scientific evidence.

The *CCAC Ethics Principles for Animal Use in Science* outlines the values and ethics expected of institutions and individuals that intend to use animals in science. Institutions and individuals are expected to commit to these principles, which first and foremost aim to ensure that animals are used only as a last resort. Adherence to this document ensures the maintenance of a strong ethical culture within Canadian institutions and confidence in the integrity of animal use in science. The *CCAC Ethics Principles for Animal Use in Science* also aims to ensure that whenever the use of animals is deemed to be necessary, the animals used in the studies will be accorded respect and will receive optimal care in compliance with the principles outlined in this document.

The *CCAC Ethics Principles for Animal Use in Science* document details the standards that are expected to be met by holders of the CCAC Certificate of GAP – Good Animal Practice®. For scientific activities conducted within or outside of Canada, protocol authors based at CCAC-certified institutions are subject to these standards. Protocol authors are also subject to any relevant legislation and regulations in the jurisdiction where the scientific activity is conducted.

# LIST OF CCAC ETHICS PRINCIPLES

## FUNDAMENTAL PRINCIPLES

### Fundamental Principle 1: Respect for Animals

Animals are valued as individuals with fundamental needs and interests.

*Section 2 CCAC Fundamental Ethics Principles, p.6*

### Fundamental Principle 2: Sufficient Benefit

Any use of animals provides sufficient benefit to humans, animals, or the environment.

*Section 2 CCAC Fundamental Ethics Principles, p.6*

### Fundamental Principle 3: Not Causing Harm

The intentional or avoidable harming of animals is morally wrong.

*Section 2 CCAC Fundamental Ethics Principles, p.6*

## PRINCIPLES OF APPLICATION

### Principle of Application 1

Sentience is to be used as the basis for decision-making concerning any potential animal-based scientific activity.

*Section 3 CCAC Principles of Application, p.10*

### Principle of Application 2

Certain activities are not to be permitted either because of the extreme level of harm likely to be experienced by the animal, or the lack of sufficient benefit.

*Section 3 CCAC Principles of Application, p.10*

### Principle of Application 3

Animals are only to be used when there are no valid non-animal means of achieving approved research, educational, or regulatory goals.

*Section 3 CCAC Principles of Application, p.12*

### Principle of Application 4

The expected outcomes of any scientific activity involving animals are to be sufficiently beneficial to humans, animals, or the environment.

*Section 3 CCAC Principles of Application, p.12*

### Principle of Application 5

The most relevant and reliable models or model systems are to be selected to achieve the approved research, educational, or regulatory goals, based on evidence of their validity for the intended purpose.

*Section 3 CCAC Principles of Application, p.13*

### Principle of Application 6

Rigorous scientific principles, and research, teaching and training, and testing practices are to be implemented to ensure high-quality animal-based scientific activities.

*Section 3 CCAC Principles of Application, p.14*

### Principle of Application 7

Potential negative welfare impacts are to be minimized, and positive welfare states are to be ensured.

*Section 3 CCAC Principles of Application, p.15*

### Principle of Application 8

Captive environments are to provide animals with the opportunity to experience a life worth living.

*Section 3 CCAC Principles of Application, p.16*

### Principle of Application 9

Animals are to be trained to cooperate with procedures in a manner that respects their welfare.

*Section 3 CCAC Principles of Application, p.17*

### Principle of Application 10

Animals are to be given the opportunity to enjoy a life after their scientific use.

*Section 3 CCAC Principles of Application, p.17*

### Principle of Application 11

Killing an animal, even using a humane killing method not intended to have any negative welfare impact, is to be considered as a harm to that animal.

*Section 3 CCAC Principles of Application, p.18*

# 1 INTRODUCTION

The *CCAC Ethics Principles for Animal Use in Science* replaces the *CCAC policy statement: ethics of animal investigation* (CCAC, 1989). It has been developed by a subcommittee of experts in ethics, philosophy, animal welfare, veterinary science, and a variety of other scientific disciplines, and has undergone both an expert and public review. Indigenous leaders reviewed the document; however, consultation with Indigenous communities and organizations about these principles has not yet taken place. The CCAC recognizes that the principles reflect dominant non-Indigenous value systems and acknowledges the importance of aligning with and respecting Indigenous perspectives, values, and protocols as outlined in *Towards Reconciliation: 10 Calls to Action to Natural Scientists Working in Canada* (Wong et al., 2020). Thus, the CCAC recognizes the need for engagement with Indigenous communities and organizations in any future revision of this document.

## 1.1 OBJECTIVE

The *CCAC Ethics Principles for Animal Use in Science* outlines the values and ethics expected of institutions and individuals that intend to use animals in science. Institutions and individuals are expected to commit to these principles, which first and foremost aim to ensure that animals are used only as a last resort. Adherence to this document ensures the maintenance of a strong ethical culture within Canadian institutions and confidence in the integrity of animal use for science. The *CCAC Ethics Principles for Animal Use in Science* also aims to ensure that whenever the use of animals is deemed to be necessary, the animals used in the studies will be accorded respect and will receive optimal care in compliance with the principles outlined in this document.

## 1.2 SCOPE

The *CCAC Ethics Principles for Animal Use in Science* is intended for anyone involved in animal-based science in Canada. It lays out the values and ethics for the oversight and conduct of Canadian animal-based scientific activities, whether carried out in Canada or elsewhere. It is to be used to resolve ethical dilemmas that may arise in the practice of animal-based science, and to guide considerations around the ethical implications of proposed animal-based grants, programs, and animal use protocols.

Both institutions and individuals have a responsibility to implement the *CCAC Ethics Principles for Animal Use in Science*, recognizing that it is a privilege, not a right, to engage in animal-based science. Any discussion of the ethics of animal-based science will be influenced by current institutional and individual knowledge, cultures, values, and preferences, which may be subject to change over time.

### 1.2.1 Role of Institutions

As the national organization responsible for overseeing animal use in science, the CCAC expects its ethics principles to be followed by institutions carrying out animal-based scientific activities and organizations

that: fund animal-based science (e.g., funding agencies, charities); require animal-based science data (e.g., regulators responsible for determining the safety and efficacy of health and consumer products); or use the results of animal-based science (e.g., publishers, journal editors). Additionally, professional bodies representing individuals using animals for scientific activities are expected to implement the *CCAC Ethics Principles for Animal Use in Science* within their organizations.

Implementation of the ethics principles is mandatory for animal-based activities carried out by Canadian institutions, regardless of the location of the activities (i.e., in the laboratory, in the field, in Canada, or in another country). In particular, the principles are to be used by local animal care committees in considering the ethical acceptability of animal-based scientific activities.

### 1.2.2 Role of Individuals

All personnel involved in animal-based science are expected to be well acquainted with the CCAC's ethics principles and to implement the principles fully within their daily activities. Protocol authors are expected to use the principles in considering the ethical acceptability of animal-based scientific activities and all scientific staff are expected to adhere to them when carrying out any procedures on animals. Veterinarians are expected to use these principles when considering how to ensure the physical and psychological well-being of animals within the institution's animal care and use program and any other animals within their oversight, and when advising on best practices for animal-based procedures (CALAM, 2020). Animal care personnel are expected to follow these principles in their day-to-day care of animals in their charge.

## 1.3 SENTIENCE

The principles espoused in this document apply to any animal where there is sufficient evidence that they are sentient. Animals who are sentient have the capacity to experience positive and negative welfare states, and hence are susceptible to harm incurred before, during, and after a scientific activity. To date, the acquisition of sentience has been generally assumed for all vertebrate animals following birth or hatching. Taxonomic boundaries for sentience in invertebrates are difficult to define. However, there is increasing scientific evidence that some invertebrates may be sentient (Birch et al., 2021; Andrews et al., 2024).

Although it is difficult to define developmental boundaries for sentience, there is evidence of sentience for unborn animals of some species during the latter stages of their development, which places them at risk of experiencing negative welfare states (Mellor and Diesch, 2006; Kollmansperger et al., 2023). In addition, scientific evidence shows that procedures carried out on some embryonic and fetal forms at early stages of development may result in lasting harm for the animals if the developing animals are allowed to live beyond the first two-thirds of their prenatal development (Campbell et al., 2014). When fetal forms are the organisms of interest, maintaining the pregnant individuals in a positive welfare state is also paramount, both to protect the best interests of the gestating or incubating animals and to minimize the impact of maternal stress on the fetuses (Campbell et al., 2014).

# 2 CCAC FUNDAMENTAL ETHICS PRINCIPLES

The CCAC's fundamental ethics principles presuppose that the scientific goals in question are worthy of consideration and cannot be achieved ethically by any other means than with the use of an animal (i.e., the use of an animal is necessary). If the goal can be achieved without the use of an animal, it is not ethical to use an animal to carry out the activity in question.

## 2.1 FUNDAMENTAL PRINCIPLE 1: RESPECT FOR ANIMALS

**Animals are valued as individuals with fundamental needs and interests.**

All vertebrate and some invertebrate animals are recognized as likely to be sentient, with the ability to experience pleasure, pain, fear, suffering, and other emotions (Andrews et al., 2024). Thus, respect for animals is paramount, with full respect for animals equating to no human interference with an animal's intrinsic nature or dignity, unless it is in the animal's best interests (Coghlan, 2024). Ethically, pursuing any scientific activity involves respecting the intrinsic nature of the animals involved, recognizing their unique characteristics and their autonomy, rather than imposing a human-centric view of them. Animals are to be recognized as being motivated to pursue their own ends in their own way and are to be allowed to do so, all else being equal.

## 2.2 FUNDAMENTAL PRINCIPLE 2: SUFFICIENT BENEFIT

**Any use of animals provides sufficient benefit to humans, animals, or the environment.**

Sufficient benefit means that any potential gains are achievable, realistic, and otherwise currently unattainable without the use of animals. Benefits are generally described in terms of better health interventions for animals or humans, successful conservation, greater knowledge of living systems, improved animal-based practices, and safer technologies. Humans, animals, and the environment are expected to benefit from animal-based scientific activities.

Sufficient benefit needs to be understood in relation to the harms incurred to secure it, not just the nature or value of the benefit. The more significant the harms to the affected individuals, the higher the threshold required for when a benefit is sufficient to warrant the harms. Some harms may be so great as to preclude there ever being a benefit sufficient to warrant using animals (DeGrazia and Beauchamp, 2020).

## 2.3 FUNDAMENTAL PRINCIPLE 3: NOT CAUSING HARM

**The intentional or avoidable harming of animals is morally wrong.**

All things being equal, animals used in scientific activities will not be subject to any harms that can be avoided. Any harm or potential harm requires justification, with a plan of actions to be taken to minimize any negative welfare impacts likely to be experienced by the animals involved.

## 2.4 HOW THE THREE FUNDAMENTAL ETHICS PRINCIPLES WORK TOGETHER

Given that animals will continue to be used in science for the foreseeable future, there is an ethical obligation to act in a manner that balances the principle of respect for animals with the principles of sufficient benefit and not causing harm.

- **Respect for animals (Fundamental Principle 1)** requires that the intrinsic nature and autonomy of the animals both balance and limit what qualifies as ethical use when weighed against potential benefits. Harms may not necessarily result in pain or distress but may impact the animal's integrity or quality of life beyond what is reasonable, given any potential benefit.
- **Sufficient benefit (Fundamental Principle 2)** cannot be determined in the absence of either the respect for animals or not causing harm principles. Benefits can only be considered sufficient if, on balance, they outweigh any harms potentially incurred by the animals involved, including limitations to the animals' intrinsic nature and autonomy.
- **Not causing harm (Fundamental Principle 3)** requires that the whole or part of the scientific activity be rejected if the procedures involved can be achieved without the use of an animal. Where causing harm, or potential harm, is unavoidable in achieving the potential gains from a scientific activity, those potential gains need to result in sufficient benefit (DeGrazia, 2002; Rollin, 2009).

## 2.5 THE THREE RS AND THE CCAC'S THREE FUNDAMENTAL ETHICS PRINCIPLES

The *CCAC Ethics Principles for Animal Use in Science* replaces the *CCAC policy statement: ethics of animal investigation* (CCAC, 1989), which was based on the Three Rs tenet of Russell and Burch (1959) – replacement, reduction, and refinement. Although often termed Three Rs principles, Russell and Burch never intended the Three Rs to be a set of ethics principles for animal research; rather, they proposed it as a framework for encouraging a more humane conduct of animal-based scientific activities. While the Three Rs continue to provide a strong framework for the mitigation of animal use and associated harms (Schuppli et al., 2004), the Three Rs tenet does not ensure that animal-based scientific activities are morally acceptable (Beauchamp and DeGrazia, 2020; Fenton, 2019a; Orlans, 2002; Schuppli et al., 2004). For example, it provides no guidance on whether the general scientific use or particular instances of scientific use of animals is ethical, on the ethical treatment of animals prior to or after their use, or on whether animals have an interest in continued living that ordinarily overrides killing them. The Three Rs tenet also requires the additional ethics principle of sufficient benefit to address the value of the scientific activity or ethical considerations beyond necessary use and avoidable harm (Strech and Dirnagl, 2019). Thus, the Three Rs tenet requires supplementation through the CCAC's remaining fundamental ethics principles: respect for animals and not causing harm.

Russell and Burch (1959) wrote that principles of humane experimental technique are to be considered in the following order: replacement, reduction, and refinement. Thus, when designing any scientific activity, it is important to first consider whether the activity or a portion of it can be carried out without the use of sentient animals. Where sentient animals can be replaced, they should be. Reduction concerns decreasing the number of sentient animals used overall: minimizing the numbers of animals used and hence the overall amount of harm. Further reduction of animal use may be achieved by employing sound experimental design and statistical analysis, underlining the importance of minimizing the numbers of animals likely to experience harm. Although considered last, refinement of both the procedures to be carried out and the conditions under which the animals are to be housed and cared for further reduces the burden of harm.

Where a scientific activity is deemed to have sufficient benefit, the principle of respect for the animal's moral significance and the principle of not causing harm require that the Three Rs principle of refinement be prioritized over reduction. Since animals are to be treated as individuals, morally, a severe level of harm to a single animal matters more than inflicting a lower level of harm to several animals (Wendler, 2022). In other words, viewing animals as individuals means animals are not to be used beyond previously agreed-upon welfare impact thresholds (i.e., cumulative endpoints) simply to reduce the total number of animals used (Fenwick and Griffin, 2013). Setting limits for harms to an individual animal (refinement) requires establishing an endpoint beyond which the scientific activity will not be permitted to proceed. The limit set may be because of the conditions in which the animal is to be held, the impact of the activity itself, or the impact of previous activities (which can cause cumulative stress effects; e.g., Bateson, 2016; Poirier et al., 2019; Young et al., 2019; Polanco, 2021). Neither financial cost nor convenience may be used as a justification when deciding on cumulative endpoints.

Thus, the CCAC's fundamental ethics principles and the following CCAC principles of application both incorporate and build on Russell and Burch's Three Rs tenet and honour the original intention of the Three Rs: to promote humane science.

# 3 CCAC PRINCIPLES OF APPLICATION

The CCAC's eleven principles of application are intended to assist in meeting the CCAC's three fundamental ethics principles. Principle of Application 1 is intended to provide the moral basis on which decisions will be made concerning the use of animals, and aims to put in place safeguards to minimize harms. Principle of Application 2 is intended to ensure that animals are not used for scientific activities that violate the three fundamental ethics principles. Principle of Application 3 is intended to ensure that the use of animals is necessary. If so, then adherence to Principles of Application 4, 5, and 6 is intended to ensure that there is sufficient benefit to be gained from the proposed animal use. If not, the animal use is unethical. If, however, there is a good prospect of sufficient benefit to be gained, then the Principles of Application 7-11 provide an animal-centric focus aimed at minimizing harms and encouraging positive welfare for the animals. While Principle of Application 1 specifically calls on the use of a precautionary approach in determining sentiency, the principles of application take a precautionary approach throughout in that the best interests of the animals to be used are to limit and balance what qualifies as ethical use.

The principles of application carry the expectation that advances will continue to be made in better meeting the CCAC's three fundamental ethics principles and the highest ethics standards. Implementation of each of these principles of application requires adherence to relevant CCAC policies and guidelines documents that may expand on the minimum requirements below.

The principles of application aim to support decision-making for the following groups:

- **funding agencies and charities supporting animal-based science**, during priority-setting for (and strategic allocation of) funds, which includes:
  - defining national science and infrastructure priorities
  - crafting calls for proposals
  - preparing research proposals and articulating testable hypotheses
  - assembling review panels
  - reviewing proposals for scientific merit and funding
- **regulatory agencies requiring animal-based science data** (e.g., for the efficacy and safety of consumer and health products), to reduce and ultimately replace animal-based regulatory testing
- **curriculum developers**, to replace or strictly limit animal-based methods for teaching or training
- **senior administrators of Canadian institutions**, to maintain a strong culture of ethical animal care and use within the institution
- **protocol authors** during the preparation of animal use protocols
- **institutional animal care committees** in their oversight of the care and use of animals, including but not limited to:
  - the development of policies, standards, guidelines, and standard operating procedures for animal-based scientific activities

  - the review of animal use protocols
  - post-approval monitoring
- **journal editors, peer reviewers, and publishers** in considering the acceptability of scientific papers for publication

## 3.1 PRINCIPLE OF APPLICATION 1

**Sentience is to be used as the basis for decision-making concerning any potential animal-based scientific activity.**

This principle of application reflects the fundamental principles of respect for animals and not causing harm. It is consistent with the Three Rs (i.e., replacement).

The presumption of sentience is foundational for ethical decision-making concerning any proposed animal-based scientific activities. Sentient animals have the capacity to experience positive and negative welfare states, and are hence susceptible to harm incurred before, during, and following a scientific activity.

Accurate attribution of sentience relies on understanding whether an animal has the capacity for subjective experience. Sentience can be challenging to evaluate, relying on the assessment of an animal's behavioural and physiological responses to an external stimulus.

Awareness of the emerging literature on developmental biology and sentience in relation to fetal, incubating, and otherwise developing organisms, as well as invertebrate animals' capacities, will inform decisions concerning whether an animal is to be included in national and local oversight of animal-based scientific activity.

Scientific uncertainty may not be used as a justification for approving practices that would not normally be permitted in species known to be sentient. A precautionary approach is to be used in cases where scientific evidence would suggest animal sentiency (Birch, 2017).

## 3.2 PRINCIPLE OF APPLICATION 2

**Certain activities are not to be permitted either because of the extreme level of harm likely to be experienced by the animal, or the lack of sufficient benefit.**

This principle of application reflects all three fundamental principles: respect for animals, not causing harm, and sufficient benefit.

Institutions and individuals are expected to identify the limits beyond which a scientific activity becomes unethical, including cases where non-animal methods exist or where excessive numbers of animals are used. Some activities do not provide sufficient potential benefit to justify the use of animals. Using animals for activities without sufficient potential benefit denies the moral significance of an animal's interests, thus violating the principle of respect for animals. In addition, harms, in particular severe harms or those with high welfare impacts, deny the moral significance of an animal's interests.

Activities that are not permissible include:

- Category of Welfare Impact E animal use protocols for teaching or training activities

- Category of Welfare Impact E animal use protocols for research or testing activities, unless in matters related to public health emergencies, where no alternative approaches are possible, or the circumstances are extraordinary
- more than one severe welfare impact procedure in the lifetime of an animal
- cumulative severe welfare impact experiences for any animal
- activities involving animals who have been assessed as having poor welfare, except where the studies are aimed at improving their welfare status

A precautionary approach is to be employed when making decisions involving the use of an animal. Any harm likely to be experienced by the animal is not to be underestimated and potential benefits are not to be exaggerated. This means that any animal-based scientific activity where there is insufficient benefit or excessive harm is unacceptable. Institutions are expected to maintain and add to a list of activities that will not be permitted, based on their animal welfare assessments.

Activities that were banned by the *CCAC policy statement: ethics of animal investigation* (CCAC, 1989) and continue to be prohibited include:

- the utilization of muscle relaxants or paralytics (curare and curare-like) alone, without anesthetics, during surgical procedures
- traumatizing procedures involving crushing, burning, striking, or beating, in unanesthetized animals

Activities that were restricted by the *CCAC policy statement: ethics of animal investigation* (CCAC, 1989) and continue to require independent external evaluation to justify their use include:

- burns, freezing injuries, fractures, and other types of trauma investigation in anesthetized animals, concomitant to which must be acceptable veterinary practices for the relief of pain, including adequate analgesia during the recovery period
- staged encounters between predator and prey or between conspecifics where prolonged fighting and injury are probable

Activities listed in the *CCAC policy statement: ethics of animal investigation* (CCAC, 1989) requiring particular caution by investigators, animal care committees, and grant review committees and referees, and continue to be permitted only under exceptional circumstances include:

- experiments involving withholding pre- and post-operative pain-relieving medication
- paralyzing and immobilizing experiments where there is no reduction in the sensation of pain
- electric shock as punishment or negative reinforcement
- extreme environmental conditions such as low or high temperatures, high humidity, modified atmospheres, etc., or sudden changes therein
- experiments studying stress and pain
- experiments requiring the withholding of food and water for periods incompatible with the species-specific physiological needs; such experiments should have no detrimental effect on the health of the animal
- injection of Freund's complete adjuvant

## 3.3 PRINCIPLE OF APPLICATION 3

**Animals are only to be used when there are no valid non-animal means of achieving approved research, educational, or regulatory goals.**

Principle of Application 3 is informed by the fundamental principles of respect for animals and not causing harm. It is consistent with the Three Rs (i.e., replacement).

Institutions and individuals are expected to seek out emerging possibilities for replacing animal use, particularly for ethically objectionable scientific activities. Where non-animal approaches can be substituted, it is expected that the use of animals will be refused.

Failing to replace animals whenever possible is unethical. Hence, the first consideration when designing any scientific activity is whether the activity's objectives can be achieved without the use of animals. This is in line with replacement (Russell and Burch, 1959) and at the heart of all international regulations concerning the use of animals in science (e.g., EC, 2010; OLAW, 2015).

Barriers of financial cost and convenience to the use of non-animal approaches, whether actual or perceived, are not valid reasons for using an animal (e.g., familiarity with the animal model but not the non-animal alternative; publication history; financial, infrastructural, or geographical barriers). When animal use is deemed to be necessary, this means there is currently no other way to adequately answer the scientific questions of interest. Where animals are not necessary to achieve the objectives of the scientific activity, they are not to be used.

## 3.4 PRINCIPLE OF APPLICATION 4

**The expected outcomes of any scientific activity involving animals are to be sufficiently beneficial to humans, animals, or the environment.**

Principle of Application 4 is informed by the fundamental principle of sufficient benefit.

Institutions and individuals are expected to ensure that any scientific activity will benefit humans, animals, or the environment. Justification for using animals requires that there is the "prospect of substantial and otherwise unattainable benefits" (Landi, 2020). Assessing the benefits is a critical part of determining acceptable animal use.

Potential benefits are demonstrable (i.e., applicable, non-trivial, and verifiable) and realistic but may be challenging to determine on an individual project basis. In general, benefits are achieved incrementally, each small gain building on the previous knowledge base (Griffin et al., 2014). Some gains may be realized in a relatively short time frame, whereas others may only be realized after many years. The less certain the potential benefit or the ability to reasonably guarantee benefit based on available evidence, the greater the impetus required to pursue this knowledge via non-animal means.

The outcome of individual scientific activities is expected to contribute gains in the form of increased knowledge (Beauchamp and DeGrazia, 2020). In assessing the potential benefits, it is useful to consider the outcomes of the scientific activity, who will use those outcomes, and how they will be used (EC, 2018). Whether the knowledge obtained benefits a particular area of interest will be understood retrospectively through systematic reviews or other types of meta-analyses (Bahadoran et al., 2020; Farré et al., 2022; Hooijmans et al., 2014; de Vries et al., 2014) and may require adjustments to the scientific strategies (including stopping any animal-based activities) if benefits have not been achieved.

For research projects, the expected outcomes have generally been subject to scientific merit review by funding agencies; however, it cannot be taken for granted that all funding agency application documents or peer reviews address the full range of evidence or methodological considerations necessary to robustly evaluate the likely societal impact of an animal-based scientific activity, the rigour of the scientific activity, and the impact on the animals themselves. Ensuring scientific merit review organizations have policies and processes in place to address impact is essential; if this is not the case, institutions are responsible for establishing an independent mechanism to do so. For regulatory testing, animal care committees are required to determine whether the study director has communicated with the relevant regulatory authority to ensure that an animal-based test is necessary. Similarly, for teaching and training activities, institutions are required to have policies and processes in place to assess whether the use of an animal is warranted (i.e., the pedagogical merit of an activity is to be established) independently of the animal care committee.

Protocol authors are expected to ensure that the benefits to be obtained are maximized. This could be achieved, for example, by tissue sharing if the animals would otherwise be humanely killed at the end of the study or by including the animal in a teaching activity, which would reduce the overall number of animals used, provided that the activity does not add substantially to the cumulative welfare impact for the animal or violate Principles of Application 7 and 10.

## 3.5 PRINCIPLE OF APPLICATION 5

**The most relevant and reliable models or model systems are to be selected to achieve the approved research, educational, or regulatory goals, based on evidence of their validity for the intended purpose.**

This principle of application is informed by the fundamental principle of sufficient benefit.

Institutions and individuals are expected to choose the most relevant and reliable model or model system for the scientific activity in question. This means that the results obtained are more likely to be robust and, hence, will contribute to the body of scientific knowledge, including support of any learning objectives. If this is not the case, the lives of any animals used will have been wasted.

Animal models may be spontaneous, naturally occurring, or they may be induced to reproduce or simulate conditions found in humans or other species of interest. The selection of the animal model or model system requires an awareness of the relevant literature, so that animal models or model systems are not selected based on historical use (Swearengen, 2018; Robinson et al., 2019; Storey et al., 2021). Indigenous or local knowledge may be valuable for selecting the animal model or system of interest and is particularly relevant to field studies. For teaching or training activities, the use of the most relevant model contributes to a successful learning outcome.

Validity of the model requires an understanding of the limitations of the animal model, including differences in species, sex, and welfare from the species of interest that might affect external validity (i.e., whether the results can be generalized) (Beery and Zucker, 2011; Garner et al., 2017; Kafkafi et al., 2018; Cait et al., 2022). For teaching or training activities, validity requires an understanding of the model's limitations in meeting the learning outcomes for the activity.

## 3.6 PRINCIPLE OF APPLICATION 6

**Rigorous scientific principles, and research, teaching and training, and testing practices are to be implemented to ensure high-quality animal-based scientific activities.**

This principle of application is informed by the fundamental principle of sufficient benefit.

Institutions and individuals are expected to ensure that animal-based scientific activities are carried out according to rigorous practices, so that sufficient benefit can be attained and biases minimized (Abdel-Sattar et al., 2014; van Wilgenburg et al., 2013). The benefits to be gained from a scientific activity are the product of the importance of the objectives and the probability of achieving those objectives (EC, 2018). Neither financial cost nor convenience is to be used as a barrier to scientific rigour.

Anyone using animals in science is required to be qualified (i.e., appropriately trained and competent). Institutions are expected to assure the competence of everyone involved to ensure that the animal-based activity will be carried out with adequate scientific rigour.

The ethical use of animals requires any scientific activity to be carried out with due care and attention at every stage: development, modification, conduct, reporting, and publication or dissemination (Schwab et al., 2022). Individuals, institutions, and the scientific community at large are required to ensure that any research, teaching and training, and testing activities meet best practices and are carried out honestly, accountably, openly, and fairly.

At a minimum, institutions and individuals are responsible for scientific rigour in the following areas:

- **proposing and conducting research** through:
  - sound experimental design, appropriate to test or address clearly articulated research questions or hypotheses, ensuring validity (construct, internal, and external) of the scientific activity (Würbel, 2017), and adherence to the PREPARE guidelines (Smith et al., 2018)
  - reduction of bias (e.g., masking or blinding, randomization) (e.g., Bailoo et al., 2014; Hirst et al., 2014)
  - careful preparation of any substances to be tested (OECD, 1998)
  - housing and husbandry conditions appropriate for the individual animal and the species (Cait et al., 2022)
  - training and habituation of animals (Prager et al., 2019)
  - effective monitoring for humane intervention points and for approved scientific endpoints (Hawkins et al., 2019)
- **recording, analyzing, and interpreting data** through:
  - careful and appropriate data collection, management, and analysis (Lazic et al., 2018; Head et al., 2015; Holman et al., 2016)
  - maintenance of complete and accurate records of data, methodologies, and findings, in a manner that will allow verification or replication of the work by others
- **reporting and publishing data and findings** through:
  - adherence to reporting guidelines (i.e., making available the necessary components of the methodology used so that study findings can be reproduced (Hooijmans et al., 2010; Percie du Sert et al., 2020; NRC, 2011; Landis et al., 2012; Osborne et al., 2018))

- submission of a checklist alongside an article for publication to increase the quality of reporting (Han et al., 2017)
- publication on open access platforms (e.g., Tri-Agency Open Access Policy on Publications, Final NIH Policy for Data Management and Sharing, and Ag Data Commons), as well as publishing "negative" results, to limit publication bias and improve translatability
- sharing of animal data on platforms to reduce the number of animals the scientific community needs to address research questions (e.g., Open data commons for spinal cord injury and TOSI: Promoting Open Science Across Canada and Beyond | The Neuro – McGill University)

- **selecting and conducting regulatory tests** through:
  - adherence to international harmonization approaches to minimize the number of animals used and the potential for harm (e.g., OECD Testing of Chemicals, and ICH Guidelines)
  - stimulation of the development and timely incorporation of scientifically justified alternative methods and strategies in the testing and assessment of substances to replace, reduce, or refine the use of vertebrate animals (Government of Canada, 2023)

## 3.7 PRINCIPLE OF APPLICATION 7

**Potential negative welfare impacts are to be minimized, and positive welfare states are to be ensured.**

This principle of application is informed by the fundamental principles of respect for animals and not causing harm. It is consistent with the Three Rs (i.e., refinement and reduction).

Institutions and individuals are expected to ensure that any potential harms are minimized, both for individual animals and for cohorts of animals. This requires assessing the welfare impacts of scientific activities prospectively and retrospectively to determine whether harms have been reduced, unanticipated harms have arisen, and positive welfare experiences have been achieved.

The intentional infliction of severe harm is unethical. Hence, Category of Welfare Impact E animal use protocols are not acceptable for any teaching or training activities and are only permissible for research or testing activities in which no alternative approaches are possible and the circumstances are extraordinary, such as in matters related to public health emergencies. Where death has been the historic scientific endpoint, activities require compelling justification. Personnel planning and carrying out these scientific activities are required to research and implement the earliest scientifically valid endpoints, and to provide supportive therapies.

Harms may result from procedures carried out on the animals, or from husbandry conditions (including any housing and transportation).

Personnel are required to be knowledgeable about the animal species, including typical behaviour and needs (both in the natural environment and in the laboratory), and any individual differences, thus minimizing negative welfare states and ensuring positive welfare states (see Principle of Application 8).

Current thinking about the potential for animal harm includes recognizing that what affects an animal's welfare depends on the animal's sensory capacities, physiological traits, social capacities, and cognitive and behavioural abilities (e.g., Mason, 2010; Nussbaum, 2011; Rollin, 2009). Animals are to be treated with sensitivity to the capacities, traits, and abilities typical for their species and developmental stage, as well as their

individual preferences. Scientific activities are to be planned with the animal's characteristics and needs in mind, working to achieve cooperation and positive welfare experiences (Sørensen et al., 2021).

Minimization of harm is a requirement common to most ethical frameworks, especially where costs to animal subjects are high (Rollin, 2009; Schuppli et al., 2004). Russell and Burch urged that we "set our sights high in removing inhumanity" (Russell and Burch, 1959). Avoiding and reducing harm supports their intended emphasis on replacement and reduction before considering refinement.

## 3.8 PRINCIPLE OF APPLICATION 8

**Captive environments are to provide animals with the opportunity to experience a life worth living.**

This principle of application reflects a commitment to the principle of respect for animals and recognizes the moral importance of animals' basic needs and interests. This is consistent with the Three Rs (i.e., refinement).

Institutions and individuals are expected to provide animals with an environment that goes beyond meeting their basic needs and not harming them. Animals are expected to be afforded some degree of control over their environment. Hence, the ethical framing of animal-based science is not simply to minimize harm. Russell and Burch argued that we must "aim at well-being rather than at mere absence of distress", which, for them, included the experience of pleasant emotions (Russell and Burch, 1959).

For a significant portion of their lives, animals used in science are not actively part of a scientific activity. Hence, this principle requires that good faith efforts be made to maintain an acceptable quality of life for animals before, during, and after any scientific activity. It requires consideration of the animal as an individual and (where appropriate) their social and "cultural" milieu (Fitzpatrick and Andrews, 2022).

Emphasizing positive experiences contributes to the overall welfare of the animal throughout their life as a scientific subject (Mellor et al., 2020). A life worth living is achieved by maximizing positive and minimizing negative experiences from the perspective of the animal (Mellor, 2016; Fenton, 2019b), so that the net sum of their life experiences is positive.

Maintaining positive welfare states within a captive environment requires adherence to the five domains of animal welfare: provision of good nutrition, appropriate physical environment, health, and behavioural interactions, all resulting in a positive mental state (Mellor et al., 2020). Each of these domains is impacted by the way humans treat animals. Financial cost and convenience are not to be used as a barrier to providing animals with a captive environment suitable for their needs.

Conventional practices, such as those related to the housing and husbandry of animals, whether before, during, or after use in a scientific activity (Lewejohann et al., 2020), are thus required to be guided primarily by considerations that optimize welfare rather than by practicality, precedent, and affordability. It is important to think critically about conventional practices, evaluating whether they are essential to successful scientific activities, and the degree to which they can be refined to improve welfare.

## 3.9 PRINCIPLE OF APPLICATION 9

**Animals are to be trained to cooperate with procedures in a manner that respects their welfare.**

This principle of application reflects a commitment to the fundamental principle of not causing harm, given the negative welfare impacts that can arise from forcing an animal to comply with procedures. It also reflects the fundamental principle of respect for animals. It is consistent with the Three Rs (i.e., refinement).

Institutions and individuals are expected to employ animal training methods that promote positive emotional states, with the aim of eliminating or decreasing an animal's fear or stress response to procedures (Wolfensohn and Lloyd, 2013; Laule et al., 2003). These methods include passive processes (i.e., habituation) and planned active ones (i.e., systematic desensitization, counter-conditioning, or positive reinforcement training).

From a welfare perspective, securing the cooperation of subjects is morally preferable to forcible compliance or coercion. From a scientific perspective, securing the cooperation of subjects means that the animals are less stressed as they have some level of control over the situation, which has an impact on any data collected (Gouveia and Hurst, 2013). The impact of cooperative subjects on the data collected justifies the expectation that animals used in scientific activities are to be trained to cooperate in a manner that respects their welfare. Meeting this principle of application requires personnel involved in caring for the animals or carrying out procedures to understand the species, their typical behaviours, and needs, as well as the individual animal's needs and preferences, to best minimize harm and promote both physical and psychological well-being (Fenton, 2019b). Neither financial cost nor convenience is to be used as a barrier to securing animals' cooperation.

Training can be a form of enrichment for the animals (Baker, 2016; Coleman and Novak, 2017; Fernandez, 2022), and as such, may contribute to providing positive experiences and a life worth living (see Principle of Application 8). Overriding an animal's resistance to cooperate (that is, their sustained dissent) requires adequate justification before the animal-based scientific activity may take place. In other words, the integrity and autonomy of the animal will be balanced with any potential harm or benefit. Whenever possible, animals used in science are expected to be offered the opportunity to consent to procedures (Fenton, 2018).

## 3.10 PRINCIPLE OF APPLICATION 10

**Animals are to be given the opportunity to enjoy a life after their scientific use.**

This principle of application reflects the ethical importance of animals' interests, which are captured in the three fundamental principles, especially respect for animals.

Institutions and individuals are expected to consider whether animals can be given a future beyond their use in a scientific activity. A plan for the fate of the animals at the end of any scientific activity is required before the activity takes place. Animals bred for use in scientific activities and no longer needed may be considered candidates for retirement, lifetime care, rehoming, or release into an ecologically suitable environment (following professional assessment of the impact on the animal and the environment), rather than being humanely killed. Only a minority of animals used in scientific activities currently fall within these categories (i.e., being considered candidates for retirement, lifetime care, rehoming, or release into an ecologically suitable environment); however, it is expected that protocol authors and animal care committees will evaluate the possibilities for the animals after their scientific use, without placing barriers due to financial cost and convenience.

The fate of animals bred or used for scientific activities will be determined by consideration of the animal's interests, while respecting government regulations. For example:

- animals who have been genetically modified cannot be released outside the animal facility (Government of Canada, 2018)
- nonhuman primates may be retired to sanctuaries, depending on import regulations and provided that there are sufficient provisions for their specialized care (CFIA, 2009)
- companion animals, such as cats and dogs, may be rehomed, providing that the new home is appropriate for their needs, and they have been conditioned for a human household environment
- other domesticated animals (e.g., rats, mice) may be rehomed, provided that the new location can fulfil their needs satisfactorily
- wild animals may be released back into an ecologically suitable environment, provided that the relevant federal, provincial, and territorial wildlife agencies have been consulted, and relevant permits obtained with adherence to all applicable local, provincial or territorial, and federal legislation following the assessment of conditions to maximize the animals' survival and minimize impacts on other species and the environment

Animals may also be transferred to other animal use protocols at the completion of a scientific activity, provided that the lifetime cumulative welfare impact is assessed (i.e., level of welfare impacts over time or the degree of sensitization of animals to repeated activities) (e.g., Bateson, 2016; Poirier et al., 2019; Young et al., 2019; Polanco et al., 2021). This is to avoid animals being transferred to other animal use protocols instead of being humanely killed, when humane killing would be the lesser harm.

For animals experiencing negative welfare, or those who are surplus to the requirements of a scientific activity and cannot be rehomed, see Principle of Application 11.

## 3.11 PRINCIPLE OF APPLICATION 11

**Killing an animal, even using a humane killing method not intended to have any negative welfare impact, is to be considered as a harm to that animal.**

This principle of application reflects the ethical importance of animals' interests captured in the three fundamental principles: respect for animals, not causing harm, and sufficient benefit.

"The killing of laboratory animals is often referred to as euthanasia, which literally means 'a good death'. Since the use of the phrase euthanasia carries connotations of the ending of suffering for the benefit of the individual animal, which is not always the reason that laboratory animals are killed, it is more accurate to use the word killing in the laboratory setting" (NC3Rs, n.d.).

Institutions and individuals are expected to present strong justification for killing animals, even where humane killing methods will be used, as all things being equal, it violates two of the three fundamental principles (respect for animals and not causing harm); hence, morally, there needs to be sufficient benefit to justify humanely killing an animal used in a scientific activity. Animal care committees are required to ensure that animals are not being humanely killed simply to satisfy matters of financial cost and convenience, while ensuring that animals are not left to suffer or languish in impoverished environments. It is also necessary to recognize when humane killing would be the lesser harm (i.e., in situations of unrelievable negative welfare states).

Clear scientific endpoints and humane intervention points are required to be explicitly described in an animal use protocol and approved by the animal care committee prior to the commencement of the scientific activity. Humane intervention points (welfare endpoints) are intended to avoid negative welfare states for the animals and may require animals to be humanely killed if negative welfare states cannot be avoided or alleviated by other means. Procedures approved prior to the commencement of a scientific activity are required to be updated as the activity progresses, when unexpected outcomes are associated with significant negative welfare impacts.

Scientific activities requiring the animal to be humanely killed at the end of the activity require strong justification, including the selection of a method that is least likely to result in negative welfare states. In other words, if an animal's death is necessary, they are required to be given the best death possible. Organs and tissues are expected to be harvested and shared to avoid humanely killing additional animals (i.e., the potential benefits from such animal use are to be maximized).

Scientific activities deliberately requiring death as an endpoint (i.e., requiring the animal to reach death as part of the activity) are unacceptable, except for matters related to public health emergencies, where no alternative approaches are possible, and the circumstances are extraordinary. Scientific activities that have the potential to result in lethal outcomes therefore require compelling justification as they may involve considerable suffering for the animal. The expectation is that surrogate endpoints will be identified so that animals do not suffer (i.e., they are humanely killed by methods that minimize harms before they become moribund) and where extreme endpoints are unavoidable, animals are monitored carefully and offered supportive treatments (e.g., warmth, fluids).

Breeding programs are required to be run efficiently, thus minimizing the number of excess animals to be humanely killed and reducing the number of animals used overall. It is expected that animal strains, particularly those created by genetic modification, will be cryopreserved when not needed (thus avoiding overbreeding to maintain a genetically distinct line and avoiding genetic drift), and made available to other research groups whenever possible.

When killing any animals, including those who are surplus to a scientific activity or breeding program and cannot be transferred to another animal use protocol or are unsuitable for lifetime care, rehoming, retirement, or release into an ecologically suitable environment, humane killing methods, aimed at minimizing harm, are to be used.

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# GLOSSARY

**Euthanasia** – Killing an animal when the reason for terminating the animal's life is for the benefit of the individual animal (this term is being phased out by the CCAC; **humane killing** will be used in all new and revised CCAC documents).

**Humane killing** – The intentional ending of an animal's life using methods that demonstrate respect for the animal and minimize negative welfare impacts.

**Killing** – Refers in general to the termination of an animal's life; used when an animal's life is ended by a non-approved method, or they die as the result of involvement in a scientific activity without any intervention to ensure the animal is humanely killed.

**Not causing harm** – A CCAC fundamental principle whereby the intentional or avoidable harming of animals is morally wrong.

**Reduction** – Strategies that will result in fewer animals being used to obtain sufficient data to answer the scientific question, or in maximizing the information obtained per animal, thus potentially limiting or avoiding the subsequent use of additional animals, without compromising animal welfare.

**Refinement** – Modification of housing, husbandry, or scientific procedures to minimize the negative welfare impacts experienced by an animal or provide a positive welfare experience for an animal.

**Replacement** – Methods that avoid or replace the use of animals in an area where they would otherwise have been used.

**Respect for animals** – CCAC fundamental principle whereby animals are valued as individuals with fundamental needs and interests.

**Scientific activity** –All aspects of any research, teaching, training, or testing activities.

**Sentience** – Capacity to experience positive and negative welfare states, and therefore to be susceptible to harm occurring before, during, and after a scientific activity.

**Sufficient benefit** – CCAC fundamental principle whereby any use of animals provides sufficient benefit to humans, animals, or the environment.

**Three Rs** – Principles of replacement, reduction, and refinement in animal-based science, as first explained by W.M.S. Russell and R.L. Burch in 1959 in *Principles of Humane Experimental Technique*.