CCAC
CCPA

Canadian Council on Animal Care
Conseil canadien de protection des animaux

CCAC guidelines:
Categories of welfare impact

Date of Publication: February 2024

ISBN: 978-0-919087-99-6

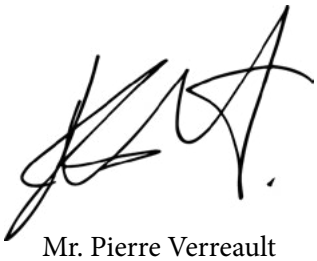
190 O'Connor St., Suite 800
Ottawa, Ontario, K2P 2R3

www.ccac.ca

ACKNOWLEDGEMENTS

The Canadian Council on Animal Care (CCAC) Board of Directors is grateful for the expertise contributed by the members of the CCAC Subcommittee on Categories of Welfare Impact and for their engagement throughout the guidelines development process. In addition, the board is grateful to all those who provided critical input during the two review periods. We would also like to acknowledge the contributions of both the CCAC Standards Committee and the CCAC Assessment and Certification Committee members, who provided important guidance to the subcommittee. We would like to thank the Secretariat project team for their excellent work, as well as Dr. Stéphane Ménard for the review of the French translation of the document. The CCAC also acknowledges its funders, the Canadian Institutes of Health Research and the Natural Sciences and Engineering Research Council of Canada. The CCAC could not continue to deliver on its current mandate without their support.

Ms. Catherine Rushton
Chair, CCAC Board of Directors



Mr. Pierre Verreault
CCAC Executive Director

CATEGORIES OF WELFARE IMPACT SUBCOMMITTEE

Dr. Andrew Winterborn (Chair), Queen's University
Dr. Denna Benn, Ontario Ministry of Food and Rural Affairs
Dr. Renée Bergeron, University of Guelph
Dr. Phil Byrne, Fisheries and Oceans Canada
Dr. Marc Cattet, Government of Yukon
Dr. Mark Fry, University of Manitoba
Dr. Sherry Hannon, Feedlot Health Management Services
Dr. Elisabeth Ormandy, Animals in Science Policy Institute
Dr. Shelley Pruss, University of Alberta
Ms. Evelina Smith, Sherbrooke, Québec
Ms. Suzanne Smith, McGill University

TABLE OF CONTENTS

PREFACE 1

1. INTRODUCTION 2

1.1 The Five Categories of Welfare Impact 2
1.2 Assigning Categories of Welfare Impact to Scientific Activities 3

2. PROSPECTIVE ASSIGNMENT 4

2.1 Process for Prospectively Determining the Category of Welfare Impact 4
2.2 Factors that Influence the Prospective Category of Welfare Impact 4
2.2.1 Step 1 – Attributes of the Environment 5
2.2.2 Step 2 – Attributes of the Animals 5
2.2.3 Step 3 – Attributes of the Scientific Procedures 8
2.3 Process for Evaluating Overall Prospective Category of Welfare Impact 10
2.4 Summary of General Principles for Prospectively Assigning Categories of Welfare Impact 11

3. RETROSPECTIVE ASSIGNMENT 12

3.1 Process for Retrospectively Determining the Category of Welfare Impact 12

REFERENCES 13

GLOSSARY 18

Categories of welfare impact

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 guidelines: Categories of welfare impact* is part of a series of general guidelines documents for the ethics and care of all animals used in scientific activities, including wild animals in the field or brought into scientific facilities, and animals owned by third parties that are used in science. General guidelines streamline information for protocol authors, animal care committees, facility managers, veterinarians, technicians, and animal care personnel to help facilitate improvement in both the care given to animals and the manner in which scientific activities are carried out.

This specific document acknowledges that the welfare of animals used in science can be impacted in diverse ways. It describes how expected welfare impacts to animals should be determined and summarized into a descriptive category, and then confirmed retrospectively.

This guidelines 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 Canada 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.

1 INTRODUCTION

1.1 THE FIVE CATEGORIES OF WELFARE IMPACT

There are five categories of welfare impact. Each category is assigned an alphabetical label[1] with category A capturing positive welfare impacts, and categories B through E reflecting different gradations of negative impact. In general, the negative welfare impact of a scientific activity is determined by its severity (i.e., the magnitude of pain or distress it causes), the frequency with which it is carried out, and its duration (including time to make a full recovery). For ease of application when determining the Category of Welfare Impact, either the frequency or the duration of the impact should be considered, whichever has the higher impact. The categories of welfare impact are therefore defined by severity and frequency or duration (in order from least to most impact on the animals in question; see Table 1, "Defining Categories of Welfare Impact Based on the Severity and Frequency or Duration of the Impact"):

A – Positive welfare impact

- Animal welfare is improved

B – Mild negative welfare impact

- Low severity, low frequency or short duration

C – Moderate negative welfare impact

- Low severity, medium-high frequency or medium-high duration; OR
- Medium severity, low-medium frequency or short-medium duration

D – High negative welfare impact

- Medium severity, high frequency or long duration; OR
- High severity, low frequency or short duration

E – Severe negative welfare impact

- High severity, medium-high frequency or medium-long duration

1 The CCAC previously used Categories of Invasiveness to describe the impact of a scientific activity on animals. While there are five letters describing the categories of welfare impact, as in the previous policy describing the categories of invasiveness, the two systems should not be considered equivalent, due to the shift to an animal-centric focus with categories of welfare impact.

Table 1 Defining Categories of Welfare Impact Based on the Severity and Frequency or Duration of the Impact

Severity	Frequency or Duration	Category of Welfare Impact
Positive	Any	A – Positive impact
Low	Low or Short	B – Mild negative impact
Low	Medium	C – Moderate negative impact
Low	High or Long	
Medium	Low or Short	
Medium	Medium	
Medium	High or Long	D – High negative impact
High	Low or Short	
High	Medium	E – Severe negative impact
High	High or Long	

1.2 ASSIGNING CATEGORIES OF WELFARE IMPACT TO SCIENTIFIC ACTIVITIES

The expectation is that categories of welfare impact will be assigned to protocols both prospectively, before undertaking any animal-based scientific activity, and retrospectively, after the scientific activity is complete. The prospective assignment of categories of welfare impact should therefore be part of a continuous learning and review process rather than a conclusion, as it is important to verify predictions rather than simply assume them to be true. The retrospective categories of welfare impact determination can ultimately be used to improve the accuracy of the prospective process.

This document is focused on recognizing the experiences of individual animals used in scientific activities and capturing how these experiences impact their welfare. Because the process is focused on the experiences of individual animals, if different subgroups of animals within a single protocol experience different levels of welfare impact, then each subgroup must be assigned to the appropriate category. As many categories as required will be used to describe the animals' experiences with the protocol (as opposed to assigning all of the animals in the protocol to the highest category experienced by one individual animal). In practice, individual animal tracking may be challenging in large groups. In such cases, group-level assessments or averaging are appropriate, with the expectation that any animals that deviate significantly from the group are noted and reported during the retrospective analysis.

2 PROSPECTIVE ASSIGNMENT

This section describes how, prior to any scientific activity (i.e., prospectively), animal use protocols must be assigned as many categories of welfare impact as required to capture the experiences of all animals on the protocol.

2.1 PROCESS FOR PROSPECTIVELY DETERMINING THE CATEGORY OF WELFARE IMPACT

The Category of Welfare Impact for each animal or subgroup of animals must be determined during the evaluation of each protocol. The steps outlined below are a formalization of the thought processes that are often currently applied when preparing and reviewing protocols. This systematic process aims to facilitate a more comprehensive account of the welfare impacts on animals used in Canadian science that is consistent between institutions.

Categories of welfare impact must be assigned based on a series of factors (described in Section 2.2, "Factors that Influence the Prospective Category of Welfare Impact"). Protocol authors should combine available scientific evidence with their experience and knowledge to determine the expected impacts of the scientific activity on animal welfare. Note that the relative impact of each factor can vary between protocols. Therefore, each protocol should be evaluated based on its unique variations in environmental, animal, and procedural factors that collectively will have a welfare impact on the animals. Implementing potential refinements for each factor should be the goal of every protocol. Finally, the animal care committee is responsible for confirming and approving the Category of Welfare Impact.

The following three broad categories of welfare-impacting factors must be considered when assessing the welfare impact of a scientific activity:

1) attributes of the environment;
2) attributes of the animals themselves;
3) attributes of the scientific procedures.

The following 16 sections describe how various factors within these three categories should be assessed, and how all of this information can be summarized into overall categories of welfare impact.

2.2 FACTORS THAT INFLUENCE THE PROSPECTIVE CATEGORY OF WELFARE IMPACT

Within the context of a specific scientific activity, some factors may not be applicable, may have a neutral welfare impact, or may be unknown. In such cases, they should be acknowledged as 'unknown' or 'not applicable' and not incorporated into the final determination of the overall prospective Category of Welfare Impact.

2.2.1 Step 1 – Attributes of the Environment

2.2.1.1 Housing and Husbandry

Holding animals in a scientific setting has an impact on their welfare. Confinement confers an impact even if the animals are healthy, phenotypically normal, and not subject to scientific procedures (e.g., Balcombe et al., 2004; Castelhano-Carlos and Baumans, 2009; Hannibal et al., 2016; Cait et al., 2022). Consequently, animals kept in scientific settings may be assigned a Category of Welfare Impact anywhere between B and E for this factor, depending on the conditions and length of time they are held. To assist in assigning a score for this factor, institutions are encouraged to create standard operating procedures (SOPs) based on relevant scientific literature, welfare assessments, health records, and retrospective Category of Welfare Impact determinations. These SOPs should also incorporate relevant CCAC standards and describe the minimally expected enrichment provision.

Animals held in significantly enriched conditions (e.g., semi-naturalistic cages for rats (Makowska and Weary, 2016)) that have been desensitized to benign husbandry procedures through positive reinforcement may have a preliminary assignment of Category of Welfare Impact B. Conversely, animals held in conditions that adversely affect their welfare to a greater extent, and those that experience aversive husbandry procedures (e.g., solitary caging of a social species), should have a higher category assigned as appropriate. This increase in the Category of Welfare Impact should be judged based on the impact of husbandry procedures and the environment's ability (or lack thereof) to meet the animals' behavioural needs; 'natural' environments should not be assumed to have a lesser welfare impact.

If wild animals are brought into captivity, a preliminary Category of Welfare Impact C or higher should be assigned. For field studies involving wild animals (i.e., animals only studied in the wild), this factor should be scored as 'not applicable'.

2.2.1.2 Relocation and Transportation

Transportation is widely regarded as a likely source of negative animal welfare impact (e.g., Broom, 2005; Gregory, 2008; Arts et al., 2012). The extent of the welfare impact will vary according to many factors, including but not limited to stocking density, developmental stage, microclimate, duration of travel, animal condition, and loading or unloading practices (Schwartzkopf-Genswein et al., 2012). The Category of Welfare Impact should take into account the frequency and duration of transportation, and the combined severity of any other transport-related factors. Transportation may cause motion sickness (e.g., Santurtun and Phillips, 2015). As transport may have a Category of Welfare Impact between B and E, it should be assigned based on available evidence. Note that this assessment includes the transport of animals from commercial animal suppliers to a scientific facility, from one scientific facility to another, and from one location to another as part of a scientific activity.

2.2.2 Step 2 – Attributes of the Animals

2.2.2.1 Species, Strain, and Sex of the Animals

The species, strain, and sex of an animal will partially determine the welfare impact of a given procedure. For example, handling fish out of the water (e.g., Brydges et al., 2009) or performing a procedure on nocturnal animals during the light phase (e.g., Abou-Ismail et al., 2008) can increase the negative welfare impact. Similarly, strain- and sex-based criteria should also be considered, as phenotypes can vary widely within

species and even between sexes. Anxiety levels are known to differ between mouse strains (e.g., Griebel et al., 2000), and sex differences in pain sensitivity or reactivity are common across many strains of rats and mice (e.g., Mogil et al., 2000). Many other examples exist where the expected impact of a procedure is influenced by the species, strain, and sex of the animals. These three attributes should therefore be incorporated into Category of Welfare Impact assessments, as appropriate.

2.2.2.2 Age and Developmental Stage of the Animal

If adult animals of the same age are used, this factor should be considered neutral in terms of affecting the overall Category of Animal Welfare Impact. However, if juvenile or elderly animals are used, increasing the Category of Welfare Impact may be warranted as they are typically more vulnerable than healthy adults. The potential welfare impact to these age groups is greater, even though the risk to both groups is qualitatively different. In some cases, juvenile animals may be more sensitive to stressors than adults (Romeo, 2010). Juveniles may also be at risk of having their development compromised, potentially in a way that affects their long-term welfare (e.g., Isgor et al., 2004; Chaby et al., 2013). Additionally, the relative physiological cost for elderly animals to respond to stressors increases (e.g., Hughes, 2008), while their ability to recover from stressors decreases (e.g., McEwen and Morrison, 2013; Lupien et al., 2009). There may be sensitive periods (e.g., pregnancy (Christian, 2012)) when animals are vulnerable to increased negative welfare impacts. These age-related concerns may vary between species and experimental contexts, so prudent judgment is required in assessing this factor (e.g., potential developmental compromises may not matter if the animals won't live long enough for them to become apparent). If animals of varying ages are used simultaneously, additional consideration should be given to increasing the Category of Welfare Impact for this factor based on the skill and knowledge of the person doing the monitoring and the potential increased difficulty of monitoring animals.

2.2.2.3 Whether the Animal is Domesticated or Non-Domesticated

Non-domesticated animals are thought to be more affected by scientific activities and housing than similar domesticated animals (all else being equal). There are three main reasons for this: first, many wild adaptations (e.g., flightiness, increased vigilance) have been eliminated or reduced through domestication over time (Price, 1999). Reduced welfare impacts on domesticated animals are the result of decreased reactivity to humans and handling (e.g., Hughes et al., 1976; Ericsson et al., 2014) and less aggression towards conspecifics (e.g., Künzl et al., 2003). It is important to note that domestication is a gradual process that has occurred over several generations (Price, 1999), so animals only a few generations removed from the wild might be considered habituated, but not domesticated. Second, domesticated animals typically experience early life exposure to humans, which reduces their fear and stress responses throughout their life (e.g., Pedersen and Jeppesen, 1990; Feenders and Bateson, 2011), something that non-domesticated animals may not experience. Third, the act of catching wild animals (including confining them and separating them from social partners) has a large welfare impact (e.g., Dickens et al., 2009) that is not typically experienced by domesticated animals. Therefore, if the animals used are domesticated, this factor may be scored as a neutral welfare impact.

2.2.2.4 Inherent Welfare Status of the Animal

In some cases, animals may have compromised welfare before the commencement of the scientific activity. For example, this may be due to a deleterious genetic mutation (e.g., Buehr et al., 2014), an induced disease

state (e.g., cachexia (DeBoer, 2009)), a neuropathic pain model (e.g., Jaggi et al., 2011), or any other manipulation that generates a compromised physical or psychological phenotype. The welfare impact for this factor should be considered neutral for healthy, phenotypically normal animals, and be reflected in the assignment of a higher Category of Welfare Impact if any pre-existing conditions exist.

2.2.2.5 Energy Status or Body Condition

Body condition scoring is a common method used to quantify the body reserves or fat accumulation, and general health status of an animal. Scoring scales can vary, but most commonly consist of a 1 – 5 scale, where 1 is emaciated, 3 is well-conditioned, and 5 is obese. Body condition scoring is a valuable tool for welfare assessment and has been implemented for many different species (e.g., mammals, from mice (Ullman-Culleré and Foltz, 1999) to cattle (Roche et al., 2009), birds (Gregory and Robins, 1998), reptiles (Rawski and Józefiak, 2014), and fish (Nash et al., 2006)). Animals at either end of the body condition scale may face additional health and welfare impacts, and therefore a Category of Welfare Impact A should be assigned to animals who are in the ideal body condition for their breed or strain, sex, and life stage (typically a 3 out of 5).

Potential metabolic demands should be considered in addition to body condition, which indicates energy reserves. To provide a few examples, animals may face increased metabolic demands from climate conditions, seasonal processes (e.g., migration), specific life-stage events (e.g., smoltification in anadromous fish), or if they are pregnant, lactating, or otherwise caring for offspring. These may compound the welfare impact a scientific activity has on the animals and should be captured in the welfare impact assigned to this factor.

2.2.2.6 Social Disruption

Generally, it is acknowledged that keeping social animals in social groups benefits their welfare. However, social groups can be a source of negative welfare impact, particularly when these groups are unstable, do not occur naturally, or are otherwise disrupted. Changes in social groupings can lead to increased aggression, anxious behaviour, and biomarkers of stress that can last for several days while a dominance hierarchy is established (Hannibal et al., 2016). In fact, one paradigm for studying chronic stress is to constantly change animals' social partners, resulting in continually repeating the period of social upheaval and hierarchy establishment (e.g., Sterlemann et al., 2008). For wild animals, even temporary removal of territorial individuals may lead to increased stress or aggression when that animal is returned. Consequently, if the scientific activity requires mixing social groups or disrupting a stable social network (e.g., through removal of the dominant animal), the welfare impact should be thoroughly considered. For this factor, a stable social group with no signs of social stress (e.g., aggression, withdrawal, self-harm, displacement) should be assigned a neutral impact.

Social housing of an asocial species may also cause welfare issues. It is crucial to understand the natural biology and behaviour of the species when assigning a welfare impact for this factor.

2.2.2.7 The Previous Experiences of the Animal (Cumulative Lifetime Experiences)

Consideration of an animal's cumulative lifetime experiences is important when determining welfare impact. It is important to note that even mild or moderate procedures can have a negative cumulative impact on the welfare of an animal (Wolfensohn et al., 2015), and that consideration of cumulative effects also applies to animals who are held long-term but used infrequently (e.g., reptiles held for annual teaching

exercises). Furthermore, animals may find a single procedure increasingly aversive when repeated multiple times (e.g., Rushen, 1986; Boulanger Bertolus et al., 2015). All the available information about the prior experiences of an animal should therefore be used to inform the current risk to their welfare. This information may come from welfare assessments (*CCAC guidelines: Animal welfare assessment* (CCAC, 2021)), health records (*CCAC guidelines: Husbandry of animals in science* (CCAC, 2017)), previous protocols, or personal knowledge of individual animals' histories. The *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022) provide more information and a framework for helping to assess cumulative welfare impacts. It is also important to note that animals may have had positive experiences that can factor into this assessment. Generally, this factor should be considered neutral if the animals have no previous scientific experiences to take into consideration.

2.2.2.8 Known Aspects of Individual Animals

Temperament can have a large influence on the welfare impact each animal is likely to experience (e.g., Coleman, 2012). Generally, there will be a distribution of temperaments within a population, even within the same species or strain, where some animals will be calmer or bolder (or conversely, more anxious or fearful) than others (e.g., Walker and Mason, 2011). It can be difficult to predict the temperaments of newly acquired animals. However, scientific team members or animal care personnel may come to recognize the temperaments of certain individual animals, particularly those they have worked with on prior protocols. In such cases, this information should be taken into consideration by assigning a higher level of welfare impact to animals known to be more timid, anxious, or fearful. If the temperaments of the animals are unknown in advance, this factor may be scored as 'not applicable'.

2.2.3 Step 3 – Attributes of the Scientific Procedures

2.2.3.1 The Scientific Procedures

The expected level of pain and distress associated with a procedure can reasonably be predicted by those with experience, including the protocol authors and members of the animal care committee. Note that all animals included in the CCAC's mandate are assumed to have the capacity to feel pain and to experience distress. The anticipated level of pain and distress should take into account any potential long-term consequences of the procedure (e.g., Cattet et al., 2008), and not only acute impacts to welfare.

Similar procedures can have markedly different impacts on animal welfare, depending on refinements to the methodology. These can take many forms, but the quality of each refinement should be judged based on the potential or demonstrated efficacy of reducing or eliminating pain and distress. For example, providing post-surgical analgesia should reduce the impact of a surgery, but the magnitude of this benefit depends on the drug and dose provided (e.g., Roughan and Flecknell, 2003). Similarly, housing animals in their social groups is not only beneficial for their welfare generally (e.g., Rault, 2012; Patterson-Kane et al., 2002; Novak and Suomi, 1991), but keeping them with their social partners improves outcomes following a procedure (e.g., Johansson and Ohlsson, 1996; Detillion et al., 2004). To assist in assessing this factor, examples of scientific procedures and surgical procedures have been provided in the associated document, "Categorizing Example Procedures".

2.2.3.2 Monitoring

Peri- and post-procedural monitoring is an essential activity to ensure animal welfare (e.g., Weary et al., 2006), as increased monitoring can reduce potential welfare impacts through early detection. The frequency and intensity of monitoring should be commensurate with the expected impact of the procedure and should be determined as part of the process to establish humane intervention points (*CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* (CCAC, 2022)). Similarly, the quality of the monitoring should be taken into account. For example, a surgical procedure conducted using advanced monitoring technologies (see the CCAC guidelines on experimental procedures (part B – analgesia, anesthesia, and surgery) (in prep.)) should be given a better score than one done without these additional resources.

The act of observing animals may also have a negative impact (e.g., if the cage lid has to be removed or the animals have to be handled; even the increased presence of a human can have a negative impact). The extent of this impact is likely to be species- or even individual-specific. While there are tools that can mitigate this impact (e.g., cameras, advanced movement-tracking software), generally, a minimum Category of Welfare Impact B should be assigned when in-person monitoring is to be performed.

2.2.3.3 Humane Killing Method

The method of killing can have a profound impact on an animal's welfare. The *CCAC guidelines on: euthanasia of animals used in science* (CCAC, 2010b) and the *Additional information on effects of euthanasia methods on research results: Addendum to the CCAC guidelines on euthanasia of animals used in science* (CCAC, 2010a) set the standards for acceptable humane killing methods in Canada and provide guidance as to when each method is permissible. In terms of evaluating the welfare impact of this factor, a neutral impact would only be appropriate if the animals are not going to be killed. If they are going to be killed, the impact should be scored in-line with the 'acceptability' of the method as noted in the documents above (i.e., acceptable, conditionally acceptable, not acceptable), relating to the risk that animals may experience pain and distress prior to loss of conscious awareness (i.e., not all of the methods used in practice should be considered 'humane').

2.2.3.4 The Setting in Which the Scientific Activity is Being Conducted

Scientific activities taking place within a clean, controlled setting appropriate to the species and procedures being done should be scored as neutral. However, the potential impact of some procedures may vary based on the physical location of the activity, the technology available, the capacity to intervene in the case of adverse events, and the ability to separate animals. For example, performing surgery in the wild or on a farm, as opposed to in a laboratory, means that there are additional challenges in terms of maintaining asepsis, providing adequate pain relief, monitoring medium-to-long-term recovery, maintaining body temperature, etc. (Hawkins, 2004), all of which may have greater impacts on welfare. Similarly, if aversive procedures are performed on animals in the presence of other animals, the animals who experience the procedure later in the process may be impacted to a greater extent because of the distress cues given off by animals who experience the procedure first (e.g., Boissy et al., 1998; Inagaki et al., 2014).

2.2.3.5 Skill and Experience of the Personnel

The skill and experience of the personnel are a critical part of the animal's experience. While all personnel involved are expected to be competent at handling animals and performing the approved procedures, those with more experience are likely to perform the procedure more efficiently with fewer complications and be better at dealing with any problems that may arise. Note that the level of experience refers to an individual's experience with the specific procedures or with establishing an animal model, in addition to general competence and experience working with the animals in question. If the procedure is novel and technically challenging (e.g., the laboratory or protocol author has not performed it before on the species in question), the animal care committee should recommend that a pilot study be conducted to inform: 1) the expected level of welfare impact; 2) the skill of the person performing the procedure; and 3) potential refinements. When procedures are conducted as part of a training exercise or otherwise by an early learner, a higher score should be assigned. If the person performing the procedure is highly skilled and experienced, this factor should have a neutral impact.

2.2.3.6 Whether or Not the Animal Has Been Habituated or Trained

In some cases, animals may be habituated to a procedure or reoccurring handling event, or trained through positive reinforcement to actively participate in a procedure (e.g., Bassett et al., 2003; Coleman et al., 2008). If implemented appropriately, habituation and training reduce the following impacts: fear, anxiety, and distress associated with procedures; the need to separate animals from their social group to perform a procedure; the need for anesthesia or physical restraint; and aggression towards human handlers (e.g., habituation: Yoshida et al., 2016; Leiner and Fendt, 2011; training: Laule et al., 2003; Prescott et al., 2004; Laule, 2010). Therefore, if animals are habituated, or trained animals are participating in a procedure for a positive reward (and no form of deprivation was used in training), this factor should be considered a Category of Welfare Impact A, which may reduce the welfare impact of the procedure itself. If animals are untrained and not habituated, this factor should not be considered.

2.3 PROCESS FOR EVALUATING OVERALL PROSPECTIVE CATEGORY OF WELFARE IMPACT

Once preliminary categories of welfare impact have been assigned to each step, they are summarized into an overall score for each protocol (or each distinct group of animals within a protocol). For the purposes of this summary, each of the three steps (animals, environment, and procedure) should be considered important areas of potential welfare impact. However, depending on the nature of the scientific activity, any of the three different areas of welfare impact could factor most prominently. Therefore, if any one of these areas of welfare impact has overwhelming importance because the welfare impact is much larger than for the other areas, that area should be given disproportionately more weight in determining the overall prospective Category of Welfare Impact. This summary must be evaluated through the lens of the protocol author's and the animal care committee's collective knowledge and experience, adjusted as necessary, and accompanied by a transparent rationale, as required. In particular, comparison of the prospective and retrospective categories of welfare impact should be a constructive learning exercise which will help inform future prospective categories of welfare impact.

To assist in summarizing welfare impacts into an overall score for each group of animals, an associated automated tool has been developed, the Automated CoWI Scoring Summary. **It is not mandatory to use the tool.**

2.4 SUMMARY OF GENERAL PRINCIPLES FOR PROSPECTIVELY ASSIGNING CATEGORIES OF WELFARE IMPACT

1) There are many potential avenues for animal welfare to be affected when the animals are involved in a scientific activity, broadly captured by looking at:
   - attributes of the environment
   - attributes of the animals
   - attributes of the scientific procedures

   These three categories have been further broken down into 16 different factors for consideration when determining the categories of welfare impact:
   - housing and husbandry
   - relocation and transportation
   - species, strain, and sex of the animals
   - age and developmental stage of the animal
   - whether the animal is domesticated or non-domesticated
   - inherent welfare status of the animal
   - energy status or body condition
   - social disruption
   - the previous experiences of the animal (cumulative lifetime experiences)
   - known aspects of individual animals
   - the scientific procedures
   - monitoring
   - humane killing method
   - the setting in which the scientific activity is being conducted
   - skill and experience of the personnel
   - whether or not the animal has been habituated or trained
2) The assigned Category of Welfare Impact must reflect the cumulative lifetime experience of the animals. This is especially important for animals who are used repeatedly on different protocols or are held long-term.
3) Each protocol should be divided into groups of animals who experience approximately equal welfare impacts. As many categories as needed must be assigned to any given protocol. Thus, protocols must not be assigned a single Category of Welfare Impact based on the highest impact that any one animal may experience. For example, if the experience of control animals differs from animals in the treatment groups, this must be indicated by assigning two (or more) different categories of welfare impact to the protocol. If there are multiple different scientific treatments being tested, they only need to be assigned different categories of welfare impact if the animals' experiences quantitatively differ (i.e., each treatment group doesn't need to be assigned separately if the impacts are equal in magnitude).
4) The animal care committee is ultimately responsible for ensuring the appropriate categories of welfare impact are assigned.

3 RETROSPECTIVE ASSIGNMENT

This section describes how the categories of welfare impact must be reassessed after the scientific activity has taken place, most often at the time of protocol renewal or completion.

3.1 PROCESS FOR RETROSPECTIVELY DETERMINING THE CATEGORY OF WELFARE IMPACT

A suggested framework for retrospectively determining the Category of Welfare Impact is to consider each factor described in Section 2 and evaluate whether the predicted welfare impact of that factor was accurate. It is likely that most of the factors will have been assessed accurately and the protocol author and animal care committee should focus the retrospective assessment on factors where the welfare impact was different than expected. Ideally, review of this information will coincide with the protocol renewal process. The animal care committee is ultimately responsible for confirming the retrospective Category of Welfare Impact assigned by the protocol author.

The purpose of the retrospective process is to answer the question, "did what was predicted to happen actually happen?" The retrospective Category of Welfare Impact should therefore be based on the prospective Category of Welfare Impact, and modified (either up or down) as necessary, using information from the following sources:

- Husbandry and health records, including mortality records and animal incident reports (see *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017))
- Environmental monitoring records (*CCAC guidelines: Husbandry of animals in science* (CCAC, 2017))
- Welfare assessment records (see *CCAC guidelines: Animal welfare assessment* (CCAC, 2021))
- Post-approval monitoring reports (see *CCAC policy statement for: senior administrators responsible for animal care and use programs* (CCAC, 2008))
- Protocol author self-reports (see *CCAC policy statement on: terms of reference for animal care committees* (CCAC, 2006))

The goal of this step is for the protocol author to reflect on this information and determine whether the welfare impact to the animals was greater than, equal to, or lesser than expected, and modify the category as appropriate. If the welfare impacts are greater than were expected, this should be communicated to the animal care committee (e.g., during the protocol renewal process), and additional follow-up may be warranted (see *CCAC guidelines: Animal welfare assessment* (CCAC, 2021)). If the protocol's welfare impact was higher than expected, yet not so large as to warrant a shift in the Category of Welfare Impact, the areas of unanticipated welfare impact should still be noted and corrected or accounted for in the future. If everything went according to plan and the welfare impact was as expected, deferring to predicted outcomes is acceptable. Finally, instances where the impact was less than expected should be highlighted for the animal care committee as a method of improving prospective assignment and promoting the Three Rs.

REFERENCES

More information about documents marked "in prep." can be found in the Guidelines section of the CCAC website.

Abou-Ismail U., Burman O., Nicol C. and Mendl M. (2008) Let sleeping rats lie: does the timing of husbandry procedures affect laboratory rat behaviour, physiology, and welfare? *Applied Animal Behaviour Science* 111:329-341.

Arts J., Kramer K., Arndt S. and Ohl F. (2012) The impact of transportation on physiological and behavioral parameters in Wistar rats: implications for acclimatization periods. *Institute for Laboratory Animal Research Journal* 53:E82-98.

Balcombe J., Barnard N. and Sandusky C. (2004) Laboratory routines cause animal stress. *Journal of the American Association for Laboratory Animal Science* 43:42-51.

Bassett L., Buchanan-Smith H., McKinley J. and Smith T. (2003) Effects of training on stress-related behaviour of the common marmoset (*Callithrix jacchus*) in relation to coping with routine husbandry procedures. *Journal of Applied Animal Welfare Science* 6:221-233.

Boissy A., Terlouw C. and Le Neindre P. (1998) Presence of cues from stressed conspecifics increases reactivity to aversive events in cattle: evidence for the existence of alarm substances in urine. *Physiology and Behavior* 63:489-495.

Boulanger Bertolus J., Nemeth G., Makowska J. and Weary D. (2015) Rat aversion to sevoflurane and isoflurane. *Applied Animal Behaviour Science* 164:73-80.

Buehr M., Hjorth P. and Hansen A. (2014) Genetically modified laboratory animals – what welfare problems do they face? *Journal of Applied Animal Welfare Science* 6:319-338.

Broom D. (2005) The effects of land transport on animal welfare. *Revue scientifique et technique-Office international des epizooties* 24:683-691.

Brydges N., Boulcott P., Ellis T. and Braithwaite V. (2009) Quantifying stress responses induced by different handling methods in three species of fish. *Applied Animal Behaviour Science* 116:295-301.

Cait J., Cait A., Scott R., Winder C. and Mason G. (2022) Conventional laboratory housing increases morbidity and mortality in research rodents: results of a meta-analysis. *BMC Biology* 20:15.

Canadian Council on Animal Care – CCAC (2006) *CCAC policy statement on: terms of reference for animal care committees*. Ottawa ON: CCAC (accessed on 2024-01-11).

Canadian Council on Animal Care – CCAC (2008) *CCAC policy statement for: senior administrators responsible for animal care and use programs*. Ottawa ON: CCAC (accessed on 2024-01-11).

Canadian Council on Animal Care – CCAC (2010a) *Additional information on effects of euthanasia methods on research results: Addendum to the CCAC guidelines on euthanasia of animal used in science*. Ottawa ON: CCAC (accessed on 2024-01-11).

Canadian Council on Animal Care – CCAC (2010b) *CCAC guidelines on: euthanasia of animals used in science*. Ottawa ON: CCAC (accessed on 2024-01-11).

Canadian Council on Animal Care – CCAC (2017) *CCAC guidelines: Husbandry of animals in science*. Ottawa ON: CCAC (accessed on 2024-01-11).

Canadian Council on Animal Care – CCAC (2021) *CCAC guidelines: Animal welfare assessment*. Ottawa ON: CCAC (accessed on 2024-01-11).

Canadian Council on Animal Care – CCAC (2022) *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints*. Ottawa ON: CCAC (accessed on 2024-01-11).

Castelhano-Carlos M. and Baumans V. (2009) The impact of light, noise, cage cleaning and in-house transportation on welfare and stress of laboratory rats. *Laboratory Animals* 43:311-327.

Cattet M., Boulanger J., Stenhouse G., Powell R. and Reynolds-Hogland M. (2008) An evaluation of long-term capture effects in ursids: implications for wildlife welfare and research. *Journal of Mammalogy* 89:973-990.

Chaby L., Cavigelli S., White A., Wang K. and Braithwaite V. (2013) Long-term changes in cognitive bias and coping responses as a result of chronic unpredictable stress during adolescence. *Frontiers in Human Neuroscience* 7:328.

Christian L. (2012) Psychoneuroimmunology in pregnancy: immune pathways linking stress with maternal health, adverse birth outcomes, and fetal development. *Neuroscience and Biobehavioral Reviews* 36:350-361.

Coleman K. (2012) Individual differences in temperament and behavioural management practices for nonhuman primates. *Applied Animal Behaviour Science* 137:106-113.

Coleman K., Pranger L., Maier A., Lambeth S., Perlman J., Thiele E. and Schapiro S. (2008) Training rhesus macaques for venipuncture using positive reinforcement techniques: a comparison with chimpanzees. *Journal of the American Association for Laboratory Animal Science* 47:37-41.

Detillion C., Craft T., Glasper E., Prendergast B. and DeVries A. (2004) Social facilitation of wound healing. *Psychoneuroendocrinology* 29:1004-1011.

DeBoer M. (2009) Animal models of anorexia and cachexia. *Expert Opinion on Drug Discovery* 4:1145-1155.

Dickens M., Earle K. and Romero M. (2009) Initial transference of wild birds to captivity alters stress physiology. *General and Comparative Endocrinology* 160:76-83.

Ericsson M., Fallasharoudi A., Bergquist J., Kushnir M. and Jensen P. (2014) Domestication effects on behavioural and hormonal responses to acute stress in chickens. *Physiology & Behaviour* 133:161-169.

Feenders G. and Bateson M. (2011) Hand-rearing reduces fear of humans in European starlings, *Sturnus vulgaris*. *PLoS ONE* 6:e17466.

Gregory N. (2008) Animal welfare at markets and during transport and slaughter. *Meat Science* 80:2-11.

Gregory N. and Robins J. (1998) A body condition scoring system for layer hens. *New Zealand Journal of Agricultural Research* 41:555-559.

Griebel G., Belzung C., Perrault G. and Sanger D. (2000) Differences in anxiety-related behaviours and in sensitivity to diazepam in inbred and outbred strains of mice. *Psychopharmacology* 148:164-170.

Hannibal D., Bliss-Moreau E., Vandeleest J., McCowan B. and Capitation J. (2016) Laboratory rhesus macaque social housing and social changes: implications for research. *American Journal of Primatology* 79:e22528.

Hawkins P. (2004) Bio-logging and animal welfare: practical refinements. *Memoirs of National Institute of Polar Research, Special Issue* 58:58-68.

Hughes C., Settle A. and Boice R. (1976) Four indices of domestication in Norway Rats. *Bulletin of the Psychonomic Society* 8:171-174.

Hughes J. (2008) Anaesthesia for the geriatric dog and cat. *Irish Veterinary Journal* 61:380.

Inagaki H., Kiyokawa Y., Tamogami S., Watanabe H., Takeushi Y. and Mori Y. (2014) Identification of a pheromone that increases anxiety in rats. *Proceedings of the National Academy of Sciences of the United States of America* 111:18751-18756.

Isgor C., Kabbaj M., Akil M. and Watson S. (2004) Delayed effects of chronic variable stress during peripubertal-juvenile period on hippocampal morphology and on cognitive and stress axis functions in rats. *Hippocampus* 14:636-648.

Jaggi A., Jain V. and Singh N. (2011) Animal models of neuropathic pain. *Fundamental and Clinical Pharmacology* 25:1-28.

Johansson B. and Ohlsson A-L. (1996) Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. *Experimental Neurology* 139:322-327.

Künzl C., Kaiser S., Meier E. and Sachser N. (2003) Is a wild mammal kept and reared in captivity still a wild animal? *Hormones and Behavior* 43:187-196.

Laule G. (2010) Positive reinforcement training for laboratory animals. In: *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals*, 8th Edition (Hubrecht R. and Kirkwood J., eds.), Chapter 16, pp.206-216. Oxford GB: Wiley-Blackwell.

Laule G., Bloomsmith M. and Schapiro S. (2003) The use of positive reinforcement training techniques to enhance the care, management, and welfare of primates in the laboratory. *Journal of Applied Animal Welfare Science* 6:163-173.

Leiner L. and Fendt M. (2011) Behavioural fear and heart rate responses of horses after exposure to novel objects: effects of habituation. *Applied Animal Behaviour Science* 131:104-109.

Lupien S., McEwen B., Gunnar M. and Heim C. (2009) Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience* 10:434-445.

Makowska J. and Weary D. (2016) The importance of burrowing, climbing and standing upright for laboratory rats. *Royal Society Open Science* 3:160136.

McEwen B. and Morrison J. (2013) The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. *Neuron* 79:16-29.

Mogil J., Chesler E., Wilson S., Juraska J. and Sternberg W. (2000) Sex differences in thermal nociception and morphine antinociception in rodents depend on genotype. *Neuroscience and Biobehavioral Reviews* 24:375-389.

Nash R., Valencia A. and Geffen A. (2006) The origin of Fulton's condition factor – setting the record straight. *Fisheries* 31:236-238.

Novak M. and Suomi S. (1991) Social interaction in nonhuman primates: an underlying theme for primate research. *Laboratory Animal Science* 41:308-314.

Patterson-Kane E., Hunt M. and Harper D. (2002) Rats demand social contact. *Animal Welfare* 11:327-332.

Pedersen V. and Jeppesen J. (1990) Effects of early handling on later behaviour and stress responses in the silver fox (*Vulpes vulpes*). *Applied Animal Behaviour Science* 26:383-393.

Prescott M., Morton D., Anderson D., Buckwell A., Heath S., Hubrecht R., Jennings M., Robb D., Ruane B., Swallow J. and Thompson P. (2004) Refining dog husbandry and care. Eighth report of the BVAAWF/FRAME/RSPCA/UFAW joint working group on refinement. *Laboratory Animals* 38 (Suppl 1):1-94.

Price E. (1999) Behavioural development in animals undergoing domestication. *Applied Animal Behaviour Science* 65:245-271.

Rault J-L. (2012) Friends with benefits: social support and its relevance for farm animal welfare. *Applied Animal Behaviour Science* 136:1-14.

Rawski M. and Józefiak D. (2014) Body condition scoring and obesity in captive African side-necked turtles (*Pelomedusidae)*. *Annals of Animal Science* 14:573-584.

Roche J., Friggens N., Kay J., Fisher M., Stafford K. and Berry D. (2009) Invited review: body condition score and its association with dairy cow productivity, health, and welfare. *Journal of Dairy Science* 92:5769-5801.

Romeo R. (2010) Adolescence: a central event in shaping stress reactivity. *Developmental Psychobiology* 52:244-253.

Roughan J. and Flecknell P. (2003) Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *European Journal of Pain* 7:397-406.

Rushen J. (1986) Aversion of sheep to electro-immobilization and physical restraint. *Applied Animal Behaviour Science* 15:315-324.

Santurtun E. and Phillips C. (2015) The impact of vehicle motion during transport on animal welfare. *Research in Veterinary Science* 100:303-308.

Schwartzkopf-Genswein K., Faucitano L., Dadgar S., Shand P., Gonalez L. and Crowe T. (2012) Road transport of cattle, swine, and poultry in North America and its impact on animal welfare, carcass and meat quality: a review. *Meat Science* 92:227-243.

Sterlemann V., Ganea K., Lieble C., Harbich D., Alam S., Holsboer F., Müller M. and Schmidt M. (2008) Long-term behavioural and neuroendocrine alterations following chronic social stress in mice: implications for stress-related disorders. *Hormones and Behavior* 53:386-394.

Ullman-Culleré M. and Foltz C. (1999) Body condition scoring: a rapid and accurate method for assessing health status in mice. *Laboratory Animal Science* 49:319-323.

Walker M. and Mason G. (2011) Female C57BL/6 mice show consistent individual differences in spontaneous interaction with environmental enrichment that are predicted by neophobia. *Behavioural Brain Research* 224:207-212.

Weary D., Niel L., Flower F. and Fraser D. (2006) Identifying and preventing pain in animals. *Applied Animal Behaviour Science* 100:64-76.

Wolfensohn S., Sharpe S., Hall I., Lawrence S., Kitchen S., and Dennis M. (2015) Refinement of welfare through development of a quantitative system for assessment of lifetime experience. *Animal Welfare* 24:139-149.

Yoshida K., Mimura Y., Ishihara R., Nishida H., Komaki Y., Minakuchi T., Tsurugizawa T., Mimura M., Okano H., Tanaka K. and Takata N. (2016) Physiological effects of a habituation procedure for functional MRI in awake mice using a cryogenic radiofrequency probe. *Journal of Neuroscience Methods* 274:38-48.

GLOSSARY

Affective state – a psychologically experienced state that can be positive or negative to the subject and may vary in both intensity and duration.

Conspecifics – animals belonging to the same species.

Cumulative endpoints – the points at which individual animals should be considered to have reached their lifetime maximum involvement in scientific activities.

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

Domesticated – adapted over several generations (as by selective breeding) from a wild state to life in close association with and to the benefit of humans.

Habituation – a decrease in response to a stimulus after repeated presentations.

Humane intervention points – the pre-established criteria (e.g., observable health impacts, physiological changes, behavioural signs) that indicate when an intervention (e.g., supportive care, analgesia, euthanasia) should occur in order to reduce welfare impacts to a level that has been approved by the animal care committee.

Husbandry – all aspects of the care and management of animals in scientific facilities: laboratory, farm, and aquatic.

Mortality – loss of life; death.

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

Procedure – the part of the scientific activity specifically related to data collection (research and testing), or hands-on demonstration or interaction with animals (teaching and training). For example, this would not include routine husbandry activities such as cage cleaning.

Protocol author – the person who is ultimately responsible for the work performed under the protocol. Frequently, this person is the primary investigator, but may also be the course instructor or testing lead. The protocol author may delegate tasks to other members of the scientific team (e.g., graduate students, postdoctoral fellows), but must always be considered responsible for the protocol.

Scientific activity – includes all aspects of any research, teaching, training, or testing activities.

Scientific endpoints – the earliest points at which the approved objectives of the scientific activity can be achieved while also ensuring that the welfare impacts experienced by the animals are minimized. When the scientific endpoints are reached, the approved live animal use is complete.

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

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

Veterinarian – the person ultimately responsible for the welfare of the animals. Veterinarians should be independent of the scientific team.

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.