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Canadian Council on Animal Care
Conseil canadien de protection des animaux

CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints

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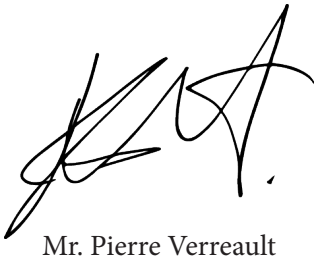

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Identification of scientific endpoints, humane intervention points, and cumulative endpoints

PREFACE

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

The *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* is part of a series of general guidelines documents that outline principles for the ethical care and use of all animals in science. This series streamlines information for investigators, study directors, instructors, animal care committees, facility managers, veterinarians, and animal care personnel to help facilitate improvement in both the care given to animals and how experimental procedures are carried out. More specific information on humane interventions can be found in the CCAC guidelines developed for specific types of animals.

This guidelines document applies to all animals used for scientific purposes, including wildlife brought into laboratory animal facilities and third-party-owned animals that are used off-site (e.g., at commercial farms or shelters).

These guidelines describe current standards and processes for identifying scientific endpoints, humane intervention points, and cumulative endpoints. The individual guideline statements in the document have been developed based on expert peer advice and current interpretation of scientific evidence.

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

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

LIST OF GUIDELINE STATEMENTS IN THIS DOCUMENT

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

2. PROCESS FOR SETTING AND MONITORING SCIENTIFIC ENDPOINTS AND HUMANE INTERVENTION POINTS

Guideline 1

The scientific endpoints, humane intervention points, and monitoring regime must be described in a protocol and approved by an animal care committee before commencing any animal-based scientific activity. This information should be easily accessible to everyone working with the animals.

Section 2.1 Before Starting the Scientific Activity, p.7

Guideline 2

When there is insufficient evidence to establish scientific endpoints prospectively, pilot studies must be conducted to identify the earliest point that the scientific activity can be terminated. Pilot studies must focus on determining welfare-appropriate endpoints, not on generating useable scientific data. The results of the pilot must be presented to the animal care committee before the protocol proceeds.

Section 2.1.1 Choose the Scientific Endpoints, p.7

Guideline 3

Animals must be monitored for the duration of the protocol. Interventions must be applied when animals reach a humane intervention point. To safeguard animal welfare, the chosen humane intervention points and scientific endpoints may need to be adjusted during a scientific activity; changes to these points should be incorporated as amendments to protocols.

Section 2.2 During the Scientific Activity, p.10

Guideline 4

A review of the effectiveness of the humane intervention points and scientific endpoints should occur when a scientific activity is completed. Any potential refinements should be included in future protocols (including renewals) and standard operating procedures (SOPs).

Section 2.3 After the Scientific Activity is Complete: Retrospective Analysis, p.12

3. CUMULATIVE ENDPOINTS

Guideline 5

Cumulative endpoints must be considered for all animals held long-term and for animals that have multiple scientific experiences, as described by the policy set by the animal care committee. These animals must have lifetime experience records that are updated as necessary and reviewed at regular intervals. The current welfare status of each animal should also be assessed regarding its continued use in science (including teaching and training) before protocol renewal or approval of the use of each animal in a new protocol.

Section 3.1 General Guidance on Decision-Making Regarding Cumulative Endpoints, p.14

Guideline 6

In certain types of studies (e.g., longevity studies), cumulative endpoints must inform the scientific endpoints as there is the potential for the cumulative endpoints to be reached before a desired scientific endpoint.

Section 3.2.3 Aging and Longevity Studies, p.17

1 INTRODUCTION

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

Animals may have their welfare compromised during scientific activities (i.e., research, teaching, training, and testing) conducted in the pursuit of benefits to humans, animals, or the environment. These guidelines aim to provide information so that animal care committees, animal health professionals, researchers, and instructors can work collaboratively to reduce potential welfare impacts through the judicious use of scientific endpoints, humane intervention points, and cumulative endpoints.

The term 'scientific endpoints' describes the earliest points at which the stated objectives of the scientific activity will be reached (e.g., collection of data or biological materials over a predetermined time, achieving learning outcomes). Protocol authors have an ethical responsibility to identify the earliest possible scientific endpoints to reduce the welfare impact to the animals; this includes recognizing when the scientific activity is not working as intended and thus should be halted. There remains a clear need for validation of early predictors of the scientific endpoint in many research models. Protocol authors must stay current with validated scientific endpoints in their field of study and include them in their protocols. Further, protocol authors are encouraged to refine outcomes that advance validated endpoints within their area of expertise whenever possible by attempting pilot or parallel studies with careful observation and welfare assessments.

In contrast, the term 'humane intervention points' describes criteria (i.e., observable health impacts, physiological changes, or behavioural signs) that when met, require an intervention to address negative welfare states. This term is used instead of 'humane endpoints' to indicate that action is to be taken to protect animal welfare, but this action is **not necessarily euthanasia**. Thus, possible interventions progress in range from changing an animal's physical or social environment; providing supportive care (e.g., suspend handling, give hydration and nutritional support); treating infections with antibiotics; providing pain relief; removing individual animals from the scientific activity temporarily (or permanently if their condition fails to improve); to euthanasia if the welfare impact has exceeded the expected severity, or the scientific benefit no longer justifies the welfare impact. For consistency with the Three Rs, welfare-protecting interventions must occur as early as possible. These intervention points must be documented and tailored to the specific protocol through careful consultation between the protocol author and those charged with attending to the well-being of the animals, and modified as needed. Humane intervention point monitoring may require specialized training, or awareness of any specific concerns, related to the design of the scientific activity, and welfare-monitoring personnel must be deemed competent.

It is important to note that in addition to anticipated humane intervention points, there may be unexpected negative outcomes (e.g., unrelated illness, life support systems failure, unexpected adverse effects of the scientific activity). These may or may not be related to the specific scientific procedure and may warrant

humane interventions before scientific endpoints are achieved. Welfare-monitoring personnel need to be skilled, flexible, and adaptive to ensure humane intervention points are applied in these circumstances.

Appropriately chosen humane intervention points can improve research quality and reproducibility by avoiding secondary complications and pathologies (e.g., increases in blood pressure, gastrointestinal distress, unexplained loss of weight or body condition, changes to blood glucose levels, abnormal behaviour). **Humane intervention points should be reproducible and minimize welfare impacts.** Thus, humane intervention points should be:

- objective and measurable (to reduce ambiguity or observer subjectivity);
- detectable prior to the onset of negative welfare states; and
- based on specific observable health impacts, physiological changes, or behavioural signs[1].

In light of current scientific evidence, these guidelines have been expanded to acknowledge the psychological impact of scientific procedures and other 'whole life' experiences (e.g., social isolation, marking for identification, under-stimulating environments) on animals. Thus, regarding limits to the long-term or repeated involvement of individual animals in scientific activities, these guidelines use the term 'cumulative endpoints'. This term describes the threshold values when procedures should be discontinued and the use of the animals in scientific activities ended, preferably before unexpected welfare impacts are apparent. This threshold value is determined by considering the aggregate impact of all welfare-impacting procedures an animal has experienced over its lifetime (see the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021)). All previous welfare impacts must be considered when thinking about an animal's cumulative lifetime experiences. To ensure that compromises to animal welfare do not exceed those approved by an animal care committee, those involved with the scientific activity and animal care have an ethical obligation to identify as early as possible animals that are no longer coping with life as a scientific subject (e.g., failure to groom, abnormal appearance, loss of appetite, stereotypic behaviour, exaggerated responses to routine procedures, continually fighting restraint).

Animals should not be held indefinitely without a clear purpose and defined plan. If animals will be placed on holding protocols between scientific activities, their prospective use should be reviewed periodically to ensure that they are not being held unnecessarily. Furthermore, the time spent on holding protocols must be included in cumulative endpoint assessments.

It is essential that humane intervention points and scientific endpoints are defined, established, and written into every animal care committee-approved protocol before commencing any scientific work (see *CCAC policy statement on: terms of reference for animal care committees*). It is also important to note that these guidelines apply to all scientific activities within the CCAC's mandate, regardless of the expected welfare impact (see *Requirement for Submitting an Animal Protocol: Addendum to the CCAC policy statement on terms of reference for animal care committees* (CCAC, 2020)).

1 As opposed to only listing negative affective states (e.g., fear and anxiety). Validated indicators are used to infer affective states, and thus a clear description of the indicator should be used to identify the situation and intervention (*CCAC guidelines: Animal welfare assessment* (CCAC, 2021)).

2 PROCESS FOR SETTING AND MONITORING SCIENTIFIC ENDPOINTS AND HUMANE INTERVENTION POINTS

The guidance provided in this section follows typical steps for planning and conducting a scientific activity. It is intended to offer a framework for effective implementation and assessment of scientific endpoints and humane intervention points.

Implementation of scientific endpoints and humane intervention points should follow a cyclical path (Figure 1). Specifically, once the planning and conduct of the scientific activity are complete, there should be reviews of the effectiveness of the chosen scientific endpoints and humane intervention points (see Section 2.3, "After the Scientific Activity is Complete: Retrospective Analysis"). The goal of this review is to incorporate refinements in future iterations of the scientific activity (e.g., when renewing a protocol or planning another trial with similar procedures). Additional guidance regarding cumulative or long-term animal use can be found in Section 3, "Cumulative Endpoints". Examples of implementing scientific endpoints and humane intervention points in specific contexts can be found in Appendix 1.

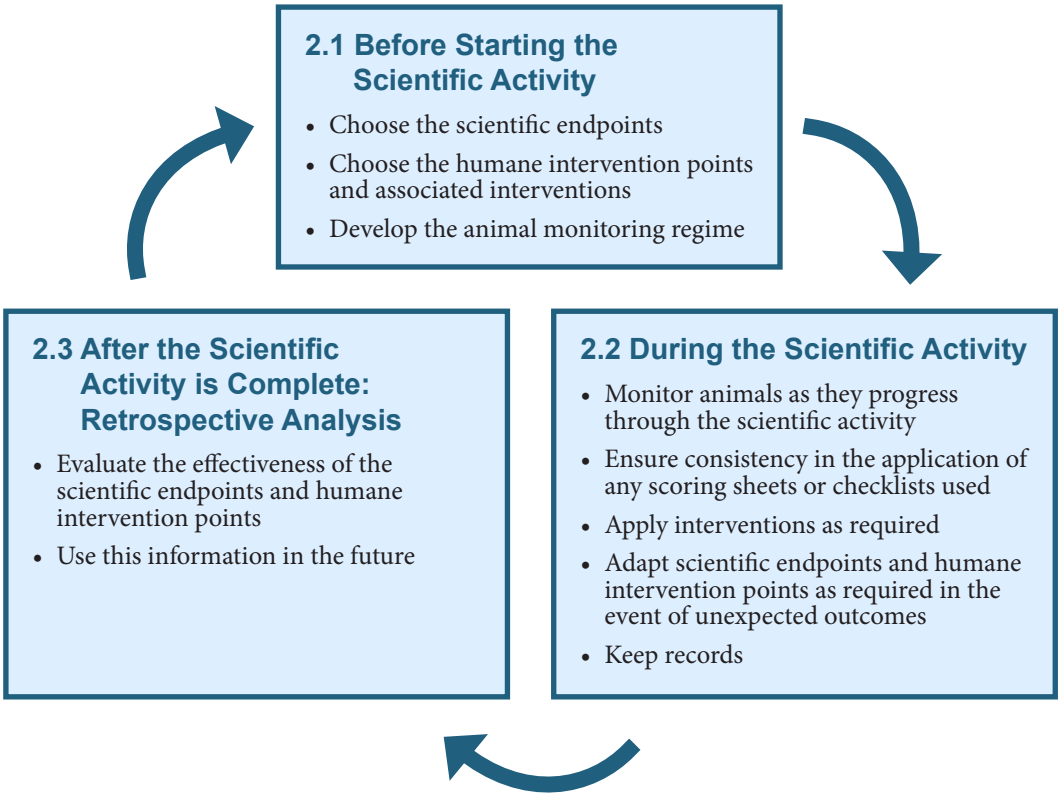

Figure 1: Overview of the process for setting and implementing scientific endpoints and humane intervention points

2.1 BEFORE STARTING THE SCIENTIFIC ACTIVITY

Guideline 1

The scientific endpoints, humane intervention points, and monitoring regime must be described in a protocol and approved by an animal care committee before commencing any animal-based scientific activity. This information should be easily accessible to everyone working with the animals.

2.1.1 Choose the Scientific Endpoints

Guideline 2

When there is insufficient evidence to establish scientific endpoints prospectively, pilot studies must be conducted to identify the earliest point that the scientific activity can be terminated. Pilot studies must focus on determining welfare-appropriate endpoints, not on generating useable scientific data. The results of the pilot must be presented to the animal care committee before the protocol proceeds.

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

The protocol author is responsible for proposing the scientific endpoints, as they are in the best position to determine when the goal of the scientific activity will be reached. However, protocol authors should be expected to justify their chosen scientific endpoints by pointing to relevant scientific literature, pilot studies, correspondence with colleagues, or previous work in their laboratory. In the absence of evidence, a reasonable justification should be made that is consistent with the definition of 'scientific endpoint' above. Where there is insufficient knowledge available to establish endpoint criteria, pilot studies must be conducted to identify the earliest scientific endpoint that will result in minimal welfare impacts for the animals in the full study. Protocol authors should also keep up with all developments of earlier scientific endpoints for their specific field of study (e.g., Collymore et al., 2018; Oliveira et al., 2017). Protocol authors are strongly encouraged to confirm earlier scientific endpoints in the experimental process through validation studies whenever possible (i.e., improve best practices).

Scientific endpoints are based on the scientific question being asked, the objective of the scientific activity, and the approved limits of impact to animal welfare. Scientific endpoints can be described in different terms depending on the specific scientific activity. For example, these endpoints may be described temporally (i.e., a specified duration), in health-related terms (e.g., a limit to disease progression), or in terms of specific objectives (e.g., students achieving learning outcomes, animals completing a certain number of behavioural tests, reaching maximum attainable growth of a tumour). In some cases, analyzing data as the experiment proceeds (rather than at the end) could result in the determination of an earlier scientific endpoint.

Scientific endpoints and their impacts on animal welfare will vary across many factors such as species, procedures, and protocol objectives. This means that the appropriateness of each scientific endpoint should be

weighed within the context of the protocol, and that generic approaches are unlikely to be effective (e.g., due to model, species, strain, or sex differences). In certain types of studies (e.g., longevity studies), cumulative endpoints must also be considered when determining the scientific endpoints (see Section 3.2.3, "Aging and Longevity Studies"). An example of determining scientific endpoints is provided in Appendix 1.

Ultimately, if an animal care committee thinks that the chosen scientific endpoints are not appropriate, the committee must not approve the work as described (see *CCAC policy statement on: terms of reference for animal care committees*). Studies should not be approved if the animals are not likely to reach the scientific endpoint; for example, if it is expected that the animals will have to be euthanized before the stated aims of the scientific activity can be achieved.

2.1.2 Choose the Humane Intervention Points

Definition: Humane intervention points are 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.

Rather than describing when the scientific activity will end, humane intervention points indicate when one should intervene in the scientific activity for humane reasons. Examples of humane intervention points might be 'cats not eating the test diet for more than 48 hours will be provided supplemental canned diet', 'dogs reaching a lameness score of 4 out of 5 will receive analgesia', or 'when wound breakdown occurs, treatment is needed'. Humane intervention points should be established through consultation between scientific and veterinary personnel, referencing scientific literature, pilot studies, or, if necessary, expert opinion. Appropriate interventions should minimally interfere with the scientific aims of the study; interventions that are mutually acceptable to the veterinarian and the protocol author must be sought. Generally, the veterinarian-recommended interventions should be followed unless the protocol author can demonstrate that the action will definitively compromise the integrity of the data (in which case, other interventions must be sought). Consideration should be given to the relative impacts of performing and withholding interventions (Peterson et al., 2017). Ultimately, all humane interventions must be approved by the veterinarian, and if mutually agreeable interventions cannot be found, the protocol must not be approved.

As with scientific endpoints, humane intervention points and associated interventions will be protocol specific and should be evaluated within the context of the described scientific activity. For example, some studies require an impact on animal welfare as part of the model. In such cases, protocol authors and veterinary and animal care personnel should consult with one another to ensure the scientific aims can be achieved with the minimum amount of welfare impact to the animal. In other cases, a much smaller amount of impact might be acceptable before interventions are applied. The end goal of humane intervention points should be to achieve the scientific endpoints with the least amount of impact on animal welfare. Care must also be taken to ensure that the welfare impacts of the interventions themselves are minimized (including impacts on any conspecifics housed with the target individual). It is essential that the scientific team and veterinary personnel work together to decide on humane intervention points before any work commences, so that there are no issues or delays with the appropriate course of action while an animal is experiencing compromised welfare. If the protocol author would like samples to be saved from any animals that have to be euthanized, this should be planned beforehand.

The need to describe humane intervention points applies equally to the use of animals in teaching and training situations. The expectation is that these humane intervention points are described in teaching protocols with as much rigour as in research protocols. Students and trainees are encouraged to learn how to monitor behavioural changes (e.g., decreased mobility and grooming, aggressiveness) that reflect welfare impacts in the animals they are using. However, the responsibility for monitoring and implementing any required humane interventions must always lie with the protocol author (who may or may not be the competent instructor), not the learners.

2.1.3 Organize the Animal Monitoring Regime

A clear plan must be in place regarding the documented monitoring of each animal (or group of animals, as necessary) as it progresses through the scientific activity. The protocol must clearly identify those responsible for monitoring the animals. The responsible individuals must demonstrate knowledge of the clinical signs of any expected negative health conditions or physiological changes and the behavioural signs of impaired welfare for that species. Monitoring animals is a joint responsibility shared by the scientific team, veterinarian, and animal care personnel. Planning, good communication, and transparency are key to effective monitoring and early detection of concerns. The protocol author and veterinary team should develop clear, objective welfare assessment criteria together. Once the animal care committee has approved these criteria, the protocol author must ensure the animal monitoring regime is understood by the animal care personnel and the scientific team. The veterinary personnel, facility manager, or other recognized authority are responsible for ensuring that personnel are trained and deemed competent to follow the animal welfare monitoring regime with consistent application between observers. Furthermore, it must be clear that every individual associated with a scientific activity has the responsibility to draw attention to the need to implement any required humane interventions to minimize animal welfare impacts (however, only competent personnel should perform the intervention). The initial monitoring frequency should be pre-set, with more frequent observations conducted whenever the welfare impact is expected to be high or when there is an increased risk of progressive decline (even if this requires after-hours monitoring). Note: while humane intervention point monitoring may coincide with daily animal health checks, the two are not necessarily the same and may be completed by different personnel at different times.

A scoring sheet (or checklist) is a useful tool to keep track of each animal's progress towards any humane intervention points or relevant scientific endpoints (e.g., Wolfe et al., 2018). If individual tracking is not possible, the smallest possible population unit may be tracked. Scoring sheets are useful because they satisfy several objectives: 1) they promote transparency and accountability; 2) they remove ambiguity regarding 'in the moment' intervention decisions; 3) they promote consistency between multiple observers; 4) when monitoring large numbers of animals, they ensure that scientific endpoints and humane intervention points are implemented consistently for all animals across all studies; and 5) they can be used to inform future monitoring and intervention point decisions. Rigid scoring sheets may not capture all important information, so incorporating some flexibility, for example, through an open comment section, can be beneficial.

Individuals responsible for animal monitoring must be trained to score in a consistent and accurate manner. Institutions may find it helpful to develop generic scoring guidelines that can be adapted to each scientific activity as required. Note: scoring sheets are only one method for consistently documenting monitoring; institutions can fulfill monitoring obligations in various ways (e.g., veterinary medical records). Regardless of how it is done, all monitoring must be documented.

2.2 DURING THE SCIENTIFIC ACTIVITY

Guideline 3

Animals must be monitored for the duration of the protocol. Interventions must be applied when animals reach a humane intervention point. To safeguard animal welfare, the chosen humane intervention points and scientific endpoints may need to be adjusted during a scientific activity; changes to these points should be incorporated as amendments to protocols.

2.2.1 Monitor Animals Throughout the Scientific Activity

Information about humane endpoints and planned interventions must be easily accessible to all stakeholders (e.g., located near the animals). All animals must be monitored according to the approved plan described in the protocol. If adverse circumstances arise, increased monitoring frequency may be required for some animals. Every animal's status should be recorded each time it is assessed (according to the scoring sheet, checklist, medical file, or other tool approved by the animal care committee). These records must be available to all relevant stakeholders (e.g., veterinarians, animal health technicians, facility managers, scientific team members; *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017)).

2.2.2 Ensure Consistency in the Application of the Scoring Sheet or Checklist (Inter-Observer Reliability)

Consistency is the key to effective use of the scoring sheet (or other tool that is employed), to ensure that variation in the application of the scoring sheet does not lead to unforeseen welfare states in the animals or undesirable scientific outcomes. It is imperative that, in cases where more than one individual is responsible for monitoring the animals, all individuals interpret the criteria in the same way (i.e., that they have good inter-observer reliability). Thus, in addition to being well-trained initially, individuals responsible for monitoring animals should have periodic consultations to ensure that they are observing animals in a consistent manner. Inter-observer reliability can be verified periodically by quantifying the agreement between observers; this is particularly important during weekends, holidays, or personnel illness, when persons on duty may not have the depth of knowledge that the regular technical personnel may have. A detailed record of the current animal concerns is invaluable in such cases.

2.2.3 Apply Interventions as Required

If a humane intervention is warranted, there must be no debate or uncertainty about the correct course of action for anticipated welfare compromises. Approved humane intervention points must be followed.

The person responsible for monitoring the animals may or may not be the same person applying the intervention. To some extent, this will be decided by the intervention itself (e.g., if an intervention specifically requires a veterinarian to perform it). However, the veterinarian and the protocol author should be promptly notified that an intervention has taken place.

In some cases, based on animal observations, the predetermined intervention points may need to be re-evaluated and modified during a scientific activity. Any such changes must be documented through the protocol amendment process and subsequently communicated to all stakeholders.

Occasionally, interventions may be necessary in extenuating circumstances without prior veterinary approval (e.g., euthanasia of an animal that is *in extremis*). However, interventions without prior veterinary approval should only take place in exceptional circumstances, not as a standard practice, and should be done using approved methods. The veterinarian must be informed after the fact, and this information should be used to prevent similar instances in the future.

2.2.4 Adapt Scientific Endpoints and Humane Intervention Points as Required in the Event of Unexpected Outcomes

Scientific activities do not always proceed according to the design in the approved protocol; thus, guidance is necessary to adapt to unforeseen circumstances. Protocol authors should have the discretion to implement earlier scientific endpoints as appropriate and terminate their scientific activity (e.g., if they have sufficient data earlier than planned). However, any other changes to the protocol must be approved by the animal care committee through an amendment prior to implementation.

In terms of responding to animals in a state of compromised welfare not explicitly planned for, the veterinary personnel, protocol author, and if necessary, the animal care committee chair, should consult with each other as soon as possible so that a timely intervention can be applied. In extreme cases, if the protocol author cannot be reached immediately, the veterinarian or their delegate, as the ultimate authority on animal welfare (see *CCAC policy statement on: terms of reference for animal care committees*), must apply the best intervention, based on their professional judgement.

2.2.5 Keep Records

It is important to keep monitoring records and records of the interventions that are applied to each animal (or group of animals). While the scientific activity is ongoing, records must be stored near the animals or electronically such that they can be easily accessed from the animal facility and by members of the scientific team. These records serve the following objectives: 1) demonstrate accountability for those assigned to monitor the animals; 2) provide a tool for evidence-based decision-making; and 3) inform any necessary cumulative endpoint decisions. Monitoring records can inform future monitoring plans; for example, by showing at what point an increased frequency of observation was required. Similarly, records of interventions can provide evidence for the effectiveness of those interventions by indicating whether the treated animals later reached the scientific endpoint. These records may also prove useful to scientists if, for example, any treated animals produced outlier data. Because record keeping is invaluable in the implementation of evidence-based humane intervention points in the future, records should be kept in a manner easily accessible by scientific and veterinary personnel for at least one year after disposition of the animals (*CCAC guidelines: Husbandry of animals in science* (CCAC, 2017)).

2.3 AFTER THE SCIENTIFIC ACTIVITY IS COMPLETE: RETROSPECTIVE ANALYSIS

Guideline 4

A review of the effectiveness of the humane intervention points and scientific endpoints should occur when a scientific activity is completed. Any potential refinements should be included in future protocols (including renewals) and standard operating procedures (SOPs).

2.3.1 Evaluate the Effectiveness of the Scientific Endpoints and Humane Intervention Points

Protocol authors should evaluate the effectiveness of the chosen scientific endpoint post hoc (i.e., was the desired data obtained in the originally estimated timeframe?). If the scientific endpoint was not reached as expected, specific barriers or problems should be noted, and potential solutions should be identified. Similarly, even if the scientific endpoint was reached as expected, the protocol author should be encouraged to think about how the data could have been collected with fewer negative impacts on the animals.

Whenever implemented, the effectiveness of the humane intervention points and associated interventions should be assessed by looking at the records (see Section 2.2.5, "Keep Records") and answering the following questions:

- **Timing**: Was the timing of humane intervention points appropriate to lessen the negative impact on the animals? Did animals receive an intervention when they needed it (i.e., was the monitoring frequency sufficient)? If not, why?
- **Consistency**: Were the interventions applied consistently by all the observers? If not, why?
- **Minimizing welfare impacts:** Was the intervention successful at restoring or protecting the welfare of the animals? If not, why?
- **Data integrity**: Did the humane intervention points preserve the quality and integrity of the data or other scientific outcomes? If not, why?
- **Refinements**: How can the chosen humane intervention points be refined? What data could be collected in the future to inform an improvement?

In addition, the professional opinion and insights of the individuals monitoring the animals and those applying interventions should be included. This information should be communicated to the protocol author, who should summarize the important points for the animal care committee during the protocol summary or renewal process.

If the chosen humane intervention points are deemed ineffective in minimizing animal welfare impacts, it is imperative to understand why. Should the interventions be implemented differently in the future, or should they be discontinued? In such cases, the animal care committee should be informed of the retrospective analysis results directly or through the existing protocol amendment or renewal processes, and should collaborate with the protocol author and veterinarians to find solutions.

2.3.2 Use this Information in the Future

The practice of setting scientific endpoints and humane intervention points should undergo continual refinement. These data and experiences can be used to improve animal welfare while ensuring no loss in scientific quality. The following actions must take place during the annual protocol renewal and at the end of each scientific activity:

1) Protocol authors must perform a retrospective analysis comparing the predicted welfare impacts to the actual welfare impacts experienced by the animals (see the CCAC guidelines on categories of welfare impact (in prep.)).
2) Procedures should be refined and updated to lessen welfare impacts, ideally based on evidence recorded during monitoring (% morbidity or mortality, weight loss, etc.). These refined procedures should be used on new protocols, amendments, or renewals.
3) Members of the animal care committee must use this information to: a) inform the cumulative endpoints assessment (see Section 3, "Cumulative Endpoints"); b) update SOPs as necessary; and c) evaluate the welfare impact of future protocols. For example, veterinarians or other animal care committee members may recommend a different, more humane intervention in another protocol because they have seen it used effectively in a similar context in the past.

Finally, protocol authors, veterinarians, and animal care personnel should share their successes and failures regarding scientific endpoints and humane intervention points as widely as possible (e.g., in scientific publications, at conferences, through their professional societies). Institutions should strive to create a culture that promotes the open exchange of ideas regarding humane interventions.

3 CUMULATIVE ENDPOINTS

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

An animal is considered to have reached a cumulative endpoint when it has reached a threshold in terms of the total amount of welfare impact it has experienced (Nunamaker et al., 2021). Determining when an animal has reached this threshold requires quantifying the sum of all the experiences that have impacted its welfare (e.g., Honess and Wolfensohn, 2010; Wolfensohn et al., 2015). Cumulative endpoints must be considered for all animals that have been involved in multiple scientific activities, scientific activities of long duration, or scientific activities that contain multiple procedures over time.

The assessment of the cumulative lifetime experiences of an animal should incorporate both measures of physical impact (e.g., tissue trauma, disease, malnutrition) and psychological impacts (e.g., pain, fear, anxiety), as inferred from behavioural observations or tests (Smith et al., 2018; see the CCAC guidelines on categories of welfare impact (in prep.)).

Animals should be subjected to only one severe or high welfare impact experience in their lifetime (a category of welfare impact level D or E; see the CCAC guidelines on categories of welfare impact (in prep.)). Thus, the Three Rs principle of Refinement should be prioritized over Reduction: animals should not be used beyond their cumulative endpoints simply to reduce the total number of animals used (Fenwick and Griffin, 2013). Finally, cost or convenience must not be used as a justification when deciding on cumulative endpoints.

3.1 GENERAL GUIDANCE ON DECISION-MAKING REGARDING CUMULATIVE ENDPOINTS

Guideline 5

Cumulative endpoints must be considered for all animals held long-term and for animals that have multiple scientific experiences, as described by the policy set by the animal care committee. These animals must have lifetime experience records that are updated as necessary and reviewed at regular intervals. The current welfare status of each animal should also be assessed regarding its continued use in science (including teaching and training) before protocol renewal or approval of the use of each animal in a new protocol.

Deciding when an animal has reached a cumulative endpoint should be a collaborative process involving the scientific team, veterinary and animal care personnel, and the animal care committee (e.g., Heiderstadt and Kennett, 2011; Nunamaker et al., 2021). However, since the animal care committee is ultimately responsible for overseeing all aspects of animal use, it is the final authority. The collaborative decisions should be species-specific and evidence-based when possible (or otherwise rely on professional judgement). Potential sources of evidence include records of formal welfare assessments (see the *CCAC guidelines: Animal welfare*

assessment (CCAC, 2021)); physical or health exam records; the list of procedures previously performed on the animal; and the list of planned future procedures (e.g., Smith et al., 2018).

Some examples of specific factors that warrant consideration, as applicable, are:

- species;
- early life experiences such as rearing environment and weaning age;
- the number, duration, frequency, and severity of procedures performed to date – this should also include the methods and frequency of any restraint;
- the physical and chemical characteristics of any administered compound or solution (e.g., whether the repeated injections or the injections of acidic or basic substances induced local irritation and necrosis);
- the routes, volumes, and frequencies of any compound or drug administered;
- the extent of any lasting impacts caused by any negative experiences;
- the interval between procedures – the shorter the interval (usually), the less opportunity the animal has to return to baseline;
- the animal's clinical condition and physical well-being, which should include determination of those factors that influence body weight and body condition;
- how the animal was conditioned (e.g., habituation, positive reinforcement training);
- changes in social structure or separation, single housing of social animals, and group housing of solitary or paired animals;
- the welfare of the animal, as inferred from valid behavioural or physiological indicators (see the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021)); and
- the nature and frequency of interventions and actions that will be taken to relieve any future welfare impacts.

Each institution should create cumulative endpoint SOPs or policies tailored to the species they use and the types of scientific activities they conduct. These SOPs or policies are the responsibility of the animal care committee and should include a description of which animals and which scientific activities they apply to; the criteria to be used for assessing cumulative endpoints; accountability for collecting and organizing the relevant data; the review or assessment intervals; and a decision tree or steps outlined for the decision-making process. The SOPs or policies should also state that cumulative endpoints should be considered when protocols are amended and when animals are transferred between protocols (including when animals are transferred between institutions). Additionally, as part of the regular SOP or policy review process (see *CCAC policy statement on: terms of reference for animal care committees*), institutions should evaluate the effectiveness of implementing cumulative endpoints and make adjustments as necessary.

3.2 ADDITIONAL GUIDANCE FOR COMMON CUMULATIVE ENDPOINT CONTEXTS

While the factors listed in the previous section are generally applicable, there are a number of situations where institutions may benefit from developing specific SOPs or policies to cover the application of cumulative endpoints. These common cumulative endpoint contexts include (but are not limited to) breeding animals, animals used for teaching, and aging or longevity studies.

3.2.1 Breeding Animals

Breeding animals are often held for relatively long periods (species-specific) and are commonly used to produce multiple offspring (concurrently or consecutively). Thus, their cumulative lifetime experience includes all of the aspects of mating, gestation, and parturition, as well as any routine husbandry procedures. Another important consideration is that the experiences of male and female breeders often differ dramatically (e.g., females may experience impaired welfare due to dystocia).

While age is a common factor to use when determining when breeders should be retired, it should not be used alone. Additional factors to consider, as applicable, include:

- phenotype;
- the health of the animal (e.g., body condition);
- number of previous matings or attempted matings;
- number of previous births (or egg-laying events) and interval since last birth (or egg-laying event);
- species-appropriate amount of parental investment that will be required;
- demonstrated ability to care for offspring;
- health and viability of offspring produced; and
- changing social situations.

3.2.2 Teaching and Training

Animals used for teaching and training may be purpose-bred, retired research animals, or volunteered by a third party. Typically, these animals are used intermittently throughout their lifetime, often with long periods of rest between uses. Furthermore, they may be subjected to procedures (including handling or restraint) by students in training. In all cases, each animal's lifetime experience should be considered when deciding whether or not it has reached a cumulative endpoint (as described above), not only its experiences related to the teaching or training protocols (i.e., any previous involvement in research must be considered as well). The following additional factors should be considered when deciding when to retire animals used for teaching or training:

- level of habituation to, or positive association with, humans;
- history of interactions with previous students or trainees (including the welfare impact of those interactions);
- expected skill or experience of upcoming students or trainees;
- number of previous uses and time since last use; and
- potential for welfare impact in future teaching or training activities.

Within their cumulative endpoint SOPs or policies, institutions should describe the maximum length of time that an animal (species-dependent) will be held for teaching or training purposes.

3.2.3 Aging and Longevity Studies

Guideline 6

In certain types of studies (e.g., longevity studies), cumulative endpoints must inform the scientific endpoints as there is the potential for the cumulative endpoints to be reached before a desired scientific endpoint.

Aging and longevity studies present a unique challenge for the implementation of cumulative endpoints. It can be hard to discriminate between the lifetime cumulative effects of challenges to welfare, and the independent biological effects of aging, especially when animals need to progress far enough into old age for the collection of relevant data (e.g., Black et al., 2003; Phillips et al., 2010; Ray et al., 2010). Thus, very clear and detailed cumulative endpoints defining euthanasia criteria are essential for these models. The following factors should be considered when deciding if animals involved in an aging or longevity study should be removed from the study due to reaching a cumulative endpoint:

- physiological, pathological, and behavioural changes expected to occur due to age (e.g., fragility, osteoarthritis, cognitive decline);
- potential for experimental procedures to have a larger welfare impact as animals get older;
- expected timeline for age-related changes to occur;
- potential for changing social situations to impact welfare (e.g., death of social partners); and
- ability of interventions to rectify suspected welfare impairments.

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

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APPENDIX 1
EXAMPLES

This appendix aims to demonstrate how to implement scientific endpoints, humane intervention points, and cumulative endpoints. However, it is not a definitive guide. These five examples are meant to show how the content of the previous sections can be applied. Each example provides broadly applicable information for a particular scenario while demonstrating the type of thinking that would be helpful in similar contexts. Selected references are provided as a starting point for inquiry and are not an exhaustive list.

Furthermore, these examples are not a comprehensive list of possible scenarios or species: protocol authors and animal care committees must tailor the scientific endpoints, humane intervention points, and cumulative endpoints to each animal use protocol. Thus, the ideas presented here should be adapted to specific protocols in combination with scientific evidence and professional judgement.

Finally, protocol authors, with the support of animal care committees, are encouraged to develop, validate, and disseminate new scientific endpoints, humane intervention points, and cumulative endpoints.

1. APPLYING HUMANE INTERVENTION POINTS IN MOUSE MODELS OF CANCER

A substantial number of mice are used each year in the pursuit of treatments for various cancers. As part of the study design, these animals develop tumours that inevitably impact their welfare, often before the scientific endpoint is reached. Thus, there is a need to implement humane intervention points along the way to safeguard animal welfare to the extent possible. The humane intervention points must be tailored to the expected pathology of the specific tumour model: for example, indicators for tumours that grow internally will necessitate a different approach than those that appear externally and are easily visible and measurable (e.g., Paster et al., 2009). However, all of the indicators that can be used for internal tumours (e.g., clinical signs, behavioural changes, body condition scores) can also be used for external tumours. Most of the indicators detailed below provide graded information that allows for a series of humane interventions. Adjustments, starting with early supportive care, should be made as the condition of the animals deteriorates. These adjustments may include increased monitoring, switching to a dietary supplement served on the cage floor, providing analgesia, up to euthanizing the animal. The exact series of interventions should be determined based on the nature of the protocol and the anticipated progressive welfare impacts.

1.1 External Tumours

1.1.1 Tumour Size

The two indicators specific to external tumours are related to the size of the tumours themselves: tumour volume and tumour burden. The volume of each tumour can be calculated as (length*width^2)/2 (Faustino-Rocha et al., 2013), though other similar formulae can be used, depending on the method of measurement. The final intervention point for mature mice should be when any tumour reaches approximately 1.5 cm^3 (Workman et al., 2010).

Tumour burden refers to the mass of the tumours relative to the animal's body weight. The tumour burden is expressed as a percentage of body weight and is calculated as (cumulative tumour weight of all tumours/ baseline body weight)*100. Mouse tumours weigh roughly 1 g/cm^3 (Tomayko and Reynolds, 1989), so the tumour volume values can be used to easily determine the cumulative weight of all tumours. The final intervention point should be when the tumour burden reaches approximately 10% of the animal's body weight (Wallace, 2000).

For some protocols (e.g., when delayed therapeutic effects of a treatment are expected beyond these tumour sizes), tumours reaching these sizes may not automatically necessitate euthanasia if the animal's welfare can be sufficiently maintained through the implementation of alternative humane interventions. Such an extension requires a well-developed understanding of the tumour line and how it impacts animal welfare.

1.1.2 Body Weight

Body weight loss (from a baseline or age-matched controls) is a commonly used metric that can easily be monitored and provides clear points at which various interventions can be applied, up to removing animals from a study at 20% weight loss (Workman et al., 2010). However, if not accounted for, tumour growth may mask losses to healthy body weight, meaning that body weight loss is best used to inform humane intervention points only when the mass of the tumours can be accounted for (Wallace, 2000; Workman et al., 2010).

1.1.3 Ulceration

In the past, ulceration of any degree was commonly used as a humane endpoint. However, some scientific endpoints may require keeping animals past the point of tumour ulceration. In these cases, animals must be monitored very closely and frequently as the potential for impaired welfare is high due to the loss of body fluids, infection, or increased pain and discomfort (Wallace, 2000). Factors that should be considered when applying humane intervention points after ulcer development include size of the ulcer, amount and type of discharge, inflammation around the wound, signs of self-mutilation, behavioural signs of pain, and evidence of healing or response to treatment. Thus, in addition to the humane intervention points captured by the general behaviour and health assessments described below, criteria related directly to the ulcers should be established (Workman et al., 2010). For example, animals with infected ulcers can be given antibiotics, animals with large or exudative ulcers can be put on supportive care to maintain hydration status, and animals with painful or irritating ulcers can be given analgesia. However, if animals fail to improve within a reasonable period of time, euthanasia may be warranted at the veterinarian's discretion.

1.2 Cancer Model – General Behaviour and Health Assessments

The next series of indicators are based on clinical signs and behavioural changes. They can be used to determine when animals reach humane intervention points regardless of the type or location of the tumours. Additionally, they are useful for monitoring the welfare impacts of other types of biomedical research, such as mouse models of infectious or neurodegenerative disease.

1.2.1 Body Condition Score

Body condition scores are a practical and rapidly assessed metric that can be used to inform intervention points. This indicator is advantageous over measuring body weight loss because it is independent of tumour

mass (Paster et al., 2009; Workman et al., 2010). Animals are scored on a 1-5 scale, where 3 is a normal, healthy animal (1 is emaciated and 5 is obese; see Ullman-Culleré and Foltz, 1999 for full details). Once an animal's body condition starts to decline to a 2 or lower, appropriate humane interventions should be applied, culminating in removing animals from the study when they reach a 1. Monitoring animals' food consumption can be beneficial, as prolonged reductions in food consumption may indicate that a humane intervention is necessary.

1.2.2 Welfare Monitoring

Changes in the animal's behaviour, appearance, and health can be monitored for humane intervention points as well (see the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021)). In terms of behaviour, changes in either baseline home cage behaviour or in provoked responses (e.g., to human handling) can indicate a welfare issue (e.g., Paster et al., 2009). Specifically, if animals are becoming less active, spending more time isolated from conspecifics, or are less responsive to human presence or manipulation, an intervention should be applied. Similarly, ascites (see the *CCAC guidelines on: antibody production* (CCAC, 2002)), hypothermia, deteriorating coat condition, hunched posture, increased breathing rate, and eye opening to a lesser degree are all commonly used indicators for humane intervention points (e.g., Aldred et al., 2002; Workman et al., 2010; Paster et al., 2009). Finally, animals must be monitored for additional health and welfare complications that may arise from the tumours such as ulceration and secondary infections, physical interference with necessary functions (e.g., locomotion, eating, drinking), pain upon palpation, and organ failure (e.g., Workman et al., 2010). In each case, the degree of the interventions should be commensurate with the degree of welfare impact experienced by the animal. Furthermore, the frequency of monitoring should increase as the impact on animal welfare increases.

A template for monitoring these indicators has been provided in Appendix 2; however, the criteria to be monitored, the humane intervention points, and appropriate interventions should be tailored to the specifics of each protocol.

2. APPLYING CUMULATIVE ENDPOINTS FOR ANIMALS HELD FOR LONGER TERMS

Some longer-lived animals (e.g., non-human primates, dogs, livestock in veterinary teaching hospitals) are often held for longer periods of time and can be involved in several scientific activities over their lifetime. Thus, establishing cumulative endpoints for these animals is very important, but can be challenging as the individual procedures may cause only minor welfare impacts when evaluated as a single protocol or single procedure (e.g., venipunctures for blood sampling).

As is standard CCAC practice, each group of animals within a protocol must be assigned a category of welfare impact. Furthermore, it is imperative that the welfare impact of each procedure be evaluated retrospectively so that the actual welfare impact can be determined (see the CCAC guidelines on categories of welfare impact (in prep.)). This retrospective welfare assessment should be incorporated into a record of the lifetime experience for each animal. Thus, individual animals should have a cumulative endpoint score that is updated after every procedure (or set of procedures, as appropriate for the scientific activity) throughout its life, based on the sum of its experiences to that point in time. This score can be used to determine, for example, when the animal should be retired from use completely; when a rest period is required (and what its duration should be); and possibly, when positive reinforcement training is needed (e.g., Honess and Wolfensohn, 2010; Wolfensohn et al., 2015).

2.1 Tracking Cumulative Effects

One practical implementation of calculating an animal's cumulative endpoint is to assign each animal a numerical score that corresponds to the category of welfare impact for each protocol that involves the animal in question. This numerical score allows for easier summation over an animal's lifetime. Following the renewal or completion of a protocol, the numerical score assigned to each animal should be approved by the animal care committee (or delegate), based in part on consultation with the scientific team. Each institution should create an internal SOP or policy that describes the type of protocols that would typically warrant each score, with the caveat that the final score (i.e., the actual welfare impact to the animal) can be affected by a number of factors (see the CCAC guidelines on categories of welfare impact (in prep.)), and should be confirmed retrospectively. In general, animals on the same protocol will be scored similarly, but scores may differ between individuals based on treatment groups or individual animal considerations (e.g., difficulty gaining venous access, behaviour or temperament of the individual animal). Animal care personnel and the scientific team should be responsible for identifying any animal showing fearful or otherwise negative behaviours and developing a plan to address these welfare concerns. For example, animals can be given a rest period or enter into an ongoing behavioural modification program such as counter-conditioning and positive reinforcement training. Any animals that are not responsive to these efforts should be removed from the colony or utilized in protocols of a lower welfare impact.

2.2 Multiple Procedure Scoring

In some cases, animals may undergo multiple procedures within a single protocol. In such cases, it is important to account for the welfare impact that the high frequency procedures may have, even if each occurrence has a relatively low impact (e.g., gavage for a mouse or urinary catheterization for a cow). Once a protocol with multiple procedures is given an overall protocol score, the tracking of cumulative endpoints should be as described above. Note that this numerical scoring approach is only a guide: retrospective welfare assessment and professional experience may influence the score (e.g., if more than one needle stick is required at each collection attempt, or if there are other unexpected welfare impacts or temperament issues).

2.3 Rest Periods

Allowing animals sufficient time to recover from procedures, especially those that acutely impact their welfare, is an important safeguard (e.g., Beerda et al., 1997). Therefore, corresponding rest periods are recommended after an animal experiences an impact of any given magnitude. Factors that should be used in determining the length of a rest period include the current welfare state of the animal and the frequency and severity of procedures previously done to it. The time should be counted from the end of the most recent procedure to the start of a subsequent procedure.

2.4 Cumulative Endpoints for Maximum Use

Even if an animal is regularly assessed and appears behaviourally normal (i.e., not demonstrating exaggerated responses to routine procedures, stereotypic behaviour, self-mutilation, or other signs of fear and anxiety), limits should be placed on the animal's involvement in scientific activities. As a general rule, animals should not be subjected to more than one category of welfare impact level D or E experience in their lifetime.

3. APPLYING HUMANE INTERVENTION POINTS IN LABORATORY-HOUSED FISH

Despite a dramatic increase in the use of finfish in biomedical research in recent decades, fish welfare protections continue to lag behind those of other laboratory animals. The reasons for this include, but are not limited to:

1) lack of well-defined, readily observable signs that reliably indicate poor welfare and reliable, clear indicators of pain alleviation (though advances are being made in this area (Sloman et al., 2019));
2) lack of established anesthetic or analgesic regimes, or other humane interventions, capable of reducing pain and other negative welfare states (advances are also being made in this area (Martins et al., 2019));
3) 32,000+ species of finfish with differing physiologies, life histories, environmental needs, resulting in no established, universally accepted standards for husbandry, management, and care of laboratory fish; and
4) use of wild-sourced stocks lacking genetic homogeneity or freedom-from-disease status typically available in commercial animal models.

There has been some resistance in the fish ecology research community to adopt humane interventions other than euthanizing fish in a moribund state. Justifications for this stance include incompatibility with historic research data, concern about pre-empting biologically important effects, or fear of poor translation to a wild fish's experience (if using wild-caught fish). To alleviate such concerns, animal care committees should work with fish researchers in the protocol review process to develop pilot studies where committee- or veterinarian-recommended humane interventions can be tested.

It is important to consider that fish welfare can also be influenced by routine events (including transient ones), not only the conduct of scientific procedures. Stress is a primary factor that affects the health and welfare of fish. Handling is likely to have a much more significant impact on fish than on terrestrial animals for several reasons:

1) fish are unable to breathe when removed from water;
2) fish are exposed to increased gravity and changing pressure (Ditsche and Summers, 2014), which affects blood pressure, swim bladder inflation, and alters the load on the animals' skeletons and musculature (Boglione et al., 2013);
3) fish eyes typically have little to no protection and therefore can be damaged by contact with nets, transfer vessels, counting or measuring devices, etc. (Brydges et al., 2009) – fish can also experience distress due to changes in light intensity when out of the water; and
4) the mucus or slime layer protecting the fish is usually compromised or somewhat removed during handling (Brydges et al., 2009).

Applying humane intervention points to fish is therefore uniquely challenging. Fish are typically held communally at high densities and are usually not individually distinguishable, which precludes assessment of individual fish without removing them from the water. Furthermore, fish are an extremely diverse taxa, so the threshold for each indicator needs to be species and life-stage specific. A useful approach can be to apply humane intervention points to the population (e.g., tank, cage, or pond) as a whole, rather than on each

individual (note: the tank is also likely to be the experimental unit). This approach is likely to adequately address welfare concerns because the welfare of all animals in the tank is highly correlated:

1) the animals within a tank share an identical artificial environment which is often the source of welfare issues (i.e., mechanized life support systems that control essential aspects such as oxygen levels, water temperature, and flow rates (Johansen et al., 2006; Huntingford et al., 2006));
2) the impact of stressors experienced by only a few individuals is easily transmitted to others (e.g., increased pathogen load; Conte, 2004); and
3) chronic stress associated with captivity (e.g., environmental conditions, handling, stocking densities, genetic selection, transport, nutrition) can culminate in immunosuppression and increased susceptibility to infectious disease agents (Håstein et al., 2005).

However, if for some reason, animals within a tank do not have uniform welfare (e.g., for certain species, life stages, or rearing conditions), a welfare assessment should first take place to determine the appropriateness of applying humane intervention points uniformly. Ultimately, to evaluate humane intervention points in practice, it may be prudent to treat the tank as one organism, even if animals within the tank may have had somewhat different experiences.

When necessary, observation of individual fish can be assisted through sedation (if applicable; sedation itself may alter valuable indicators such as ventilation rate) and transfer into a clear water-filled viewing vessel. Remote observations using cameras can enable monitoring of animal behaviour without disturbance. Time-series sub-sampling events provide an opportunity to gain additional information on the overall health of tank populations, as can careful examinations of sentinel fish and post-trial fish that have been euthanized. Protocol authors must use welfare checklists, scoring sheets, or other forms of record keeping (see the *CCAC guidelines: Animal welfare assessment* (CCAC, 2021)) and look for creative opportunities to get additional information on the welfare of the population and identify practically useful humane intervention points.

There are numerous non-specific indicators (e.g., Harper and Lawrence, 2011; Martins et al., 2012; Segner et al., 2012; Reed and Jennings, 2011; Smith, 2014) that suggest a fish population's welfare is deteriorating. Some indicators for humane intervention points could include:

- alterations in typical feeding behaviour (loss of appetite);
- changes to individual or group swimming behaviour (e.g., lack of orientation to flow, altered posture within the water column, darting, jumping, flashing, gasping at the water surface);
- altered responses to disturbance (evidenced by changes to normal startle response);
- changes in behavioural responses to light (either increased sensitivity or a lack of sensitivity);
- aggression (e.g., nipping, charging, bumping);
- physiological changes (e.g., respiration rate, skin colour (blanching in warm-water fish, darkening in cold-water fish));
- diminishing body condition (e.g., Clark et al., 2018);
- visible abnormalities on the body (e.g., scale loss, skin ulcers, necrotic leading edges on the fins) or the eyes (e.g., cloudy eyes); and
- morbidity or mortality rates.

No current biochemical assays are 100% reliable in reflecting stress in fish, but there are some commonly investigated indicators. For example, changes in major plasma ions have been shown to reliably predict im-

pending death in senescent adult salmon prior to observable signs of morbidity (e.g., Jefferies et al., 2011). Other stress markers include increased blood glucose, corticosteroids, and red blood cell counts (e.g., Olsen et al., 2005; Acerete, 2004; Barton, 2002; see the *CCAC guidelines on: the care and use of fish in research, teaching, and testing* (CCAC, 2005) and the *CCAC guidelines: Zebrafish and other small, warm-water laboratory fish* (CCAC, 2020b)). Adherence to best practices in scientific methods, particularly random tank assignments and the use of appropriate controls, can increase the value of biochemical welfare indicators for use as humane intervention points.

Unexpected outcomes are not uncommon in fish research and are frequently the result of suboptimal or poorly understood environmental conditions, or antagonistic interactions between individuals. Once the pre-determined humane intervention thresholds are observed, the appropriate interventions (which will be species, life stage, and facility dependent) should be applied both at the individual level if possible (e.g., separation, treatment of injured animals, or euthanasia) and the level of the experimental unit (e.g., rectifying water quality issues, treatment of diseases or parasites). Medical interventions recommended by veterinarians must be followed unless the protocol author can demonstrate that the action will definitively compromise the integrity of the data (in which case, alternative interventions must be sought). Both during and immediately after an intervention, there should be a rest period from all scientific activities for the entire tank, commensurate with the magnitude of the welfare impact of the stressor and intervention (e.g., Acerete et al., 2004), even if this is not consistent with the scientific objectives. This rest period includes a reprieve from any non-essential handling, which may require deviation from the normal teaching or research routine. Without allowing the entire population sufficient time to recover, welfare issues may compound, quickly leading to higher mortality (e.g., Pickering and Pottinger, 1989).

4. SCIENTIFIC ENDPOINTS AND HUMANE INTERVENTION POINTS FOR MICE IN LONGEVITY STUDIES

Mice are often used to model the effects of various interventions on mammalian longevity due to their shorter lifespans (Flurkey et al., 2007). However, these types of studies present unique challenges in applying humane intervention points, as age-related welfare concerns need to be addressed in a way that does not compromise the scientific objectives. There are two main approaches to protecting animal welfare when conducting scientific work with aging animals: 1) establishing earlier scientific endpoints based on clinical signs, modelled data, or biomarkers of longevity that reliably predict impending death; and 2) applying humane intervention points.

4.1 Choosing Earlier Scientific Endpoints

For most longevity studies, mice can be euthanized at a point where their longevity can be accurately predicted, but their welfare is not severely compromised. For example, Ray et al. (2010) estimated that death could be accurately predicted to within two weeks if geriatric mice are euthanized when there is a 10% reduction from average stable values in the product of body weight and core body temperature. This degree of accuracy results in an underestimation in survival time of only 2%, well within the acceptable range for most studies (Ray et al., 2010). However, it is important to note that for some models, imminent death is better predicted by hypothermia or body weight loss alone (rather than the product of both; see, for example, Trammell and Toth, 2011), so the chosen scientific endpoint must be protocol specific. Note that measuring core body temperature can have a welfare impact (depending on the method used) and may be

time-consuming, especially for larger numbers of animals. It may be more appropriate to measure this less frequently, and only begin to do so regularly once body weight begins to fall.

Alternatively, Robertson et al. (2011) submit that most interventions that influence rodent longevity induce effects that are consistent with proportional hazards models. These models are commonly used in medical research to assess the expected relationship between survival time and various factors of interest. Thus, by increasing the sample size, longevity studies can be truncated long before all animals reach the point of spontaneous death or euthanasia, and survival estimates can subsequently be accurately modelled using the censored data (Robertson et al., 2011).

Finally, protocol authors should consider using appropriate biomarkers as proxy indicators of longevity. For reference, Moeller et al. (2014) have produced a comprehensive guide to validated biomarkers of longevity across multiple inbred strains of mice, including the age at which they are most predictive. Many of the biomarkers are blood-based (e.g., red blood cell count, lymphocytes), but others are hormonal (e.g., thyroxine) or otherwise physiological (e.g., body mass index, heart rate), allowing for a choice of the most appropriate biomarker for the study. Overall, many methods allow for sufficient data to be collected before the spontaneous death of the animals. These alternatives allow for accurate conclusions regarding how most experimental treatments may influence longevity.

4.2 Applying Humane Intervention Points

As mice age, they require a series of interventions aimed specifically at addressing their changing needs. One particularly valuable way of monitoring humane intervention points in older animals is using a frailty index that quantifies accumulations in health deficits over time (Parks et al., 2012; Rockwood et al., 2017). This index incorporates activity levels, body composition, metabolic status, and hemodynamic measures into an overall frailty score that can inform various humane intervention points ranging from increased monitoring to euthanasia.

Changes in the aged animals' behaviour, appearance, or health can be monitored for humane intervention points (see Appendix 1, Section 1, "Applying Humane Intervention Points in Mouse Models of Cancer", for specific information) for many different types of research. However, there are additional changes in an animal's behaviour or physiology specifically related to aging that can indicate a humane intervention is warranted. For example, geriatric mice may have more difficulty building nests (e.g., Filali and Lalonde, 2009; Chen et al., 2005), so they should be provided with materials that are more easily manipulated or preformed nests. Aging mice may have difficulty reaching food and water on the cage lid, or may be increasingly vulnerable to injury during handling, so their changing needs should be accommodated. Finally, very close attention should be paid to body weight and body temperature, as decreases in either variable may necessitate euthanasia (Trammell et al., 2012).

5. HUMANE INTERVENTION POINTS FOR THIRD-PARTY-OWNED ANIMALS (E.G., COMMERCIAL BEEF CATTLE)

Increasingly, scientific work in Canada is being conducted with commercial animals in production settings. This presents unique challenges in establishing and overseeing humane intervention points. Although the process for choosing and applying humane intervention points for commercial animals being used for science should follow a process similar to that of laboratory animals, institutions do not have the same authority when they do not own the animals. This translates to reduced flexibility in how humane intervention

points are managed: animals in commercial production do not necessarily have to be managed according to CCAC standards[1]. Thus, it is imperative that protocol authors, institutional veterinarians, and animal care committees review and agree to the standards of care that are already in place for the animals prior to the commencement of any scientific activity (see Question 3 of the *CCAC frequently asked questions: Animal ethics and care program components*). All aspects of the scientific activity must be conducted with the consent of the owner of the animals.

Commercial beef cattle are often used in Canadian science, so the following example is focused on them. However, this process would be the same for any protocol that involves a commercial partner or other third-party-owned animals (e.g., shelter animals).

5.1 Process for Applying Humane Intervention Points in Commercial Beef Cattle

Beef cattle in commercial production should be covered by herd health management programs developed by veterinarians, in conjunction with producers, to manage all aspects of cattle health at each location (NFACC, 2013). Included in this program is a description of the process for monitoring and treating sick and injured cattle.

Before starting the scientific activity, it is important that the protocol author and the animal care committee agree with the humane intervention points put in place by the producer and herd health veterinarian (see the *CCAC guidelines on: the care and use of farm animals in research, teaching and testing* (CCAC, 2009)), in addition to any required by the scientific activity. For example, there may be instances when cattle being used for a scientific activity require a humane intervention for unrelated reasons (e.g., they become sick or lame). As long as these animals remain in use for science, their ethical treatment falls under the purview of the animal care committee, as they are responsible for the welfare of all animals used in science conducted under their institution. Thus, if committee members deem the humane intervention points unacceptable, the protocol should not be approved. In such cases, the animal care committee should encourage the protocol author to further refine the humane intervention points in collaboration with the other parties, and if no refinements can be made, to find another commercial partner. It is important to note that the institution is only responsible for the animals currently being used for science, not all the animals on-site, or animals that are no longer being used.

Scientific activities conducted on commercial beef cattle tend to have smaller welfare impacts (e.g., behavioural observations, nutritional or vaccine trials); however, the protocol author is still responsible for collaborating directly with the producer and the herd health veterinarian to ensure that all parties commit to the humane intervention points and scientific endpoints required of the scientific activity itself. If an animal requires humane interventions due to the nature of the scientific activity, the protocol author has a responsibility to ensure that the interventions are applied, and the animal's welfare is monitored until it recovers or is euthanized. Records of all humane interventions should be made available to veterinarians and the animal care committee as necessary.

1 However, each animal care committee should ensure that producers working with CCAC-certified institutions follow industry standards and meet the National Farm Animal Care Council Codes of Practice (see the *CCAC guidelines on: the care and use of farm animals in research, teaching, and testing* (CCAC, 2009)).

5.2 Selecting Humane Intervention Points

As noted in the previous examples, many of the humane intervention points for cattle are based on changes in behaviour indicating health and welfare concerns. Some behavioural changes that may signal a humane intervention point include decreased feeding or watering behaviour (e.g., Quimby et al., 2001), increased lying time and decreased standing episode frequency (e.g., Szyszka and Kyriazakis, 2013), and increased lameness (e.g., Stokka et al., 2001). Additional health monitoring parameters that may indicate a humane intervention point are external wounds, visible signs of inflammation or infection, and increased rectal temperature (e.g., Edwards, 2010). Many of these factors may already be included in the herd health management program (though the frequency of monitoring may need to be amended), so in addition to these, intervention points specifically related to the scientific activity should be added to the protocol.

5.3 Implementing Humane Interventions

There are two distinct differences in applying interventions in commercial animals as compared to laboratory animals: 1) the range of potential interventions or treatments is smaller; and 2) there are monetary incentives to avoid euthanizing animals. The range of intervention options for commercial beef cattle may be constrained by available infrastructure and resources, which should be assessed at the protocol review stage. For example, convalescent care of sick or injured cattle is promoted by the Codes of Practice (e.g., segregation, providing easier access to food and water, increased monitoring; NFACC, 2013) and is part of most herd health management programs. The effectiveness of this approach should not be limited by the number of farm personnel to monitor animals or sick pens available to house them. Treatment options are further constrained by the fact that the primary purpose of these animals is food production – any drugs given to them must be approved by the Canadian Food Inspection Agency and Health Canada. Finally, because there is an economic incentive to have animals reach slaughter, the final humane intervention point (euthanasia) may be later than it would otherwise be in research animals. In such cases, institutions should ensure that commercial research animals are receiving compensatory interventions (e.g., provision of analgesia) to safeguard their welfare as much as possible.

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

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APPENDIX 2
EXAMPLE HUMANE INTERVENTION POINT MONITORING TEMPLATE FOR MICE USED IN CANCER RESEARCH

This monitoring template is an example. It is intended to be used along with the information in the main body of this document and with Appendix 1; however, it can be modified in any way a protocol author or animal care committee requires, based on their needs.

1. CLINICAL PARAMETERS MONITORING GUIDE

CLINICAL SIGN	DEFINITION	SCORE	SCORE CRITERIA
General appearance	Condition of the animal's fur before moving the cage	0	Normal, smooth fur
		1	Ruffled fur <25% of body (excluding head)
		2	Ruffled fur 25-50% of body
		3	Ruffled fur >50% of body
Degree of eye opening	Proportion of eye visible before moving the cage	0	100% open
		1	25% closed
		2	50% closed
		3	75% closed
Breathing pattern	The rise and fall pattern of the chest cavity before moving the cage	0	No effort observed, normal
		1	Rapid breathing, no abdominal involvement
		2	Rapid, abdominal breathing
In-cage activity level	Observe animal motility when cage is picked up to be moved	0	Animal moves around when cage is disturbed
		1	Animal moves around a bit, but quickly settles down
		2	Animal barely moves from its position
Appetite	Observe supplemental feed to see if eaten	0	Whole portion eaten
		1	>50% portion eaten
		2	<50% portion eaten
Changes in normal behaviour	Observe animals within their home cage	0	No abnormal behaviour, no additional aggression, performing maintenance behaviour normally (eating and drinking, grooming, nest building, etc.)
		1	Some signs of increased aggression or abnormal behaviour
		2	Greatly increased levels of aggression, abnormal behaviour, or inactivity (during active phase)

CLINICAL SIGN	DEFINITION	SCORE	SCORE CRITERIA
Activity during handling	Observe animal's reaction to handling	0	Animal struggles to escape
		1	Animal struggles at first, but quickly quiets
		2	Animal doesn't move in hand
Body weight loss	Amount of body weight loss compared to baseline (or baseline cohorts)	0	0-4.9%
		1	5-9.9%
		2	10-14.9%
		3	15-19.9%
Body condition score	Assess body condition on a scale of 1 (emaciated or very thin) to 5 (obese)	0	≥3
		1	2
		2	<2
Dehydration	Assess skin elasticity by gently pulling skin and timing how long it takes to return to position	0	Skin returns to position in less than 2 seconds
		1	Skin returns to position in less than 5 seconds
		2	Skin returns to position in less than 10 seconds
Tumour volume or burden	Size of single tumour or total mass of multiple tumours	0	No tumours detected
		1	Tumours are present, but the size is sub-threshold
		2	Tumours have reached or exceeded maximum approved size
Ulceration	Size and characteristics of visible ulcers	0	No ulceration
		1	Small amount of ulceration, signs of inflammation
		2	Large, leaky ulcer, signs of self-mutilation

2. EUTHANASIA POINTS

In parallel with the above monitoring guide, clear euthanasia criteria should be established before the scientific activity begins. There should be a definitive indication of which clinical signs or score combinations warrant immediate euthanasia on their own (e.g., body weight loss score of 3, tumour volume score of 2). Additionally, a cumulative maximum may be set so that when a specified sum is reached across all clinical signs, the animal is euthanized. It is recommended that institutions create a general SOP describing these points that protocol authors can revise as necessary for their specific project.

3. CLINICAL PARAMETERS MONITORING TOOL

Person(s) monitoring: _____

Mouse ID: _____

Date and Time/ Initials	General Appearance (once daily)	Eye Opening (once daily)	Breathing Pattern (once daily)	In-Cage Activity Level (once daily)	Appetite (once daily)	Behavioural Indicators (once daily)	Activity Level During Handling (once daily)	Body Weight Loss (twice weekly)	Body Condition Score (twice weekly)	Dehydration	Total Score

4. TUMOUR MONITORING TOOL

Person(s) monitoring: ____________________________________

Maximum approved tumour size (or tumour burden if multiple tumours are present): ____________________________________

Measurement should be based on selected tumour volume formula. One option is: Σ [(length*width^2)/2]

Animal ID	Date/ Initials	Measurement	Ulceration

Animal ID	Date/ Initials	Measurement	Ulceration

Animal ID	Date/ Initials	Measurement	Ulceration

5. ACTION PLAN

This section should summarize the approved steps to be taken when the humane intervention point is reached. It should describe the interventions to be performed, indicate how and when these interventions should be followed up on, and list who should be notified that an intervention has occurred.

6. CONTACT LIST

This section should provide the names, roles or positions, and contact information for everyone involved with both the research and the care of the animals.

GLOSSARY

Abnormal behaviour – actions performed by an animal that are not part of the behavioural repertoire of that species in the wild.

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

Competency – the ability to effectively perform a particular task in relation to the care, maintenance, or use of animals, while ensuring the animals' welfare is protected as much as possible within the constraints of any approved studies that they are involved in. Focusing on competency rather than training acknowledges that there may be various ways of acquiring the necessary knowledge and skills and emphasizes learning outcomes. See the *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015) for more details.

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.

Discomfort – a mild form of distress.

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

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 facilities: laboratory, farm, and aquatic.

Morbidity – visible manifestation of a diseased state.

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, post-doctoral 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 impact experienced by the animals is 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.

Stereotypic behaviour – repetitive or unvarying behaviours that appear to have no purpose.

Three Rs – refer to the principles of 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.