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Conseil canadien de protection des animaux



CCAC guidelines: Animal welfare assessment

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Animal welfare assessment

PREFACE

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

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

This specific document was developed based on the recognition that animals used for scientific purposes should have good welfare, and that this requires more than ensuring they are healthy. Good welfare is characterized by maximizing animals' positive experiences while minimizing their negative ones. This approach to ensuring good welfare is already at the core of many existing practices, such as health monitoring, humane intervention point implementation, post-approval monitoring, and the assignment of categories of invasiveness. Formal welfare assessments are another tool to ensure that animals have the best possible welfare.

The *CCAC guidelines: Animal welfare assessment* applies to all animals used for scientific purposes. This includes wild animals, either brought into laboratory animal facilities or in the field, and animals owned by third parties that are used off-site (e.g., at commercial farms, or shelters). See Section 1.2 for more information on institutional responsibilities in these contexts.

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

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

SUMMARY OF THE GUIDELINES LISTED IN THIS DOCUMENT

The following list of guideline statements serves as an executive summary covering the most important aspects of animal welfare assessment. These guideline statements are included throughout this document alongside details and references that provide support and context for their implementation.

Guideline 1

The animal care committee is responsible for overseeing the implementation of welfare assessments, but the assessments themselves should be completed by a team involving protocol authors and their delegates, veterinarians, and animal care personnel. Where possible, the assessments should draw on information gathered through research, veterinary, and husbandry activities.

Section 2. General Guiding Principles, p.6

Guideline 2

Animals should be healthy and express a high prevalence and diversity of positively motivated species-typical behaviour, along with low levels of abnormal behaviour. They should neither experience negative affective states, such as pain, frustration, or fear, nor display behavioural signs of chronic anxiety or depression.

Section 2. General Guiding Principles, p.6

Guideline 3

Welfare assessments must be performed regularly for all animals. The assessments should take into account physical condition, psychological well-being, and impact of experimental procedures. When known, cumulative lifetime experiences and environmental parameters should also be included in the assessment.

Section 2. General Guiding Principles, p.7

Guideline 4

Information gathered in relation to welfare assessments should be recorded in a format accessible to investigators, veterinarians, animal care personnel, and animal care committees.

Section 2. General Guiding Principles, p.7

Guideline 5

The animal care committee should use welfare assessment summaries to identify systemic welfare risks, anticipate welfare implications, and inform future decisions concerning the ethical care and use of animals.

Section 2. General Guiding Principles, p.9

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.

1.1 DEFINITION OF ANIMAL WELFARE

Animal welfare is a concept used to characterize the physical and mental state of an individual animal and how this animal is experiencing the conditions in which it lives (OIE, 2018). Fraser (2008) has pointed out that different groups of people put emphasis on different aspects of animal welfare (i.e., biological functioning vs. affective states¹ vs. relatively natural life). However, this document utilizes affective states as the primary determinant of animal welfare (Duncan, 2006; Brydges and Braithwaite, 2008; Dawkins, 2008; Mendl et al., 2009), even though there are no direct measures of affective states in animals. Instead, welfare assessment relies on inferring affective states based on validated changes in physiology and behaviour. In particular, behavioural observations are crucial for *in situ* welfare assessment as they are practical to conduct, minimally invasive, and broadly useful across species.

Over the last few decades, significant progress has been made in understanding how behaviour and physiology can provide information on how animals experience their environment. This better understanding of animals’ mental states, combined with advancements in measurement of physiological and behavioural indicators of affective states, has led to greater consideration of how suboptimal environments and aversive stimuli can negatively affect animal welfare. Minimizing exposure to such environments and stimuli (and thus experiences of negative states such as pain, fear, and frustration) is the first step to improving animals’ welfare. Broadly, this may be achieved by preventing or reducing animals’ need to respond to perceived or real aversive stimuli (Fraser and Duncan, 1998). Positive reinforcement training may assist in this regard by reducing the aversiveness of a stimulus over time through associative conditioning (e.g., Laule et al., 2003).

More recently, it has been recognized that the prevention of suffering alone is not sufficient for good welfare. Positive experiences and affective states are core components of a good quality of life and good welfare (Boissy et al., 2007; Panksepp, 1998; Panksepp and Biven, 2012; Mellor, 2015a; Mellor and Beausoleil, 2015). Behaviour associated with positive affective states in some, but not all, species includes: play, particularly in young animals (though not always [Blois-Heulin et al., 2015]), exploration, affiliative interactions with conspecifics, mating, and specific vocalizations (Fraser and Duncan, 1998; Boissy et al., 2007; Yeates and Main, 2008). Based on current knowledge, specific behaviour patterns associated with positive affective states can

¹ Defined as psychologically experienced states that can be positive or negative to the subject and may vary in both intensity and duration.

vary greatly between even closely related species (e.g., social play indicates positive welfare in rats [Burgdorf et al., 2008], but not in mice [Richter et al., 2016]). Thus, species-specific indicators of positive welfare are beyond the scope of this document but instead can be found in the appropriate CCAC animal-type guidelines document.

Behaviour associated with positive affective states only occurs when other immediate needs are met and indicates that the animal is not deprived of important sources of pleasure (Boissy et al., 2007). Finally, promoting positive affective states (e.g., by increasing animals' agency or control over their lives or by providing them more opportunities to engage in activities they are positively motivated to do [Mellor, 2016]) can help prevent or mitigate the effect of negative events and reduce the occurrence of negative affective states (Yeates and Main, 2008).

1.2 REASONS FOR ASSESSING ANIMAL WELFARE

The reasons described below apply to all instances where a member of a CCAC-certified institution is performing scientific activities which fall under the CCAC mandate (see [Requirement for submitting an animal protocol: Addendum to the CCAC policy statement on terms of reference for animal care committees](#) [CCAC, 2018]). Thus, welfare assessments must be performed on all animals while they are owned, held, used, or interacted with by members of a certified institution.

Assessment of the welfare status of an animal is important to:

- reduce negative affective states and enhance long-term positive affective states of the animal by promoting conditions (environment, resources, husbandry) that are appropriate for the individual animal;
- improve the likelihood that any signs of pain or distress are detected as soon as possible so that relevant mitigation strategies/humane intervention points can be implemented as appropriate;
- ensure the physiological, behavioural, and psychological condition of the individual animal is suitable for achieving the desired study outcomes;
- ensure that the scientific activity remains within the bounds of the protocol, as approved by the local animal care committee;
- inform and validate the assignment of a category of invasiveness for a protocol²;
- improve (or ensure) the quality of scientific data collected from animals; and
- continuously improve our understanding of animal needs in order to optimize housing and husbandry practices.

For some species, and in some scientific contexts, evidence is not currently available to be certain the above-stated goals are being met, but they can be achieved by consistently applying new scientific knowledge as it becomes available.

When protocols involve animals owned by a third party (e.g., pets or off-site commercial livestock), there is an additional layer of oversight that needs to be accounted for (on top of the oversight already provided by

² Some animals may have compromised welfare prior to a procedure (e.g., due to genotype or disease state) and thus be more vulnerable than a non-compromised animal. The welfare impact of a procedure may also require that the category of invasiveness be reassessed when the protocol is renewed.

the veterinarian and animal care committee); owners should be empowered to advocate on behalf of their animals, and they have the right to remove their animals from participation in the scientific activity at any time. In such cases, or where there is a scientific activity involving wild animals in the field, the institution's responsibility for assessing animal welfare ends with the termination of the research or teaching activity. However, institutions are responsible for ensuring that welfare compromises that result from research or teaching activities are mitigated as much as possible.

2 GENERAL GUIDING PRINCIPLES

Guideline 1

The animal care committee is responsible for overseeing the implementation of welfare assessments, but the assessments themselves should be completed by a team involving protocol authors and their delegates, veterinarians, and animal care personnel. Where possible, the assessments should draw on information gathered through research, veterinary, and husbandry activities.

The composition and structure of the welfare assessment team should be defined by the animal care committee. This team should function with good communication amongst its members (Lambeth et al., 2013) and between itself and the animal care committee. Team members must be competent in assessing welfare and have full access to the animals.

Welfare assessments should be an integration of information collected during regular husbandry activities as well as during and after experimental procedures, with additional assessment tools incorporated. Additional assessment tools may include information gathered from physical exams, behavioural observations (during the animals' active phase when possible), animal training sessions and other activities. Thus, welfare assessment goes beyond daily health checks and is distinct from phenotyping.

Section 3 provides more information on selecting welfare indicators while Wolfensohn et al. (2015), Smith et al. (2006), Hawkins et al. (2011), and Mellor (2015b) provide examples of scoring information for a number of parameters. The European Commission Expert Working Group (2013) has also developed some practical scoring examples. For species-specific welfare assessment examples, please see the appropriate CCAC animal-type guidelines document.

Guideline 2

Animals should be healthy and express a high prevalence and diversity of positively motivated species-typical behaviour, along with low levels of abnormal behaviour. They should neither experience negative affective states, such as pain, frustration, or fear, nor display behavioural signs of chronic anxiety or depression.

Specific behaviour patterns associated with specific negative affective states can be hard to identify because they may vary greatly between even closely related species and are highly context-dependent. Thus, it is not necessary to be able to put a name to a unique affective state (e.g., frustration) to conclude that an animal displaying abnormal behaviour has compromised welfare. However, understanding the cause of the abnormal behaviour (and thus the underlying affective state) is important in correcting the problem.

It is recognized that there may be exceptions to this guideline statement, approved by an animal care committee, where pain and/or disease are being investigated. Nonetheless, humane intervention points must always be in place (*CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* [in prep.]), and efforts should always be made to improve animal welfare. The welfare of the animals should always take precedence over considerations of cost and convenience while also being mindful of human safety.

Guideline 3

Welfare assessments must be performed regularly for all animals. The assessments should take into account physical condition, psychological well-being, and impact of experimental procedures. When known, cumulative lifetime experiences and environmental parameters should also be included in the assessment.

As stated in the *CCAC guidelines on: training of personnel working with animals in science* (CCAC, 2015), “Institutions must strive to sustain an institutional culture of respect for animal life.”

Welfare assessment is a necessary component of animal-based studies, promoting both a good quality of life for the animals and reliable scientific data (Hawkins et al., 2011). Making the appropriate observations of the animals and assigning objective values to these observations allows for effective interventions that minimize any actual or potential pain, distress, or discomfort experienced by the animals (Hawkins et al., 2011; see also Appendix 1 for an example).

Consideration of animal welfare implications must encompass the whole life of the animal, with appropriate housing and care provided from birth to end of life. Although the assessment of cumulative lifetime experiences (i.e., the sum of all positive and negative welfare impacts over an animal’s lifetime [Pickard et al., 2013]) is currently challenging and not yet well-validated, there are some potentially useful indicators such as stereotypic behaviour (Gottlieb et al., 2013) and early senescence (Walker et al., 2012). There are additional potential measures of cumulative experience (e.g., feather/hair corticosteroids [Bortolotti et al., 2008; Meyer and Novak, 2012]), but these are only practically useful to those who are already assessing these factors as part of their research protocol.

The measures chosen for assessing the welfare of animals should allow for a comprehensive understanding of the factors influencing their welfare (including their cumulative lifetime experiences) in order to determine what is best for the animals based on veterinary and scientific knowledge and professional judgement. The frequency of assessment must be sufficient to detect any changes in welfare and should be commensurate with the expected welfare impact of the scientific activity.

Guideline 4

Information gathered in relation to welfare assessments should be recorded in a format accessible to investigators, veterinarians, animal care personnel, and animal care committees.

See *CCAC guidelines: Husbandry of animals in science* (CCAC, 2017), Section 12, “Record Keeping”, for details relating to maintaining and retaining records.

Records related to welfare assessment should include all information gathered on the welfare of the animals and detail actions to be taken where there are concerns. Additionally, these records provide valuable institutional information (see Guideline 5 below) and demonstrate that welfare assessments were actually completed.

Records should also identify the welfare status of the animals, as noted below, to facilitate appropriate care of animals in addition to the category of invasiveness for protocols involving those animals. These descriptions of welfare status should be considered within the context of each protocol (i.e., which category of invasiveness it was assigned) and not acted on in an identical manner across all protocols. However, the welfare status recorded should still reflect the actual experience of the animals.

When welfare compromises are expected (e.g., due to procedures approved by an animal care committee), humane interventions must occur at specific points agreed upon during preparation and review of an animal protocol. On the other hand, when welfare compromises are unexpected or go beyond what was expected, humane interventions must still be applied, but additional steps involving the animal care committee may be warranted. For example, animals experiencing a D-level category of invasiveness should have their welfare status noted as ‘severe’. However, the humane intervention implemented to mitigate this, and the level of involvement of the animal care committee, should be different than if animals on a B-level protocol were unexpectedly found to have a ‘severe’ welfare status, in which case emergency mitigation and a full investigation would be warranted (i.e., animals on the D-level protocol should already have a humane intervention plan, approved by the animal care committee, describing what action should be taken, whereas those on the B-level protocol may not have an approved plan to cover their current state). Table 1 demonstrates a framework for labelling and mitigating varying welfare statuses. Further information on setting and implementing humane intervention points can be found in *CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* [in prep.].

Table 1: Welfare Status and Required Actions

WELFARE STATUS	DESCRIPTION AND ACTIONS REQUIRED
Acceptable	No mitigation required.
Mild to moderate	Manageable welfare concerns have been identified. Pre-determined humane interventions or other mitigation strategies can be employed.
Severe	Welfare concerns have been identified that require extensive mitigation measures and close monitoring. Discussion with the animal care committee may be required to rectify the situation or terminate the protocol.
Unacceptable	Overwhelming welfare concerns have been identified, providing justification for immediate euthanasia. Discussion by the animal care committee is required to rectify the situation or terminate the protocol.

At a minimum, animal care committees must be notified of any *unexpected* instances where ‘severe’ or ‘unacceptable’ welfare states are found. They may choose to review other completed assessments, but many minor/moderate welfare concerns can be dealt with at the animal care or facility management level. Expected

welfare compromises should be mitigated through the use of humane intervention points, which are reviewed by the animal care committee during protocol review.

Guideline 5

The animal care committee should use welfare assessment summaries to identify systemic welfare risks, anticipate welfare implications, and inform future decisions concerning the ethical care and use of animals.

Data collected during welfare assessments can be used to identify risks and improve animal care in many ways, including:

- setting appropriate humane intervention points that are aligned with scientific endpoints;
- monitoring the effectiveness of chosen humane intervention points and promoting the implementation of earlier scientific endpoints;
- informing any mitigation strategies that might be necessary to improve an animal's welfare;
- identifying reoccurring issues with specific facilities or protocols;
- triggering a closer evaluation of animal housing and husbandry;
- assessing how well prospective assignment of category of invasiveness predicts the actual experience of the animals ([CCAC policy statement on: categories of invasiveness in animal experiments](#) [CCAC, 1991], which is under revision as *CCAC guidelines: Categories of welfare impact* [in prep.]);
- indicating when additional personnel training is needed; and
- triggering a halt and review of an ongoing study.

Welfare assessment records (or summaries thereof) should be reviewed by the animal care committee on at least an annual basis.

WELFARE INDICATORS

3

3.1 CONSIDERATIONS IN DEVELOPING WELFARE ASSESSMENT TOOLS

There is no direct, single method to quantify animal welfare; consequently, assessment requires attention to a number of variables (Fraser, 2008). The chosen variables, taken together, should provide an assessment of the individual's welfare, and hence, when repeatedly measured over time, provide an assessment of its quality of life.

Many welfare assessment programs (e.g., the [Welfare Quality® project](#)) focus on three types of measures for facilitating comprehensive yet feasible assessments: animal-based measures, resource-based measures, and management-based measures. Of these three types, animal-based measures should form the basis of any welfare assessment program. The incentive for using primarily animal-based measures is that they assess directly the response of the animal to its environment. Resource-based measures that examine aspects of the environment (e.g., enclosure size and features, access to litter, shelter, temperature, humidity) and management (e.g., stocking density, records assessment) do not always clearly correlate with some of the outcomes indicative of good animal welfare (Blokhuis et al., 2013). Nevertheless, resource- and management-based measures that are closely associated with animal-based measures should be considered in the welfare assessment because the quality of life of an animal is affected by husbandry practices and the environment in which it lives.

When developing welfare assessment tools, consideration must be given to addressing the following broad categories (Mellor et al., 2009): nutrition, environment/housing, health and biological functioning, and behaviour (including human-animal interactions). The indicators (measures) used should determine if the conditions: 1) promote positive and minimize negative affective states; 2) allow animals to develop normally, be healthy and free of disease and injuries; and 3) allow animals to express positively motivated behaviour. The assessment tool should be capable of addressing expected developmental changes of an animal with age. Care should also be taken to select indicators that have not been overly affected by domestication, such as reproductive traits. Although reductions in some traits that have been heavily selected for in production animals (e.g., growth, reproduction) could potentially indicate a welfare problem, these traits are not ideal welfare indicators because they may not significantly change when animals have poor welfare (Broom, 1991).

The measures selected should be validated to be reliable and practical. Furthermore, the primary measures chosen should be non-invasive or employed concurrently with ongoing procedures so that the assessment itself does not compromise animal welfare. If a potential welfare issue is found, only then should secondary, more invasive measures be considered to assess animal welfare status.

To be effective, welfare assessment tools should be developed for animals of different species, developmental stages or ages, strains or genetic modifications, and in accordance with the research or procedures performed.

When developing welfare assessment criteria, the welfare assessment team should think critically about the particular animal species/strain/disease model, the procedures being performed, etc. It is important to understand the particular species/strain/disease model and how manipulations on these particular animals

will affect their welfare before the study is initiated. Additionally, it should be recognized that some indicators of welfare lend themselves to binary or categorical outcomes that are easier to score and interpret, but provide less information (e.g., coat condition [Battini et al., 2015]), while others are much more sensitive to continuous changes in the welfare of the animals (e.g., time spent performing stereotypic behaviour). The current literature should be reviewed as a starting point for developing assessment tools.

Assessment tools often evolve, and it is important that their effectiveness be re-evaluated over time. Standardizing the terms used for describing the condition of the animals will assist in the clear identification and understanding of welfare issues (see, for example, [Mouse Welfare Terms](#)).

When choosing animal-based indicators for the assessment of an animal's welfare, the following general categories should serve as a starting point.

3.1.1 Behavioural Indicators

Behaviour can be assessed through general observations or behavioural testing (including simple tests such as scoring reaction to human approach). It includes potential indicators of negative affective states (e.g., stereotypic behaviour, self-injurious behaviour, prolonged inactivity, or writhing in pain [Mason and Latham, 2004; Rommeck et al., 2009; Meagher et al., 2013; Roughan and Flecknell, 2003; National Research Council, 2009]) and potential indicators of positive affect (e.g., play and other affiliative behaviour and some vocalizations [Boissy et al., 2007]). Ideally, behavioural indicators should be considered on an individual level. In some cases, this may not be possible. Therefore, consistent with the [CCAC guidelines: Husbandry of animals in science](#) (CCAC, 2017), records for rodents, rabbits, fish and other small animals may be at the cage or tank level; for farm animals, records may be at the pen or pasture level. However, once an animal has a health or welfare concern identified, the situation should be monitored (and records kept) independently.

Behavioural indicators should be interpreted relative to a baseline that has been predetermined for each animal during its active phase, following acclimation (i.e., upon arrival to a facility or room), and prior to any study. A change in behaviour for an animal (e.g., a normally calm animal behaving aggressively or a normally active animal being lethargic) may indicate it is experiencing poor welfare.

Each behavioural indicator should be evaluated on the basis of its frequency, severity (e.g., mild, moderate, severe), and whether or not it impedes other species-typical behaviour (Novak et al., 2012).

Observations of behaviour include both direct observations made by an observer and video-recorded observations. Each of these techniques has its advantages and disadvantages. Video recordings allow for continuous monitoring of animals in the absence of an observer, including after hours when staff are unlikely to be present. However, unless sophisticated technology is in place, the recordings must be reviewed in a timely manner by a human assessor, which can often be a time-consuming process. Direct observations by an observer are much easier and cost-effective to perform, but they can only cover limited periods of time. Furthermore, the presence of an observer may affect the behaviour of interest, especially if animals are not habituated to the observer's presence. Thus, it is important to know if a specific behaviour is induced or inhibited by the presence of an observer (or a specific observer). Regardless of which method is to be employed, the approved protocol should specifically list the persons responsible for monitoring animals' behaviour.

The scoring systems used to assess animal welfare must be tailored to the species and be used on an individual basis. For example, while hair loss is generally a sign of poor welfare, there are a number of potential

physiological or psychological causes that need to be taken into consideration when determining the cause and severity of the issue (Bellanca et al., 2014; Lutz et al., 2013; Novak and Meyer, 2009).

When negative changes are observed (that go beyond a pre-established threshold), all relevant parties should be promptly notified (e.g., research team, animal health technician, veterinarian, and behavioural or other specialist, if warranted) and a humane intervention should be applied (*CCAC guidelines: Identification of scientific endpoints, humane intervention points, and cumulative endpoints* [in prep.]). Subsequently, the animal should be monitored until its condition returns to an acceptable level. Scoring systems can also provide a useful measure of the effectiveness of an intervention or enrichment strategy. For example, a comparison with the animal's baseline score may indicate that a particular abnormal behaviour is less prevalent after an intervention. Scoring grids can also be useful in training new employees to recognize changes in animal behaviour and relating observations to the baseline behaviour of the animal.

3.1.2 Observable Health Indicators

Non-invasive health indicators can be obtained through observations of the appearance of individual animals at the enclosure level, assessment of clinical signs in individual animals, and maintenance of individual- and colony-level health records.

Examples of health indicators include posture, gait or swim pattern, coat condition, skin and mucosa condition, ocular and nasal discharge, body condition score, respiratory rate, injuries and visible lesions, changes in food and water intake, presence of inflammation, body temperature (via infra-red technology), appearance of feces and urine, facial expressions (e.g., grimace scoring), reproductive issues (such as the inability to lay eggs or deliver offspring), external parasites, and morbidity and mortality rates. This list is not exhaustive.

3.1.3 Additional Physiological Indicators

Further measurement of physiological indicators is generally more invasive than the measurement of observable health or behavioural indicators and should only be used to investigate serious welfare concerns or when these data are already being collected for other purposes (i.e., as part of an animal care committee-approved study). When physiological information is required, the least invasive method that will provide the necessary data should be used. Consideration should be given to obtaining samples from saliva, hair, urine, and feces.

Physiological indicators may include immunological functions (rates of lymphocyte proliferation or suppression of their activity, etc.), blood pressure, hematology or biochemistry blood analysis, and levels of various hormones (e.g., corticosteroids, noradrenaline). There is no single physiological indicator that can provide a clear measure of negative affective states, and deviations from baseline in either direction should be considered cause for concern.

3.2 ADDITIONAL GUIDANCE

An example demonstrating animal-based welfare indicators has been provided in Appendix 1. This example is intended to provide a general idea of how observable health indicators (e.g., appearance) and behavioural observations can be used to assess welfare. Further specific guidance can be found in each animal-type guidelines document.

4 RECORDING WELFARE ASSESSMENT INFORMATION

A detailed welfare assessment form should be developed to record and report the general welfare of the animals and detail actions to be taken if concerns arise. The form should be suited to the particular species, developmental stages or ages, strains or genetic modifications, and according to the research or procedures performed, as applicable.

Each form should provide the following general information, as applicable:

- species/animal type;
- strain/breed;
- genotype;
- type of enclosure (pen, cage, or tank);
- room and enclosure (pen, cage, or tank) identification;
- date of birth (or age) and sex;
- group size;
- protocol number;
- name of the person performing the assessment;
- date of welfare assessment;
- welfare assessment tools used;
- scientific procedures performed on the animals since the last assessment (including any restraint or capture methods);
- welfare history and previous assessment dates; and
- outcome of the assessment (welfare status of the animals and suggested mitigation strategies [if any]).

In the event that an intervention is required, documented follow-up explaining the steps taken to correct the situation (and by whom) should be made available to all relevant parties.

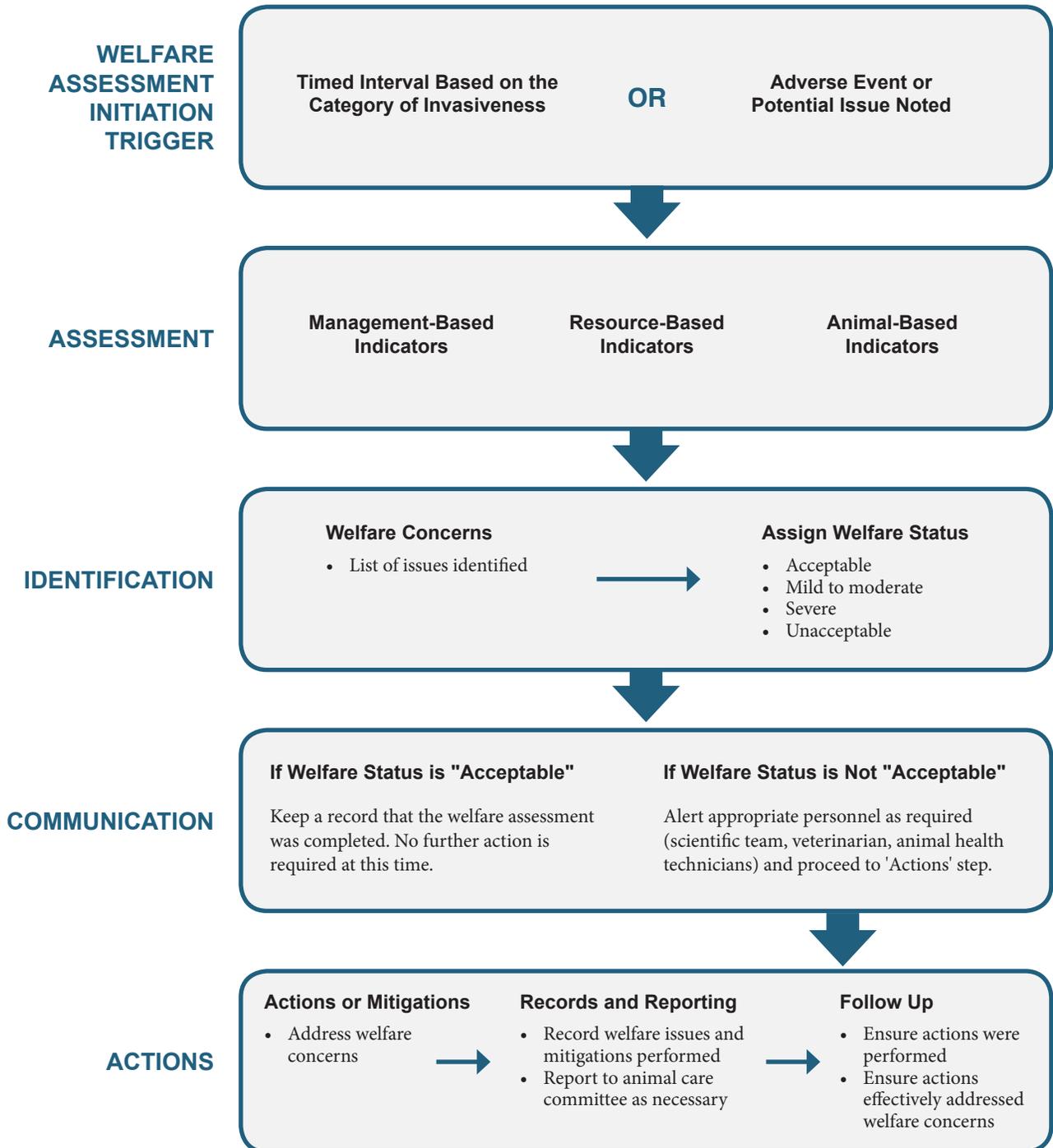
POTENTIAL OBSTACLES TO ASSESSING ANIMAL WELFARE

The measures chosen need not require veterinary diagnostic expertise or specialist animal behaviour knowledge to be accurately recorded, but the involvement of such experts in devising the welfare assessment tool should be encouraged as needed. The measures should be simple and descriptive, not requiring diagnoses in the field nor at the cage-, pen-, or tank-side. Each animal care committee should evaluate if the resources and expertise indicated on the protocol are sufficient to allow for objective animal welfare assessment of those animals. If an animal care committee does not feel confident in the stated welfare assessment plan, they should require additional resources and/or training or not approve the protocol.

As previously mentioned, there is no single indicator of animal welfare. A combination of multiple indicators is more likely to identify animal welfare issues. Thus, welfare assessments must be comprised of multiple indicators rather than a single one, whose reliability may be unknown. When possible, post-mortem findings should be used to validate welfare assessments and be included in summary reports.

Those planning and conducting welfare assessments should be aware that intra-observer consistency is important, such that a person conducting welfare assessments regularly scores the measures in the same way. Likewise, strong inter-observer reliability is crucial when multiple people are assessing the same animals (e.g., Clingerman and Summers [2012] note variation between observers for body condition scoring). Thus, welfare assessors must be well-trained, and the reliability of the welfare assessment tool should be checked periodically.

6 VISUAL SUMMARY OF WELFARE ASSESSMENT PROCESS



REFERENCES

- Battini M., Peric T., Ajuda I., Vieira A., Grosso L., Barbieri S., Stilwell G., Prandi A., Comin A., Tubaro F. and Mattiello F. (2015) Hair coat condition: A valid and reliable indicator for on-farm welfare assessment in adult dairy goats. *Small Ruminant Research* 123(2-3):197-203.
- Bellanca R.U., Lee G.H., Vogel K., Ahrens J., Kroeker R., Thom J.P. and Worlein J.M. (2014) A simple alopecia scoring system for use in colony management of laboratory-housed primates. *Journal of Medical Primatology* 43(3):153-161.
- Blois-Heulin C., Rochais C., Camus S., Fureix C., Lemasson A., Lunel C., Bézard E. and Hausberger M. (2015) Animal welfare: Could adult play be a false friend? *Animal Behavior and Cognition* 2(2):156-185.
- Blokhuis M., Miele M., Veissier I. and Jones B. (2013) Improving Farm Animal Welfare, Science and Society Working Together: The Welfare Quality Approach. 222 pp. Wageningen, The Netherlands: Wageningen Academic Publishers.
- Boissy A., Manteuffel G., Jensen M.B., Moe R.O., Spruijt B., Keeling L.J., Winckler C., Forkman B., Dimitrov I., Langbein J., Bakken M., Veissier I. and Aubert A. (2007) Assessment of positive emotions in animals to improve their welfare. *Physiology and Behavior* 92(3):375-397.
- Bortolotti G., Marchant T.A., Blas J. and German T. (2008) Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. *Functional Ecology* 22(3):494-500.
- Broom D.M. (1991) Animal welfare: Concepts and measurement. *Journal of Animal Science* 69(10):4167-4175.
- Brydges N. and Braithwaite V.A. (2008) Measuring animal welfare: What can cognition contribute? *Annual Review of Biomedical Sciences* 10:T91-T103.
- Burgdorf J., Kroes R.A., Moskal J.R., Pfaus J.G., Brudzynski S.M. and Panksepp J. (2008) Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: Behavioural concomitants, relationship to reward, and self-administration of playback. *Journal of Comparative Psychology* 122(4):357-367.
- Canadian Council on Animal Care – CCAC (1991) [CCAC policy statement on: categories of invasiveness in animal experiments](#). Ottawa ON: CCAC (accessed on 2021-03-02).
- Canadian Council on Animal Care – CCAC (2015) [CCAC guidelines on: training of personnel working with animals in science](#). Ottawa ON: CCAC (accessed on 2021-03-02).
- Canadian Council on Animal Care – CCAC (2017) [CCAC guidelines: Husbandry of animals in science](#). Ottawa ON: CCAC (accessed on 2021-03-02).
- Canadian Council on Animal Care – CCAC (2018) [Requirement for submitting an animal protocol: Addendum to the CCAC policy statement on terms of reference for animal care committees](#). Ottawa ON: CCAC (accessed on 2021-03-02).
- Clingerman K.J. and Summers L. (2012) Validation of a body condition scoring system in rhesus macaques (*Macaca mulatta*): Inter- and intrarater variability. *Journal of the American Association for Laboratory Animal Science* 51(1):31-36.

- Dawkins M.S. (2008) The science of animal suffering. *Ethology* 114(10):937-945.
- Duncan I.J.H. (2006) The changing concept of animal sentience. *Applied Animal Behaviour Science* 100(1-2):11-19.
- European Commission Expert Working Group (2013) [Examples to illustrate the process of severity classification, day-to-day assessment and actual severity assessment](#). Brussels, Belgium (accessed on 2021-03-02).
- Fraser D. (2008) *Understanding Animal Welfare: The Science in its Cultural Context*. 336pp. Oxford UK: Wiley-Blackwell.
- Fraser D. and Duncan I.J.H. (1998) 'Pleasures', 'pains' and animal welfare: Toward a natural history of affect. *Animal Welfare* 7(4):383-396.
- Gottlieb D.H., Capitanio J.P. and McCowan B. (2013) Risk factors for stereotypic behavior and self-biting in rhesus macaques (*Macaca mulatta*): Animal's history, current environment, and personality. *American Journal of Primatology* 75(10):995-1008.
- Hawkins P., Morton D.B., Burman O., Dennison N., Honess P., Jennings M., Lane S., Middleton V., Roughan J.V., Wells S. and Westwood K. (2011) A guide to defining and implementing protocols for the welfare assessment of laboratory animals: Eleventh report of the BVA/AFW/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* 45(1):1-13.
- Hurst J.L. and West R.S. (2010) Taming anxiety in laboratory mice. *Nature Methods* 7(10):825-826.
- Lambeth S.P., Schapiro S.J., Bernacky B.J. and Wilkerson G.K. (2013) Establishing 'quality of life' parameters using behavioural guidelines for humane euthanasia of captive non-human primates. *Animal Welfare* 22(4):429-435.
- Lutz C.K., Coleman K., Worlein J. and Novak M.A. (2013) Hair loss and hair-pulling in rhesus macaques (*Macaca mulatta*). *Journal of the American Association for Laboratory Animal Science* 52(4):454-457.
- Mason G.J. and Latham N.R. (2004) Can't stop, won't stop: Is stereotypy a reliable animal welfare indicator? *Animal Welfare* 13:S57-S69.
- Meagher R.K., Campbell D.L.M., Ahloy Dallaire J., Díez-León M., Palme R. and Mason G.J. (2013) Sleeping tight or hiding in fright? The welfare implications of different subtypes of inactivity in mink. *Applied Animal Behaviour Science* 144(3-4):138-146.
- Mellor D.J. and Beausoleil N.J. (2015) Extending the 'Five Domains' model for animal welfare assessment to incorporate positive welfare states. *Animal Welfare* 24(3):241-253.
- Mellor D.J., Patterson-Kane E. and Stafford K.J. (2009) *The Sciences of Animal Welfare*. UFAW Animal Welfare Series. 212pp. Oxford UK: Wiley-Blackwell.
- Mellor D.J. (2015a) Enhancing animal welfare by creating opportunities for positive affective engagement. *New Zealand Veterinary Journal* 63(1):3-8.
- Mellor D.J. (2015b) Positive animal welfare states and reference standards for welfare assessment. *New Zealand Veterinary Journal* 63(1):17-23.
- Mellor D.J. (2016) Updating animal welfare thinking: Moving beyond the "five freedoms" towards "a life worth living". *Animals* 6(3):21.

- Mendl M., Burman O.H.P., Parker R.M.A. and Paul E.S. (2009) Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science* 118(1-3):161-181.
- Meyer J.S. and Novak M.A. (2012) Minireview: Hair cortisol: A novel biomarker of hypothalamic-pituitary-adrenocortical activity. *Endocrinology* 153(9):4120-4127.
- National Research Council (2009) *Recognition and Alleviation of Pain in Laboratory Animals*. Washington D.C: National Academies Press.
- Novak M.A., Kelly B.J., Bayne K. and Meyer J.S. (2012) Behavioral disorders of nonhuman primates. In: *Nonhuman Primates in Biomedical Research*, American College of Laboratory Animal Medicine Series, vol. 1: Biology and Management, 2nd ed. (Abee C., Mansfield K., Tardif S. and Morris T., eds.), Chapter 7, pp.177-196. Boston MA: Elsevier Academic Press.
- Novak M.A. and Meyer J.S. (2009) Alopecia: Possible causes and treatments, particularly in captive nonhuman primates. *Comparative Medicine* 59(1):18-26.
- Panksepp J. (1998) *Affective Neuroscience: The Foundations of Human and Animal Emotions*. 480pp. New York NY: Oxford University Press.
- Panksepp J. and Biven L. (2012) *The Archaeology of Mind: Neuroevolutionary Origins of Human Emotions*. 592pp. New York NY: W.W. Norton.
- Pickard J., Buchanan-Smith H., Dennis M., Flecknell P., Joannides A., Lemon R., Prescott M. and Schultx W. (2013) *Review of the Assessment of Cumulative Severity and Lifetime Experience in Non-Human Primates Used in Neuroscience Research*. 161pp. London UK: Animal Procedures Committee.
- Poole T. (1997) Happy animals make good science. *Laboratory Animals* 31(2):116-124.
- Richter S.H., Kästner N., Kriwet M., Kaiser S. and Sachser N. (2016) Play matters: The surprising relationship between juvenile playfulness and anxiety later in life. *Animal Behaviour* 114:261-271.
- Rommeck I., Anderson K., Heagerty A., Cameron A. and McCowan B. (2009) Risk factors and remediation of self-injurious and self-abuse behavior in rhesus macaques. *Journal of Applied Animal Welfare Science* 12(1):61-72.
- 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(5):397-406.
- Smith J.J., Hadzic V., Li X., Liu P., Day T., Utter A., Kim B., Washington I.M. and Basso M.A. (2006) Objective measures of health and well-being in laboratory rhesus monkeys (*Macaca mulatta*). *Journal of Medical Primatology* 35(6):388-396.
- Walker M.D., Duggan G., Roulston N., Van Slack A. and Mason G.J. (2012) Negative affective states and their effects on morbidity, mortality and longevity. *Animal Welfare* 21(4):497-509.
- 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(2):139-149.
- World Organisation for Animal Health (OIE) (2018) [Introduction to the recommendations for animal welfare](#) (chapter 7.1). In: *Terrestrial Animal Health Code*, 27th ed. (accessed on 2021-03-02).
- Yeates J.W. and Main D.C.J (2008) Assessment of positive welfare: A review. *The Veterinary Journal* 175(3):293-300.

APPENDIX 1 EXAMPLE OF AN ANIMAL-BASED MEASURES PORTION OF A WELFARE ASSESSMENT: BREEDING MICE

This is an example of an animal-based measures portion of a welfare assessment program that could be implemented for a mouse breeding colony. It is not a definitive or required list. The indicators described in this appendix should be adapted to fit individual circumstances and needs as protocol-specific factors may influence, or be influenced by, some welfare indicators. Additionally, these indicators are meant to be incorporated into a comprehensive assessment program; no single indicator should be used to make judgements on the welfare of the animals.

ANIMAL ASSESSMENT: MOUSE BREEDING CAGE

	WELFARE MEASURES (appearance/health and behaviour)	MOUSE PARAMETERS	INDICATE (X) ABNORMAL PARAMETERS	ADDITIONAL NOTES/ OBSERVATIONS	ANIMAL ASSESSMENT: acceptable → severe (based on frequency/intensity of abnormal observations)
ADULTS	Appearance <ul style="list-style-type: none"> Evaluating for injuries or disease, pain, stress, and ability to self-care 	<ul style="list-style-type: none"> Body condition score (accounting for pregnancy) Lameness Skin colour/piloerection/coat condition Posture Physical injuries/wounds/growths Urine/feces Discharge or blood (eyes, nose, prepuce, vagina) Prolapse Abdominal distention (accounting for stage of pregnancy) Facial expression (head and ear carriage, muscle tension) Respiratory rate or effort Hydration status 			
	Solitary Behaviour <ul style="list-style-type: none"> Evaluating for self-care With/if husbandry/human disturbance 	<ul style="list-style-type: none"> Activity (exploratory, vertical and horizontal movement, stereotypy) Resting/sleeping Ability to access feed and water Self-grooming/cleaning Nesting behaviour Quality and speed of ambulation when disturbed Time to reappear after hiding 			
	Social Behaviour <ul style="list-style-type: none"> Evaluating social interactions Parental behaviour 	<ul style="list-style-type: none"> Isolation or interaction with cage mates Aggression Social grooming/cleaning Nursing Pup retrieval Interaction with pups and nesting after disturbance 			

	WELFARE MEASURES (appearance/health and behaviour)	MOUSE PARAMETERS	INDICATE (X) ABNORMAL PARAMETERS		ADDITIONAL NOTES/OBSERVATIONS	ANIMAL ASSESSMENT: acceptable → severe (based on frequency/intensity of abnormal observations)
PUPS	Appearance <ul style="list-style-type: none"> Evaluating for growth and development (choose age-dependent parameters) 	<ul style="list-style-type: none"> Size Milk spot Skin colour/condition or hair growth/coat condition Developmental eye opening Physical abnormality (e.g., anophthalmia, hydrocephalus) Posture Found dead or suddenly absent Other abnormalities/injuries 		CIRCLE AGE: (PND) < 7 7-10 11-21		
	Behaviour <ul style="list-style-type: none"> Evaluating for growth and development (choose age-dependent parameters) Ambulatory pups with/if husbandry/human disturbance 	<ul style="list-style-type: none"> Nursing or eating (age-dependent) Grooming Pup-pup interactions Pup-adult interactions Aggression Gait/locomotory development Exploring/climbing (age-dependent) Resting/sleeping Appropriate location—in nest, outside nest (age-dependent) 				

REFERENCES

Spangenberg E.M.F. and Keeling L.J. (2015) Assessing the welfare of laboratory mice in their home environment using animal-based measures—A benchmarking tool. *Laboratory Animals* 50(1):30-38.

Langford D.J., Bailey A.L., Chanda M.L., Clarke S.E., Drummond T.E., Echols S., Glick S., Ingrao J., Klassen-Ross T., LaCroix-Fralish M.L., Matsumiya L., Sorge R.E., Sotocinal S.G., Tabaka J.M., Wong D., van den Maagdenberg A.M.J.M., Ferrari M.D., Craig K.D. and Mogil J.S. (2010) Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6):447-449.

Ullman-Culleré M.H. and Foltz C.J. (1999) Body condition scoring: A rapid and accurate method for assessing health status in mice. *Comparative Medicine* 49(3):319-323.

The Jackson Laboratory. *JAX® Mice Pup Appearance by Age* [poster].

SELECTED REFERENCES FOR FURTHER READING ON ANIMAL WELFARE INDICATORS

- Baxter E.M. and Edwards S.A. (2017) Piglet mortality and morbidity: Inevitable or unacceptable? In: *Advances in Pig Welfare* (Špinko M., ed.), Chapter 3, pp.73-100. Duxford UK: Woodhead Publishing.
- Beausoleil N.J. and Mellor D.J. (2015) Introducing breathlessness as a significant animal welfare issue. *New Zealand Veterinary Journal* 63(1):44-51.
- Bechard A. and Mason G.J. (2010) Leaving home: A study of laboratory mouse pup independence. *Applied Animal Behaviour Science* 125:181-188.
- Beerda B., Schilder M.B., Janssen N.S. and Mol J.A. (1996) The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. *Hormones and Behavior* 30(3):272-279.
- Benson G.J. (2004) Pain in farm animals: Nature, recognition, and management. In: *The Well-Being of Farm Animals: Challenges and Solutions* (Benson G.J. and Rollin B.E., eds.), pp.61-84. Ames IA: Blackwell.
- Botreau R., Bonde M., Butterworth A., Perny P., Bracke M.B.M., Capdeville J. and Veissier I. (2007) Aggregation of measures to produce an overall assessment of animal welfare. Part 1: A review of existing methods. *Animal* 1(8):1179-1187.
- Botreau R., Bracke M.B.M., Perny P., Butterworth A., Capdeville J., Van Reenen C.G. and Veissier I. (2007) Aggregation of measures to produce an overall assessment of animal welfare. Part 2: Analysis of constraints. *Animal* 1(8):1188-1197.
- Burghardt G.M. (2013) Environmental enrichment and cognitive complexity in reptiles and amphibians: Concepts, review, and implications for captive populations. *Applied Animal Behaviour Science* 147(3-4):286-298.
- Campbell B., Wood G. and McBride T. (1997) Origins of orienting and defensive responses: An evolutionary perspective. In: *Attention and Orienting: Sensory and Motivational Processes* (Lang P.J., Simons R.F. and Balaban M., eds.), pp.41-68. Mahwah NJ: Lawrence Erlbaum Associates Publishers.
- Carlezon Jr. W.A. (2003) Place conditioning to study drug reward and aversion. In: *Opioid Research: Methods and Protocols* (Pan Z.Z., ed.). Methods in Molecular Medicine series, vol. 84, pp.243-249. Totowa NJ: Humana Press Inc.
- Castelhano-Carlos M.J. and Baumans V. (2009) The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals* 43(4):311-327.
- Causby L.A. and Smith E.N. (1981) Control of fear bradycardia in the swamp rabbit, *Sylvilagus aquaticus*. *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology* 69(2):367-370.
- Chalfoun A.D. and Martin T.E. (2007) Assessments of habitat preferences and quality depend on spatial scale and metrics of fitness. *Journal of Applied Ecology* 44(5):983-992.

- Chauvin C., Hillion S., Balaine L., Michel V., Peraste J., Petetin I., Lupo C. and Le Bouquin S. (2011) Factors associated with mortality of broilers during transport to slaughterhouse. *Animal* 5(2):287-293.
- Clouard C., Meunier-Salaün M.-C. and Devillers N. (2011) Development of approach and handling tests for the assessment of reactivity to humans of sows housed in stall or in group. *Applied Animal Behaviour Science* 133(1):26-39.
- Conti G., Hansman C., Heckman J.J., Novak M.F.X., Ruggiero A. and Suomi S.J. (2012) Primate evidence on the late health effects of early-life adversity. *Proceedings of the National Academy of Sciences* 109(23):8866-8871.
- Creel S., Dantzer B., Goymann W. and Rubenstein D.R. (2013) The ecology of stress: Effects of the social environment. *Functional Ecology* 27(1):66-80.
- Dantzer R. (2009) Cytokine, sickness behavior, and depression. *Immunology and Allergy Clinics of North America* 29(2):247-264.
- Dantzer R. and Kelley K.W. (2007) Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity* 21(2):153-160.
- D'Eath R.B., Tolkamp B.J., Kyriazakis I. and Lawrence A.B. (2009) 'Freedom from hunger' and preventing obesity: The animal welfare implications of reducing food quantity or quality. *Animal Behaviour* 77(2):275-288.
- Feng X., Wang L., Yang S., Qin D., Wang J., Li C., Lv L., Ma Y. and Hu X. (2011) Maternal separation produces lasting changes in cortisol and behavior in rhesus monkeys. *Proceedings of the National Academy of Sciences* 108(34):14312-14317.
- Fureix C. and Meagher R.K. (2015) What can inactivity (in its various forms) reveal about affective states in non-human animals? A review. *Applied Animal Behaviour Science* 171:8-24.
- Fureix C., Walker M., Harper L., Reynolds K., Saldivia-Woo A. and Mason G. (2016) Stereotypic behaviour in standard non-enriched cages is an alternative to depression-like responses in C57BL/6 mice. *Behavioural Brain Research* 305:186-190.
- Gottlieb D.H., Capitanio J.P. and McCowan B. (2013) Risk factors for stereotypic behavior and self-biting in rhesus macaques (*Macaca mulatta*): Animal's history, current environment, and personality. *American Journal of Primatology* 75(10):995-1008.
- Gottlieb D.H., Maier A. and Coleman K. (2015) Evaluation of environmental and intrinsic factors that contribute to stereotypic behavior in captive rhesus macaques (*Macaca mulatta*). *Applied Animal Behaviour Science* 171:184-191.
- Hampshire V. and Robertson S. (2015) Using the facial grimace scale to evaluate rabbit wellness in post-procedural monitoring. *Lab Animal (NY)* 44(7):259-260.
- Hart B.L. (1988) Biological basis of the behavior of sick animals. *Neuroscience and Biobehavioral Reviews* 12(2):123-137.
- Harvey A.M., Beausoleil N.J., Ramp D. and Mellor D.J. (2020). A ten-stage protocol for assessing the welfare of individual non-captive wild animals: Free-roaming horses (*Equus ferus caballus*) as an example. *Animals* 10(1):148.

- Hawkins P., Morton D.B., Burman O., Dennison N., Honess P., Jennings M., Lane S., Middleton V., Roughan J.V., Wells S. and Westwood K. (2011) A guide to defining and implementing protocols for the welfare assessment of laboratory animals: Eleventh report of the BVA/AFW/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* 45(1):1-13.
- Herskin M.S. and Di Giminiani P. (2018) Pain in pigs: Characterisation, mechanisms and indicators. In: *Advances in Pig Welfare* (Špinková M., ed.), Chapter 11, pp.325-355. Duxford UK: Woodhead Publishing.
- Inglis I.R., Forkman B. and Lazarus J. (1997) Free food or earned food? A review and fuzzy model of contrafreeloading. *Animal Behaviour* 53(6):1171-1191.
- Jameel M.K., Joshi A.R., Dawane J., Padwal M., Joshi A.R., Pandit V.A. and Melinkeri R.R. (2014) Effect of various physical stress models on serum cortisol level in Wistar rats. *Journal of Clinical & Diagnostic Research* 8(3):181-183.
- Justice W.S.M., O'Brien M.F., Szyszka O., Shotton J., Gilmour J.E.M., Riordan P. and Wolfensohn S. (2017) Adaptation of the animal welfare assessment grid (AWAG) for monitoring animal welfare in zoological collections. *Veterinary Record* 181(6):143.
- Keating S.C.J., Thomas A.A., Flecknell P.A. and Leach M.C. (2012) Evaluation of EMLA cream for preventing pain during tattooing of rabbits: Changes in physiological, behavioural and facial expression responses. *PLoS ONE* 7(9):e44437.
- Kirkden R.D. and Pajor E.A. (2006) Using preference, motivation and aversion tests to ask scientific questions about animals' feelings. *Applied Animal Behaviour Science* 100(1-2):29-47.
- Langford D.J., Bailey A.L., Chanda M.L., Clarke S.E., Drummond T.E., Echols S., Glick S., Ingrao J., Klassen-Ross T., LaCroix-Fralish M.L., Matsumiya L., Sorge R.E., Sotocinal S.G., Tabaka J.M., Wong D., van den Maagdenberg A.M.J.M., Ferrari M.D., Craig K.D. and Mogil J.S. (2010) Coding facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6):447-449.
- Laule G.E., Bloomsmith M.A. and Schapiro S.J. (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(3):163-173.
- Mason G.J. and Latham N.R. (2004) Can't stop, won't stop: Is stereotypy a reliable animal welfare indicator? *Animal Welfare* 13:S57-S69.
- Mason G.J. and Rushen J. (2006) *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare* (2nd ed). 384pp. Wallingford UK: Cabi Publishing.
- Meagher R.K., Campbell D.L.M. and Mason G.J. (2017) Boredom-like states in mink and their behavioural correlates: A replicate study. *Applied Animal Behaviour Science* 197:112-119.
- Mendl M., Burman O.H.P., Parker R.M.A. and Paul E.S. (2009) Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science* 118(3-4):161-181.
- Milligan S.R., Sales G.D. and Khirnykh K. (1993) Sound levels in rooms housing laboratory animals: An uncontrolled daily variable. *Physiology and Behavior* 53(6):1067-1076.

- Mormède P., Andanson S., Aupérin B., Beerda B., Guémené D., Malmkvist J., Manteca X., Manteuffel G., Prunet P., van Reenen C.G., Richard S. and Veissier I. (2007) Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiology and Behavior* 92(3):317-339.
- Morton D.B. and Griffiths P.H. (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* 116(16):431-436.
- Natalini C.C. and Robinson E.P. (2003) Effects of epidural opioid analgesics on heart rate, arterial blood pressure, respiratory rate, body temperature, and behavior in horses. *Veterinary Therapeutics* 4(4):364-375.
- Newberry R.C. (1995) Environmental enrichment: Increasing the biological relevance of captive environments. *Applied Animal Behaviour Science* 44(2-4):229-243.
- Olsson I.A.S. and Dahlborn K. (2002) Improving housing conditions for laboratory mice: A review of “environmental enrichment”. *Laboratory Animals* 36(3):243-270.
- Patterson-Kane E., Pittman M. and Pajor E.A. (2008) Operant animal welfare: Productive approaches and persistent difficulties. *Animal Welfare* 17(2):139-148.
- Poirier C., Bateson M., Gualtieri F., Armstrong E.A., Laws G.C., Boswell T. and Smulders T.V. (2019) Validation of hippocampal biomarkers of cumulative affective experience. *Neuroscience & Biobehavioral Reviews* 101:113-121.
- Ray M.A., Johnston N.A., Verhulst S., Trammell R.A. and Toth L.A. (2010) Identification of markers for imminent death in mice used in longevity and aging research. *Journal of the American Association for Laboratory Animal Science* 49(3):282-288.
- Romero L.M. and Romero R.C. (2002) Corticosterone responses in wild birds: The importance of rapid initial sampling. *The Condor* 104(1):129-135.
- Roughan J. and Flecknell P.A. (2003) Evaluation of a short duration behaviour-based post-operative pain scoring system in rats. *European Journal of Pain* 7(5):397-406.
- Roughan J.V. and Flecknell P.A. (2006) Training in behaviour-based post-operative pain scoring in rats—An evaluation based on improved recognition of analgesic requirements. *Applied Animal Behaviour Science* 96(3-4):327-342.
- Rushen J. (1996) Using aversion learning techniques to assess the mental state, suffering, and welfare of farm animals. *Journal of Animal Science* 74(8):1990-1995.
- Schroeder C.A. and Smith L.J. (2011) Respiratory rates and arterial blood-gas tensions in healthy rabbits given buprenorphine, butorphanol, midazolam, or their combinations. *Journal of the American Association for Laboratory Animal Science* 50(2):205-211.
- Scott K., Laws D.M., Courboulay V., Meunier-Salaün M.-C. and Edwards S.A. (2009) Comparison of methods to assess fear of humans in sows. *Applied Animal Behaviour Science* 118(1-2):36-41.
- Sheriff M.J., Krebs C.J. and Boonstra R. (2010) Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? *General and Comparative Endocrinology* 166(3):614-619.
- Smith E.N. and Worth D.J. (1980) Atropine effect on fear bradycardia of the Eastern Cottontail Rabbit, *Sylvilagus floridanus*. In: *A Handbook on Biotelemetry and Radio Tracking* (Amlaner C.J. and MacDonald D.W., eds.), pp.549-555. Oxford UK: Pergamon Press.

- Sneddon L.U. (2009) Pain perception in fish: Indicators and endpoints. *Institute for Laboratory Animal Research Journal* 50(4):338-342.
- Sotocinal S.G., Sorge R.E., Zaloum A., Tuttle A.H., Martin L.J., Wieskopf J.S., Mapplebeck J.C.S., Wei P., Zhan S., Zhang S., McDougall J.J., King O.D. and Mogil J.S. (2011) The rat grimace scale: A partially automated method for quantifying pain in the laboratory rat via facial expressions. *Molecular Pain* 7:55.
- Tata D.A. and Anderson B.J. (2010) The effects of chronic glucocorticoid exposure on dendritic length, synapse numbers and glial volume in animal models: Implications for hippocampal volume reductions in depression. *Physiology & Behavior* 99(2):186-193.
- Waiblinger S., Boivin X., Pedersen V., Tosi M.-V., Janczak A.M., Visser E.K. and Jones R.B. (2006) Assessing the human–animal relationship in farmed species: A critical review. *Applied Animal Behaviour Science* 101(3-4):185-242.
- Walker M.D., Duggan G., Roulston N., Van Slack A. and Mason G.J. (2012) Negative affective states and their effects on morbidity, mortality, and longevity. *Animal Welfare* 21(4):497-509.
- Weary D.M., Niel L., Flower C. and Fraser D. (2006) Identifying and preventing pain in animals. *Applied Animal Behaviour Science* 100(1-2):64-76.
- Wells D.L. (2009) Sensory stimulation as environmental enrichment for captive animals: A review. *Applied Animal Behaviour Science* 118(1-2):1-11.
- Winckler C. and Willen S. (2001) The reliability and repeatability of a lameness scoring system for use as an indicator of welfare in dairy cattle. *Acta Agriculturae Scandinavica, Section A – Animal Science* 51(1):103-107.
- 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(2):139-149.
- Zanella A.J., Broom D.M., Hunter J.C. and Mendl M.T. (1996) Brain opioid receptors in relation to stereotypes, inactivity, and housing in sows. *Physiology and Behavior* 59(4-5):769-775.

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.

Animal agency – when an animal engages in voluntary, self-generated, and goal-directed behaviour. Such behaviours are rewarding and typically accompanied by positive affective states.

Aversive stimuli – things that animals will avoid or try to escape from.

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.

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

Humane intervention points – the pre-established criteria (e.g. physical, physiological, and psychological/behavioural) that indicate when an intervention (e.g. supportive care, analgesia, euthanasia, etc.) should occur in order to rectify negative welfare states.

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

In situ – taking place where the animals live.

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

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

Play – voluntary interaction of animals with objects or other animals for purposes other than meeting their needs for survival or reproduction, which results in positive welfare.

Positively motivated behaviour – behaviours that are self-rewarding, and that are generally motivated by positive affective states.

Procedure – the part of the scientific activity specifically related to data collection (research and testing), or hands-on demonstration/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.

Quality of life – the welfare of the animal throughout its entire lifespan.

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

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

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

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

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.