CCAC training module on: pain, distress and endpoints

Companion Notes

CCAC training module on: pain, distress and endpoints

Companion Notes

Slide 1 CCAC training module on: pain, distress and endpoints

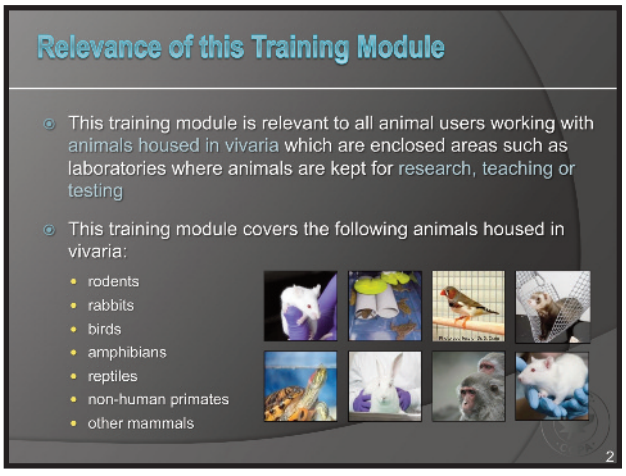
This module is devoted to understanding and minimizing pain and distress in animals housed in vivaria in research, teaching and testing.

Slide 2 Relevance of this Training Module

This training module is relevant to all animal users working with **animals housed in vivaria** which are enclosed areas such as laboratories where animals are kept for **research, teaching or testing**.

This training module covers rodents, rabbits, birds, amphibians, reptiles, non-human primates and other mammals.

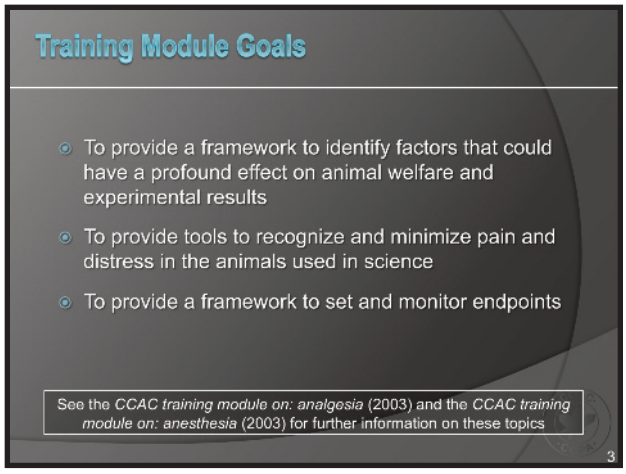
Pain in amphibians, reptiles and fish is still a poorly studied subject. However, since they have similar anatomical, physiological and neurological features to mammals and show avoidance behaviour to stimuli known to be aversive to mammals; they should be treated accordingly. There are now published guidelines on pain and analgesia in amphibians and fish.



Note: This module does not cover fish; training materials relevant to fish users are available in the Fish Stream. This module does not cover farm animals; training modules relevant to farm animal users are available in the Farm Animal Stream.

Slide 3 Training Module Goals

The main goal of this module is to provide the investigator with tools to minimize pain and distress in the animals they use experimentally. A framework is provided within which it will be possible to identify factors that could have a profound effect on animal welfare and experimental results.

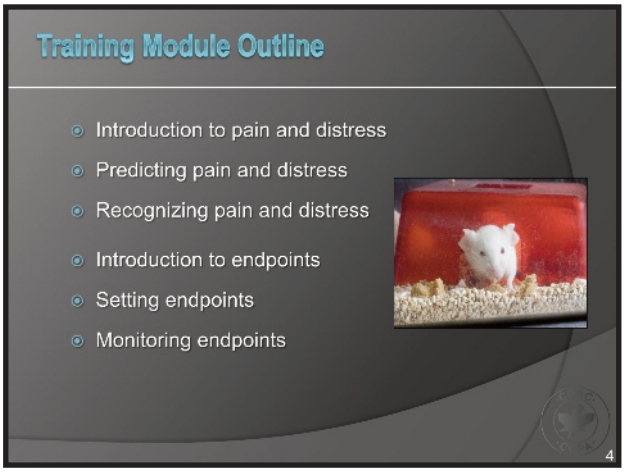


In addition, the goals of this module are to:

- define pain and distress
- discuss the prediction of pain and distress
- discuss the recognition of pain and distress
- discuss methods to assess and minimize pain and distress
- help set and monitor endpoints so that pain and distress are minimized

Slide 4 Training Module Outline

This training module will provide an overview of the following:



- introduction to pain and distress
- predicting pain and distress
- recognizing pain and distress
- introduction to endpoints
- setting endpoints
- monitoring endpoints

Slide 5 Pain and Distress

Pain is the word we use when something hurts. Pain can result from injury to tissue but may also result from threat of injury to tissue where no real tissue injury occurs. In humans, pain may be evaluated and treated based on verbal reporting on its quality and severity. How can we prevent pain in animals in the absence of this information? How do we know that animals feel pain? We share with many species much of the same neurophysiology that allows us to perceive pain. Mice are now the most commonly used model for the study of pain precisely because we share comparable neurophysiology. Presumably the ability to perceive pain to escape and learn to avoid bodily damage has been closely linked with survival and therefore has been highly conserved across species.

The International Association for the Study of Pain acknowledges the value of animals as models for the study of human pain, stating that "research using animals has contributed to remarkable gains in the understanding of the mechanisms that underlie the generation of pain and also has led to the development of new approaches to the management of pain." In short, procedures expected to cause pain in humans should be expected to cause pain in animals.

We also know that behaviours in animals that are associated with pain are decreased or eliminated with analgesics, and that animals will work to end painful stimuli or reduce their intensity. Animals will also self-administer analgesics following painful procedures. Taken together it is clear that vertebrates feel pain. In recognition of this, we are obligated to prevent unnecessary pain and distress by our national guidelines and in some jurisdictions by legislation. It is also our moral obligation to prevent unnecessary pain and distress in the animals we use in research.

Distress results from stress with which we are no longer able to cope. We experience stress when time is too short to complete our work, when something is new to us or unpredictable or perhaps when our sense of safety is threatened. Usually, we are able to adjust to these situations with no lasting effects. However, when the level of stress reaches a point where we can no longer make adjustments to effectively cope, we become distressed.

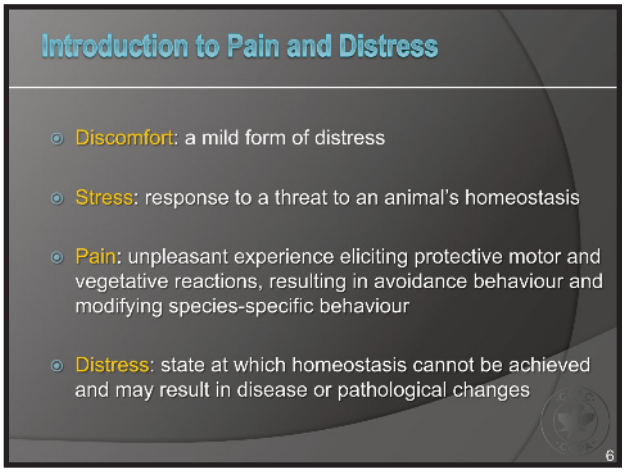
We have an obligation to prevent unnecessary pain and distress in the animals we use in research.

Additional References and Resources:

The International Association for the Study of Pain: http://www.iasp-pain.org/

Slide 6 Introduction to Pain and Distress

The following definitions of distress, discomfort and pain were developed by Canadian Council on Animal Care (CCAC) based on the Federation of European Laboratory Animal Science Associations (FELASA) definitions.



Discomfort:

Discomfort is viewed as a mild form of distress.

Stress:

Biological responses that an animal exhibits in an attempt to cope with a threat to its homeostasis (Carstens and Moberg 2000).

Pain:

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. Pain elicits protective motor and vegetative reactions, results in learned avoidance behaviour and may modify species specific behaviour (Zimmerman, 1986).

Distress:

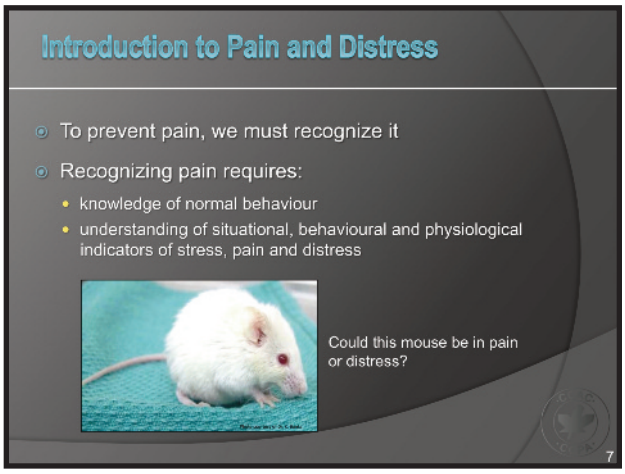
Distress is a state at which normal biological responses are no longer sufficient to achieve return to homeostasis. Normal biological functions may be disrupted as the animal must devote substantial effort or resources to challenges emanating from the environmental situation. Distress may result in disease or pathological changes.

Additional References and Resources:

Carstens E. and Moberg G.P. (2000) Recognizing Pain and Distress in Laboratory Animals. *Institute for Laboratory Animal Research Journal* 41(2):62–71.

Zimmerman M. (1986) Physiological mechanisms of pain and its treatment. *Klinische Anaesthesiol Intensivtherap* 32:1-19.

Slide 7 Introduction to Pain and Distress



In order to prevent pain we must be able to recognize it. Pain produces changes in behaviour that may be observed, however, this requires knowledge of normal behaviour. As an additional complication, species that have evolved as prey species may successfully mask signs of pain in order to avoid predation. Many laboratory species may appear to behave normally when we observe them even after painful procedures. However, if the animals are observed remotely as by a video camera, they may be sitting apart from the group, licking or scratching at an incision, stretching frequently, or showing other signs of pain.

Pain also produces physiological changes. Pain produces changes in the cardiovascular and pulmonary systems, the immune system, the gastrointestinal and urinary tracts and also has effects on food intake and body weight. Many of these changes are measurable, but alterations in these parameters may not be exclusive to painful stimuli. Use of these parameters in the assessment of pain therefore must be carefully considered in the context of behavioural changes, in addition to other measures such as response to analgesics.

Distress may be associated with pain but may also occur in the absence of pain. Like pain, distress can result from physical causes such as extreme muscular exertion or from emotional causes such as fear.

Like pain, distress produces profound changes in physiology in animals that are similar to those occurring in humans. Distress also produces changes in behaviour that may be observed, some of which are species-specific. For instance, in the evaluation of a mouse after a potentially painful procedure, the following questions may be useful:

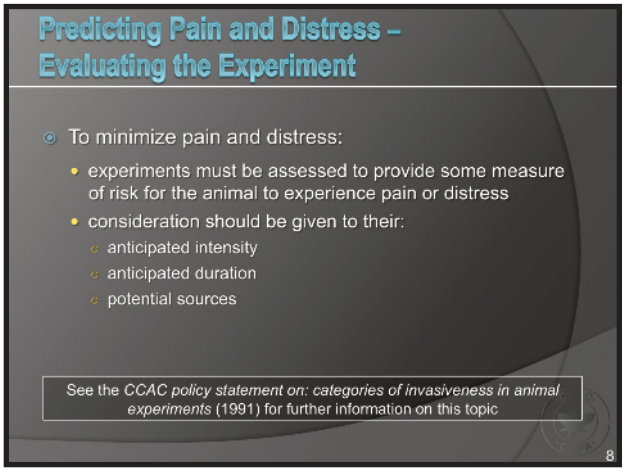
- Is the hair coat is unkempt?
- Does it have a hunched posture?
- Is it hypothermic (cool to the touch) and reluctant to move?

Knowledge of the experiment and of the species' normal behaviour is required to judge whether these behavioural and physiological changes constitute pain or distress or both.

Some seemingly neutral situations may be stressful, such as isolation for a social animal, and may progress to distress in certain individuals. Inappropriate handling and restraint of an animal unaccustomed to either may provoke intense struggling and fear with dramatic alterations in physiological parameters consistent with distress.

Pain and distress will be discussed together, and it will soon be noted that many of the behavioural and physiological changes associated with these states overlap. This is to be expected since stress is often a common factor in pain and distress. Increased understanding of situational, behavioural and physiological indicators of stress, pain and distress provides us with tools to minimize or eliminate these states with obvious ethical implications. Improvements in animal welfare are now being recognized to ameliorate the quality of science. Recognizing the profound effect of pain and distress on behaviour and many physiological parameters, it is clear that the question should not be "will treatment of pain and distress have an effect on my results?" but "what effect will pain and distress have on my results?"

Slide 8 Predicting Pain and Distress – Evaluating the Experiment



In order to minimize pain and distress, we must be able to accurately predict when they might occur. An experiment must be assessed to provide some measure of risk for the animal to experience pain or distress.

There are several means of assessing risk for pain and distress. Consideration should be given to the anticipated intensity and duration of pain or distress, and to the potential sources of pain and distress.

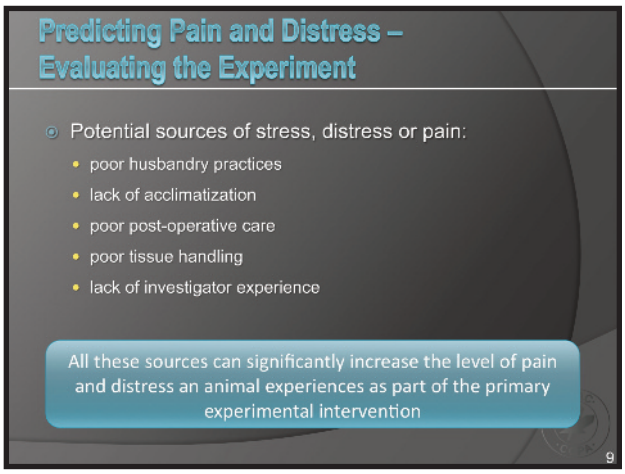
The *CCAC policy statement on: categories of invasiveness in animal experiments* (1991) requires investigators to rate their experiment on a scale for potential to produce pain or distress. In doing so, the investigator must consider the experiment for potential sources of pain or distress, their intensity and duration. In addition to these basic considerations, there are other variables that can have a significant effect on the potential for pain and distress.

Does the procedure induce only passing, momentary pain, or could it have the capacity to induce chronic pain? For example, a single intraperitoneal injection in a rat may produce only acute pain while a single intraperitoneal injection of a compound that is inherently irritating may induce chronic pain in association with inflammation of the peritoneal membrane.

Additional References and Resources:

CCAC policy statement on: categories of invasiveness in animal experiments (1991). Visit the CCAC website at www.ccac.ca to access and consult this policy statement.

Slide 9 Predicting Pain and Distress – Evaluating the Experiment



In evaluating potential sources of pain and distress, the primary focus is the experimental intervention. When investigators rate their experiments with a category of invasiveness, they are rating the experimental intervention; whether this is blood sampling, a surgery, behavioural observation or perhaps all of these. However, there are many other sources of pain and distress that should also be taken into account. Poor husbandry practices, lack of acclimatization, poor post-operative care, poor tissue handling, or low level of investigator experience can all significantly increase the level of pain and distress an animal experiences as part of the primary experimental intervention.

Consider the difference in potential for pain and distress between an animal that undergoes a laparotomy by an experienced surgeon and an animal that undergoes a laparotomy by a novice surgeon. The novice surgeon will likely inadvertently produce more trauma during the surgery due to poor tissue handling, may grasp the skin too tightly during suturing and may pull the sutures too tight, all common errors of the inexperienced surgeon. This will result in higher

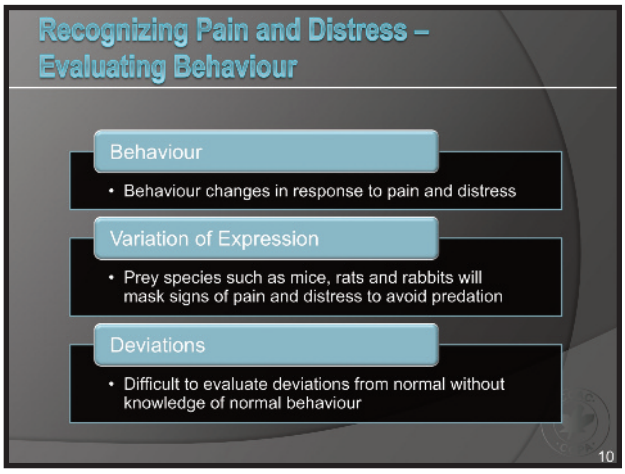
levels of pain and possibly distress in the post-operative period. We should not substitute analgesics for poor surgical technique but rather address this with careful planning and oversight provided by investigative or veterinary staff with a high level of experience.

Another parameter important to the assessment of risk for pain and distress acknowledges the particular tissue type that is subject to the experimental intervention. Some tissues have higher densities of nociceptors than others and manipulations associated with damage to these tissues should therefore be considered differently than those possessing lower densities. The brain does not have nociceptors but consideration should be given to intracranial procedures that may damage the dura or blood vessels, both tissues that do have nociceptors. A widely used risk assessment scale ranks various tissue types in descending order of sensitivity based on the degree of their innervation and nociceptor density:

- cornea
- dental pulp
- testicles
- nerves
- spinal marrow
- skin
- serous membrane
- periosteum and blood vessels
- viscera
- joints
- bones
- encephalic tissue.

In summary, when predicting risk for animals to experience pain or distress, a comprehensive approach provides the most accurate prediction and potential for targeting our focus to minimize that risk. The environment and circumstances under which the experiment is performed should be considered, together with the experience of those performing the experiment. The experimental interventions should be considered for their potential to damage tissue and the tissue type considered for its sensitivity to damage. The intensity and duration of any expected pain or distress must also be considered in the assessment process. Accurate prediction of risk for pain and distress provides us with the highest ability to plan for their eventuality and work towards their minimization.

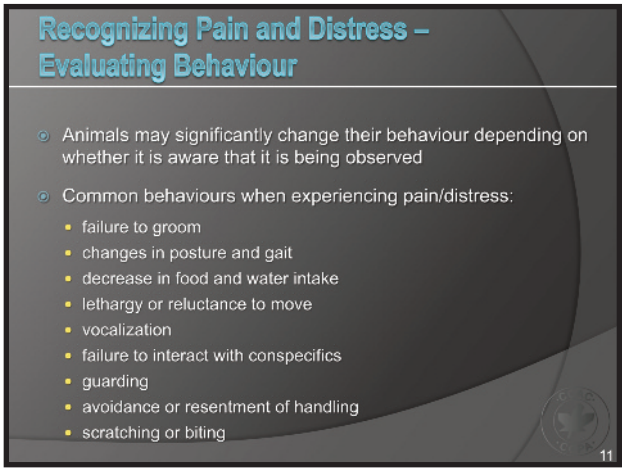
Slide 10 Recognizing Pain and Distress – Evaluating Behaviour



Animals change their behaviour in response to pain and distress. The specific changes in behaviour depend on the species, strain, and tolerance for pain of the individual animal. They also depend on the nature of the stimulus inducing these states, its intensity and duration and the circumstances under which it is applied among other factors.

Prey species such as mice, rats and rabbits will mask signs of pain and distress to avoid predation. Recognition of changes in behaviour presupposes that we have an understanding of normal behaviour in the species under observation. Without knowledge of normal behaviour, it is very difficult to evaluate deviations from normal.

Slide 11 Recognizing Pain and Distress – Evaluating Behaviour



Though we may have knowledge of common behavioural signs of pain and distress we must also take into account some limitations in interpreting them. We are potential predators to our most commonly used laboratory species (mice, rats, rabbits) making how we observe their behaviour crucially important. A rabbit may significantly change its behaviour following a painful event such as a laparotomy depending on whether it is aware that it is being observed. As a prey species, it will mask signs of pain and distress to avoid predation. If the rabbit is observed remotely however, it may engage in behaviour indicative of pain or distress and then abruptly cease these behaviours when the investigator reappears. We should therefore attempt to gather as much information as possible about the animal's environment and behaviour before we make our presence felt. Quiet observation of a mouse in its cage before the cage is disturbed can reveal important changes in behaviour that will be disrupted by handling of the cage or the mouse.

Many laboratory species are nocturnal. For ease of working, most facilities will have their lighting set to be on during the day rather than on reverse cycle (lights off during daytime hours). We will therefore be disturbing nocturnal animals from their sleep to work with them. There may also be disturbances in sleep in association with pain or distress. An animal that is awake and engaged in repetitive behaviours while its cage mates sleep may be in pain or distress. Knowledge of a species sleep-wake cycle can be an important consideration in interpreting behaviour.

Many laboratory species share common behaviour when experiencing pain and distress. These include:

- failure to groom
- changes in posture and gait

- decrease in food and water intake
- lethargy or reluctance to move
- vocalization
- failure to engage with conspecifics in social species
- guarding
- avoidance or resentment of handling
- grinding of teeth
- scratching or biting.

Slide 12 Recognizing Pain and Distress – Evaluating Behaviour

Although some behaviours associated with pain and distress are species-specific, understanding the importance of these behaviours and taking the time to look for them is a very powerful tool in recognizing pain and distress.

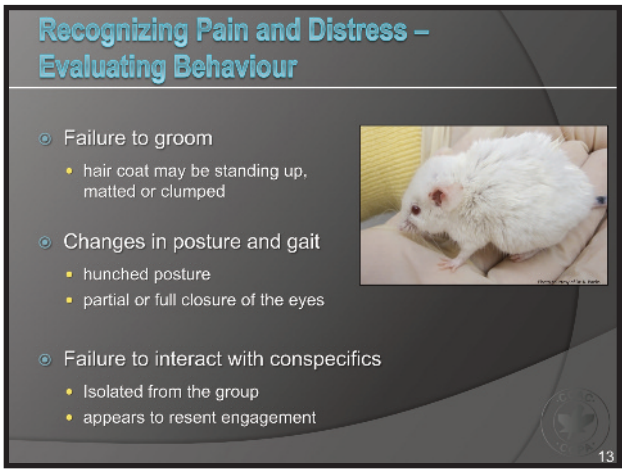
Slide 13 Recognizing Pain and Distress – Evaluating Behaviour

Failure to groom:

Grooming is an important behaviour for animals and many species invest considerable time engaged in this behaviour. Failure to groom is a very reliable early sign of pain or stress. The hair coat may be standing up (piloerection) rather than lying flat. It may be dull rather than shiny and it may be matted or clumped, particularly around the face and mouth and the anal and genital areas.

Changes in posture and gait:

Other aspects of an animal's physical appearance can also be reliable signs of pain or distress. A hunched posture and closure or partial closure of the eyes are also common indicators of these states.

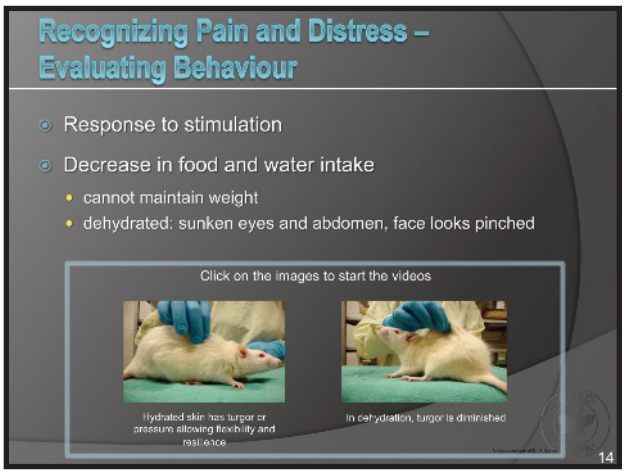


Failure to interact with conspecifics:

Many laboratory animal species are social species. These species will work to gain access to conspecifics and may develop abnormal behaviour and physiology when isolated. When social species are housed together we can often observe them engaging in mutual grooming, nest building, sleeping in groups or at play. An animal that is isolated from the group, and appears to resent engagement with a cage mate by displaying defensive behaviours such as rearing, vocalizing or biting may be in pain or distress.

Slide 14 Recognizing Pain and Distress – Evaluating Behaviour

Decrease in food and water intake:



Food and water intake is very often decreased when an animal is in pain or distress. Animals that are in pain or distress may simply not feel well enough to eat or drink sufficiently to maintain their body weight. Alternatively, the underlying cause of pain or distress may increase an animal's energy expenditure contributing to a net loss of energy resulting in weight loss. Many types of cancer that are modeled in animals may result in emaciation as they progress to latter stages of the disease. However, any experimental intervention that causes even low levels of pain or stress has the potential to result in inappetence and weight loss. Because many laboratory animal species are housed in groups or have an automatic supply of water, it can be difficult to detect changes in food and water intake. However, loss of body weight can be used as an indirect measure of a decrease in food and water intake. Animals undergoing experimental interventions with high risk for pain or distress should be closely monitored to allow accurate assessment of loss of body weight.

Body condition scoring may be a useful adjunct to measurement of body weight in models of disease where weight loss may be chronic and accompanied by emaciation and muscle wasting. In certain models of cancer where the weight of a tumour may offset loss of body weight, body condition scoring can be particularly useful. It involves assessment of the animal's physical appearance by observation and palpation at standardized locations on the body to provide a rating of body condition on a 5-point ordinal scale. (See: Appendix 1 – Body Condition Scoring).

Dehydration is detectable by simple observation and by a manual test known as the skin turgor test. In dehydration, the body experiences a deficit in water. The body's cells and the tissues they comprise shrink as they lose water. Dehydrated animals tend to have a characteristic appearance related to the loss of water in these tissues; the eyes may be sunken in the orbits, the abdomen will have a sunken appearance, and the contours of the face may appear sunken giving it a 'pinched' appearance. The mucous membranes will also be dry and the coat will tend to be dull. Animals that have experienced chronic and severe loss of body weight may show some of these signs as their body fat is metabolized for calories.

Response to stimulation:

Response to stimulation is also an invaluable tool in the assessment of behavioural signs of pain and distress. Once an animal has been observed at rest for a period of time, hopefully without alerting it to our presence, we should evaluate its response to stimulation. This is most effectively done in a step-wise manner to avoid inadvertently inducing further pain or distress if these states are already present. Slowly proceed from alerting the animal to your presence, to opening the cage and finally to handling the animal. Each of these steps

represents increasing levels of stimulation, and by simple observation we can gather very important information on the health status of the animal. It also allows us to stop at early stages of stimulation if the animal appears to be unable to cope with lower levels of stimulation. An animal that is under a high level of stress in association with pain or fear may be provoked to distress simply by being handled or having its cage opened. A mouse that has an increased respiration rate before opening the cage may progress to open mouth breathing merely from the stress of handling.

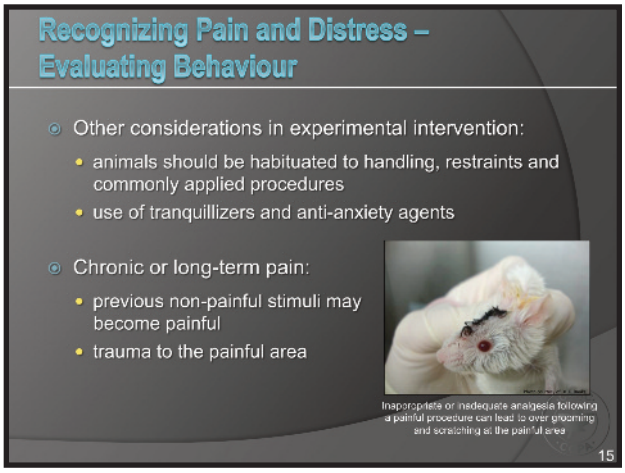
Other important clinical signs and behaviours may also be gleaned through careful handling. An animal that is cool to the touch is hypothermic and is likely also reluctant to move, both of which are grave signs. Smaller mammals such as mice and rats have very high metabolic rates and will rapidly lose body temperature when inactive and not eating. Colour of the mucous membranes or extremities in albino animals may also be assessed. Pallor is commonly associated with pain or distress but may also accompany hypothermia, anemia or other conditions.

Additional References and Resources:

Folz C.J. and Ullman-Culleré M.H. (1999) Body condition scoring: a rapid and accurate method for assessing health status in mice. *Laboratory Animal Science* 49(3):319-323.

Slide 15 Recognizing Pain and Distress – Evaluating Behaviour

Other considerations in the recognition of pain and distress relate to the circumstances of an experimental intervention. Short-term pain (e.g., an injection) may be very well tolerated, depending on the site and volume of injection and the physical characteristics of the compound injected. There may be few behavioural changes as a result of a subcutaneous injection of a non-irritating material of low volume. However, consider the same procedure applied to an animal that has just arrived from a commercial supplier and that has no experience with handling or restraint. The animal will likely be under considerable stress associated with its journey and it will likely be extremely fearful and resent handling. A rat may vocalize and attempt to bite under these circumstances. Vocalization and biting are behavioural signs of fear and distress in this situation.



Fear is an important modulator of the experience of pain and stress. Animals should be habituated to handling, restraint and commonly applied procedures to allay fear and stress related to these manipulations and to circumvent the behavioural and physiological effects of these states on experimental results. Tranquillizers and anti-anxiety agents can be useful adjuncts to analgesia or anesthesia for their capacity to reduce fear and stress.

Chronic or long term pain (e.g., arthritis, orthopedic procedure) may produce more subtle behavioural changes. Animals and people react to protracted pain through various coping mechanisms. Descending pathways from the brain to the spinal cord inhibit ascending neuronal activity relaying painful stimuli and reduce the sensation of pain. In addition, endogenous opioids (e.g., endorphins and enkephalins) are released in the central nervous system that may provide some relief of pain. These internal adaptations tend to dampen external behavioural changes associated with pain.

Chronic pain can also result in changes to how animals respond to other stimuli. Stimuli that were previously non-painful may become painful. We have likely all had the experience of a painful area being very

sensitive to stimulation such that even a light touch may be perceived as painful. Occasionally, persistent pain can cause an animal to traumatize the area that is painful.

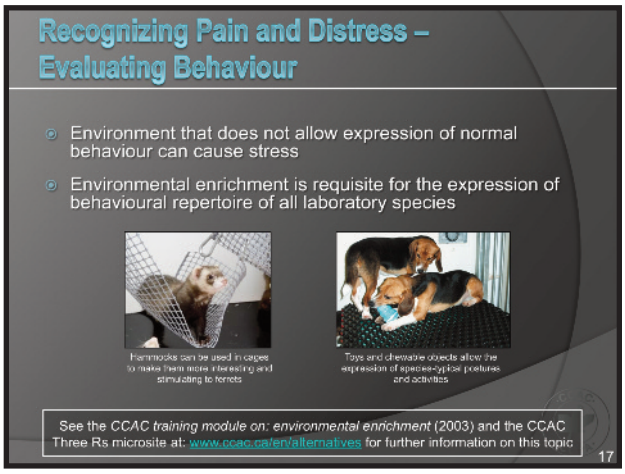
Slide 16 Recognizing Pain and Distress – Evaluating Behaviour

Response to analgesia can also be invaluable in the recognition of pain. Observation of changes in behaviour which disappear or whose frequency are diminished following administration of an analgesic is good indication that pain was the basis for the observed change in behaviour. Abdominal contractions and writhing in mice induced by intraperitoneal injection of irritating compounds are both behaviours reduced by analgesics.

More recent technologies allow for sophisticated computer-assisted analysis of a full range of behaviours that may be applied in the recognition of pain and distress. Ultrasonic vocalizations associated with pain and distress have been characterized in rodents and equipment for their measurement is now commercially available.

See the *CCAC training module on: analgesia* (2003) for further information on this topic. Visit the CCAC website at www.ccac.ca to access and consult this training module.

Slide 17 Recognizing Pain and Distress – Evaluating Behaviour



An animal may experience stress when an impoverished environment does not allow expression of the normal behavioural repertoire. This may progress to distress if additional stress is applied as part of normal husbandry or an experimental intervention. Environmental enrichment is a requisite part of the cage environment to allow for the expression of the normal behavioural repertoire of all laboratory species.

However, some individuals may require more enrichment than others and may develop species-specific non-purposeful repetitive behaviours called stereotypies. In these cases, more enrichment should be provided or other changes to the environment should be made as a stress reduction strategy. A social species that is isolated could be group-housed; in species with strong hierarchy, the cage position may be changed to allow an animal to escape being viewed by another more dominant animal. Certain experiments may require social species to be housed alone. In these cases it is our responsibility to ensure that other outlets for expression of normal behaviour are provided. The effects of deprivation of social species from contact with conspecifics should not be underestimated. Single housing of rodents has been shown to induce immunosuppression that is associated with more rapid growth of tumours.

Additional References and Resources:

See *CCAC training module on: environmental enrichment* (2003) for more information on this topic. Visit the CCAC website at www.ccac.ca to access and consult this training module.

CCAC Three Rs microsite: http://www.ccac.ca/en/alternatives/

Russell W.M.S. and Burch R.L. (1959) *The Principles of Humane Experimental Technique*. London UK: Methuen.

Slide 18 Recognizing Pain and Distress – Evaluating Behaviour

There are many excellent resources for an introduction to normal behaviour in many laboratory species that may assist the investigator new to the subject. Your local veterinary and veterinary technical staffs are also excellent resources.

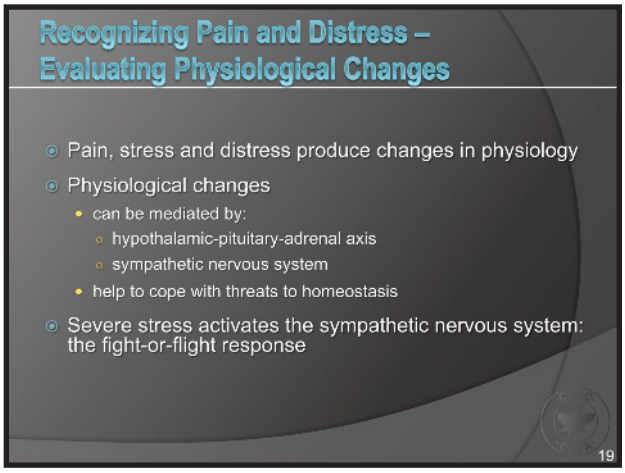
Additional References and Resources:

Assessing the Health and Welfare of Laboratory Animals: www.ahwla.org.uk/

Canadian Council on Animal Care (1993) *CCAC Guide to the Care and Use of Experimental Animals*, vol. 1, 2nd ed. Ottawa ON: CCAC. Visit the CCAC website at www.ccac.ca to access and consult this guide.

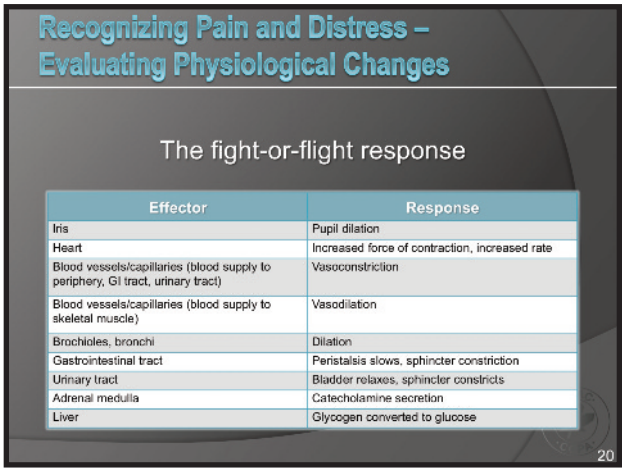
Slide 19 Recognizing Pain and Distress – Evaluating Physiological Changes

Recall that pain has a neurophysiological basis known as nociception, and that it also produces changes in the cardiovascular and pulmonary systems, the immune system, the gastrointestinal and urinary tracts. Stress can produce many of the same changes as pain. Many of these changes are mediated by the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). A painful or stressful experience can stimulate the HPA axis resulting in the release of glucocorticoids (cortisol in most mammals, corticosterone in rodents) and catecholamines (principally epinephrine) from the adrenals. The SNS, also responsive to pain and stress, releases epinephrine and norepinephrine when activated.



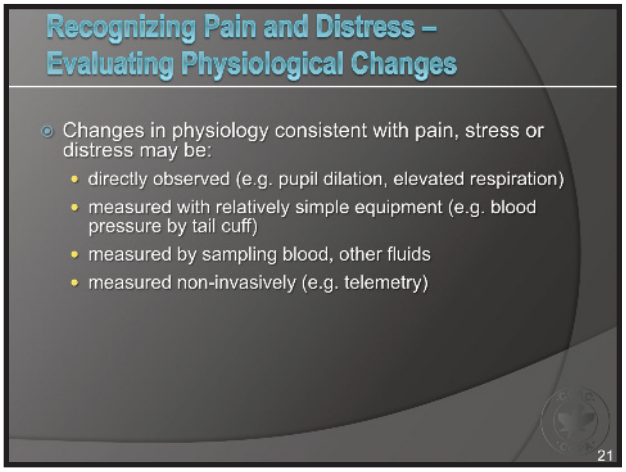
Slide 20 Recognizing Pain and Distress – Evaluating Physiological Changes

Glucocorticoids and catecholamines produce physiological changes that help the body cope with threats to its homeostasis. A severe stressor activates the SNS more robustly and will induce physiological changes collectively known as the flight-or-fight response. Alterations in blood pressure, heart rate, respiration and many other parameters allow humans and animals to mobilize their bodies to either fight or flee danger.



Slide 21 Recognizing Pain and Distress – Evaluating Physiological Changes

Some physiological indicators of pain, fear and stress may be directly observed. Dilation of the pupils and increase in respiration rate are common signs of these states and are readily observable. However, the evaluation of many physiological parameters requires obtaining blood or other samples that involve handling and restraint of the animal. These interventions may exacerbate any pain, fear or stress the animal is experiencing and produce variability in the parameter being measured. Occasionally, handling and restraint of the animal may in themselves produce larger deviations in the parameter being measured than the painful or stressful stimulus under study. This is especially true of wild species or of animals that have not been habituated to handling or restraint. Some species are more amenable to repeated handling and restraint than others and can readily learn to undergo these procedures with a minimum of apprehension or stress.

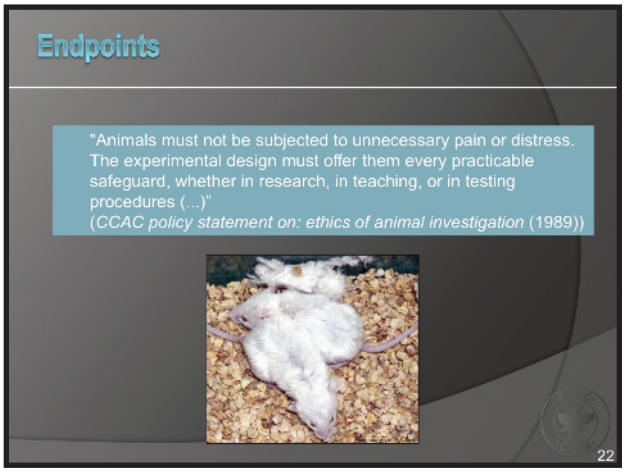


Heart rate and blood pressure may be measured by relatively simple interventions. A common method of obtaining indirect blood pressure measurements is through use of the Doppler technique employing a cuff placed around a peripheral artery. The tail artery may be used for this purpose in rodents. As an alternative, telemetry may be used to avoid the requirement of handling the animal to obtain these measurements. Transmitters are surgically implanted which transmit various physiological parameters to a remote receiver. Body temperature, heart rate, and blood pressure are commonly measured parameters using telemetry. However, telemetry obviously involves a surgical intervention, which would be expected to be associated with some measure of pain or stress itself. The question remains what effect might the intervention have on subsequent responses to other stimuli.

Recall that stimulation of the HPA axis and SNS results in elevations in glucocorticoids (cortisol or corticosterone) and catecholamines. Cortisol, corticosterone or their metabolites may be measured in the blood, urine, saliva or feces of many species. Commercial kits are available for measurement of cortisol and corticosterone in some species. Catecholamines are most commonly measured in the blood.

Through extensive connections between the autonomic nervous system, the HPA axis and the immune system, pain and stress can also affect measurable alterations in immune function. Certain populations of cytokines, which act as effectors of the immune system, are sensitive to stress and variations and their levels may be used as indication of stressful events. Chronic stress can induce immunosuppression, which can have devastating effect in models of chronic disease. Suppression of the immune system may itself be the subject of some studies as a modulator of chronic disease.

Slide 22 Endpoints

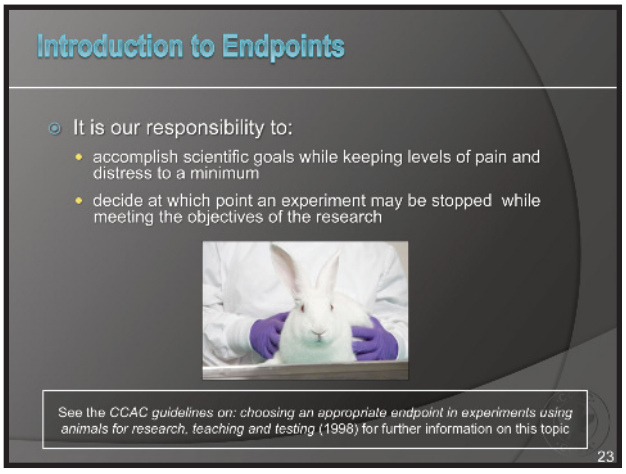


As stated in the *CCAC policy statement on: ethics of animal investigation* (1989), "Animals must not be subjected to unnecessary pain or distress. The experimental design must offer them every practicable safeguard, whether in research, in teaching, or in testing procedures (...)".

This section of the module is devoted to the issue of setting endpoints so that the pain and distress in invasive experiments is minimized.

For thorough information on pain management as a mean to minimize pain and distress, see the *CCAC training module on: analgesia* (2003) and the *CCAC training module on: anesthesia* (2003).Visit the CCAC website at www.ccac.ca to access and consult the policy statement and these training modules.

Slide 23 Introduction to Endpoints



Causing some level of pain or distress is unavoidable in some research programs. Sometimes we must induce disease in order to study disease. Alternatively, through knowledge of the underlying pathways of the pathogenesis of disease, we may be able to study perturbations in these pathways and avoid induction of the disease itself. Nevertheless, it remains our responsibility to search for ways to accomplish the specific goals of a research program while maintaining the level of pain or distress any experimental experiences within it to an absolute minimum.

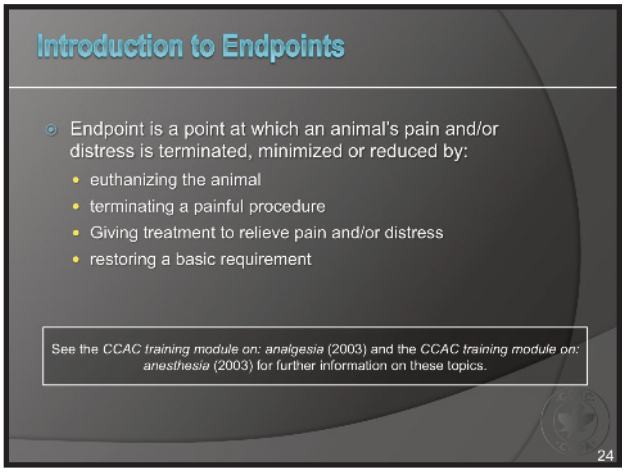
Anticipating when pain may occur and formulating a pain management plan are important components of minimizing pain. Adjunctive treatments such as fluid therapy, provision of a supplemental heat source, of a highly palatable source of nutrition, of softer contact bedding may be other strategies for minimization of pain and distress. But most important, the scientific goal should be determined so that pain

and distress are minimized by planning ahead of time at what point an experiment may be stopped, to avoid pain and distress that would occur if the experiment continued. Stopping the experiment at a point that pre-empts the collection of useful data would render the experiment useless. The point at which an experiment is stopped must therefore be compatible with the objectives of the research.

See the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998) for further information on this topic. Visit the CCAC website at www.ccac.ca to access and consult this guidelines document.

Slide 24 Introduction to Endpoints

The term "endpoint" can be defined as the point at which an experimental animal's pain and/or distress is terminated, minimized or reduced by taking actions such as humanely killing the animal, terminating a painful procedure, or giving treatment to relieve pain and/or distress. An endpoint might also involve restoring a basic requirement e.g. withdrawing an unpalatable experimental diet and restoring a palatable diet at a certain level of weight loss, or restoring group housing for a social species where isolation is determined to cause distress.



Additional References and Resources:

- *CCAC training module on: analgesia* (2003).
- *CCAC training module on: anesthesia* (2003).

Visit the CCAC website at www.ccac.ca to access and consult these training modules.

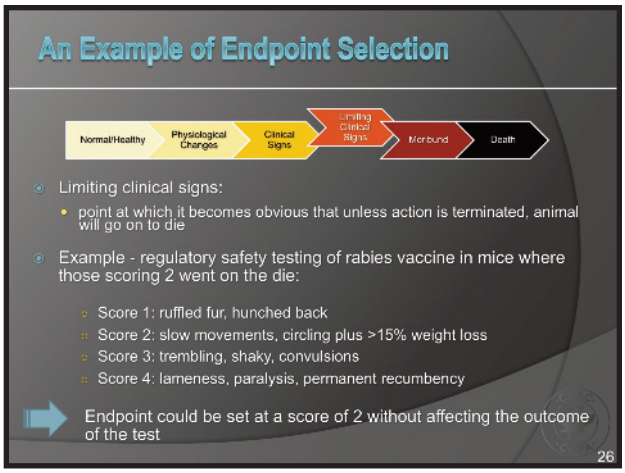
Slide 25 Selecting Endpoints

The *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998) states: "In experiments involving animals, any actual or potential pain, distress, or discomfort should be minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research. Selection of this endpoint by the investigator should involve consultation with the laboratory animal veterinarian and the animal care committee." This statement acknowledges that there are both ethical and scientific considerations that go into determination of an appropriate endpoint. Arriving at that decision involves the principal investigator and those individuals at the institution given the responsibility for the care and judicious use of experimental animals.

See the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998) for further information on this topic as well as *CCAC training module on: analgesia* (2003)

and the *CCAC training module on: anesthesia* (2003). Visit the CCAC website at www.ccac.ca to access and consult this guidelines document and training modules.

Slide 26 An example of Endpoint Selection



In selecting endpoints, it is useful to consider the experiment in terms of the various stages of discomfort, pain and distress that it may produce. The various stages of an animal's condition in a generic invasive experiment can be depicted on a scale from "normal" through "moribund" to death at the other end of the scale.

When an invasive procedure is performed that has possible lethal effects, there is generally a progression of events that occur in the experimental animal. Its condition deteriorates from being a "normal" healthy animal. In studies that involve infection, cancer, or arthritis, for example, as the condition progresses there may be increasing pain and distress. Eventually the condition of the animal may reach a point where it becomes obvious that unless action is taken to terminate the condition, the animal will go on to die. This point on the scale is called the "limiting clinical signs".

An example of limiting clinical signs is in regulatory safety testing of the rabies vaccine in mice. The traditional endpoint for this test has been death in the control animals (and perhaps in some dilutions of vaccinated animals). However, using a 4 stage clinical scoring system:

Score 1: ruffled fur, hunched back

Score 2: slow movements, circling, and than 15% weight loss

Score 3: trembling, shaky, convulsions

Score 4: lameness, paralysis, permanent recumbency

The researchers found that all mice progressing to score 2 went on to die. The observations ascribed to a score of 2 were the most significant predictors of further deterioration in the animal's condition, and a score of 2 was the earliest point at which those signs appeared. Therefore the experimental endpoint could be set at a score of 2 rather than waiting until the mice died, without affecting the outcome of the test.

When the limiting signs of the intervention are unknown, a pilot study using a few animals under close observation may provide the information to allow the earliest endpoint to be found.

The example of testing of rabies vaccines also raises the issue of the balance between scientific and ethical considerations in selecting an endpoint. If the scientific objectives are not met because an experiment was terminated too early, then the study and the animals' lives are wasted. The endpoint should not change the outcome or invalidate the results. The objective is to maintain the validity of the experiment, while holding any pain and distress to a minimum.

The example of testing of rabies vaccines makes use of observational study of the mice to identify behaviours that were associated with rabies infection and its various stages. Every experiment will likely have a distinct

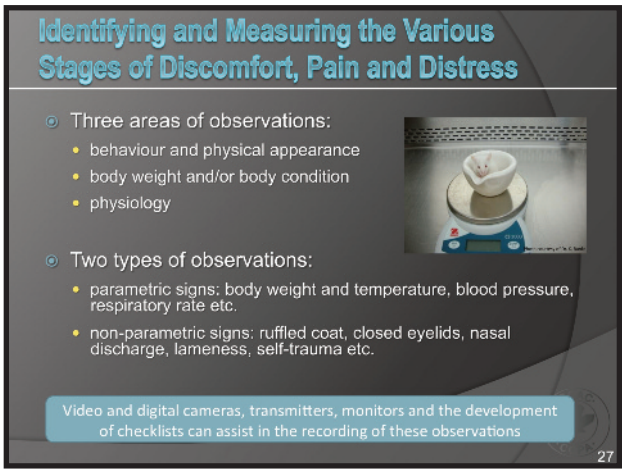
set of behaviours and/or physiological changes that may be used to identify animals at various stages of severity of their condition. These may be used as tools in the selection of the endpoint. A study of the pain of castration in lambs will use different observations than a study of bacterial infection in mice. Nevertheless the approach to making (and recording) the observations will be much the same.

Slide 27 Identifying and Measuring the Various Stages of Discomfort, Pain and Distress

For most studies, there are three areas in which observations of the animal should be made:

- behaviour (both when the animal is at rest and when it is stimulated) and physical appearance
- body weight and/or body condition (and related changes in food and water intake)
- physiology (e.g., body temperature, heart rate, respiratory rate)

Of these, measuring and recording changes in physical appearance, behaviour and body weight should be considered for almost every endpoints assessment.



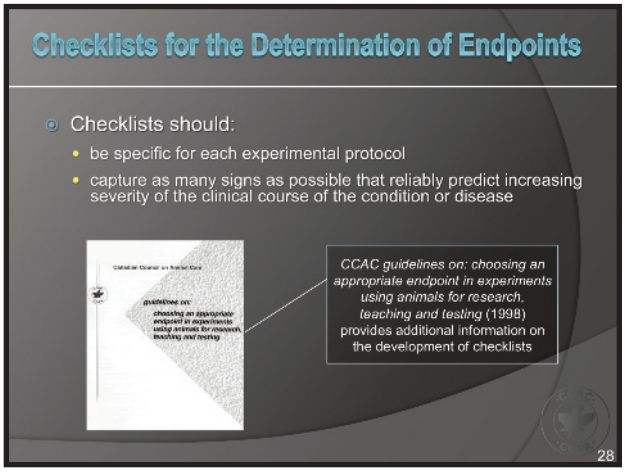
There are two types of observations that can be made:

- parametric signs (numerical or continuous scale indicating deviation from normal). These observations may include: body weight; body temperature; blood pressure; respiratory rate; activity level.
- non-parametric signs (presence or absence of signs). These observations may include ruffled coat, closed eyelids, nasal discharge, lameness, hunched posture, recumbent, circling, vocalisation, self-trauma, dyspnea, seizures.

An interval scale may be created for an observation (for parametric signs) with increasing deviation from normal defined, to allow for a parameter to be monitored over time as an animal progresses through increasing levels of severity of their condition. This approach helps ensure that the observations are as objective as possible. An endpoint can then be pre-set at a specific stage of severity as defined by a specific score on the scale.

Some technologies that can assist in the recording of these observations include video cameras, digital cameras, implantable telemetry transmitters, locomotor activity monitors, and computer assisted behaviour monitors. However, simple observations may be sufficient (as in the rabies vaccine example) and preclude the need for specialized equipment. Digital and video cameras may now be purchased inexpensively and can be extremely useful in capturing images of animals approaching or at endpoints or displaying specific clinical signs or behaviours that may be used as training aids.

Slide 28 Checklists for the Determination of Endpoints



In some cases, the development of checklists may be required to determine appropriate endpoints. Several scaled observations and non-parametric observations may be compiled to create a checklist (See: Appendix II – Example of a Checklist for the Determination of Endpoints). To be effective, the checklist should be specific for each experimental protocol, and should capture as many signs as possible that reliably predict increasing severity of the clinical course of the condition or disease. Once each sign's score is rated, all scores are then added together. Endpoints are predetermined at a specific total score. By repeatedly applying the checklist over several time points, the clinical course may be accurately tracked. Interventions such as fluid therapy, provision of a supplementary heat source, or palpable foods may also be provided at predetermined total scores.

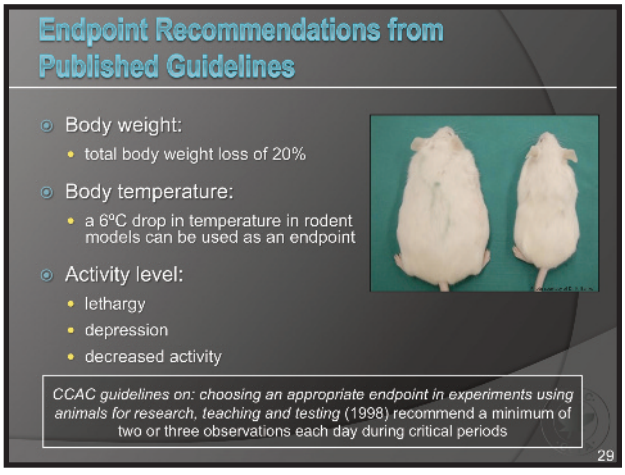
The *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998) provides additional information on the development of checklists. Visit the CCAC website at www.ccac.ca to access and consult this guidelines document.

Additional References and Resources:

Lloyd M.H. and Wofenshohn S.E. (1998) Practical use of distress scoring systems in the application of humane endpoints. In: *Humane Endpoints in Animal Experiments for Biomedical Research, Proceedings of the International Conference*. November 22-25 1998, Zeist, The Netherlands (Hendriksen C.F.M. and Moron D.B., eds). London UK: Royal Society of Medicine Press, pp.48-53.

Slide 29 Endpoint Recommendations from Published Guidelines

Body weight:



As an indirect measure of food and water intake, body weight is an easily measured, objective data point amenable to the determination of endpoints. The rate, duration and extent of weight loss are all important in the determination of endpoints. Weight loss of 20% of total body weight is identified as an endpoint in the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998), as well as the UK Coordinating Committee on Cancer Research (UKCCCR) *Guidelines for the Welfare of Animals in Experimental Neoplasia*. A number of other endpoints for animal models of cancer research are also presented in the latter publication. For example, the total mass of a tumour should not exceed 10% of the normal bodyweight of the tumour-bearing animal.

Body condition scoring may be necessary to identify loss of body weight in cancer studies where weight loss may be offset by the growth of the tumour or where metabolic changes result in muscle wasting with lesser deviation in body weight such as diabetes models.

Body temperature changes:

A number of studies have shown that body temperature can be used an endpoint in rodent models of infectious disease (bacterial and viral). As an example, in a mouse model of influenza, animals whose body temperature dropped more than 6°C went on to die, and so a 6°C drop in body temperature was proposed as an endpoint in studies of this nature.

Change in activity level:

Lethargy, depression, and decreased activity levels accompany many disease conditions (mediated in part by the actions of cytokines released in response to infection, inflammation or tissue injury). In rodents, observing this decrease in activity may require observing them during the dark phase of the room's lighting system.

How often should the observations be made?

The *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998) recommend a minimum of two or three observations each day during critical periods, and more frequently if necessary to ensure that no animal's condition progresses past the set endpoint. In pilot studies, continuous monitoring using video equipment can be helpful in identifying critical times.

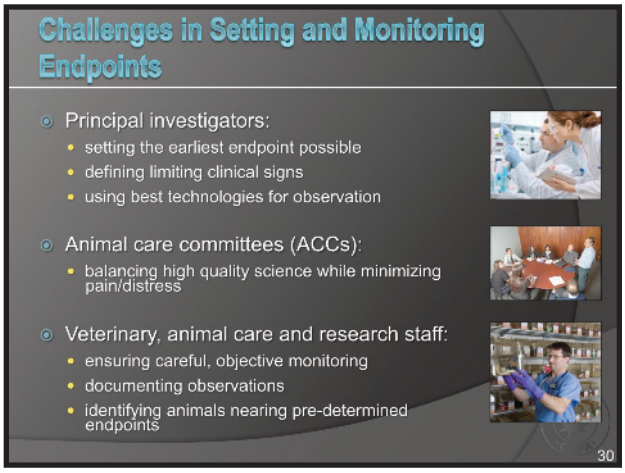
Additional References and Resources:

CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing (1998). Visit the CCAC website at www.ccac.ca to access and consult this guidelines document.

Workman P., Balmain A., Hickman J.A., McNally N.J., Rohas A.M., Mitchison N.A., Pierrepoint C.G, Raymond R., Rowlatt C., Stephens T.C., Wallace J. and Straughan D.W. (1988) UKCCCR Guidelines for the welfare of animals in experimental neoplasia. *Laboratory Animals* 22(3):195-201.

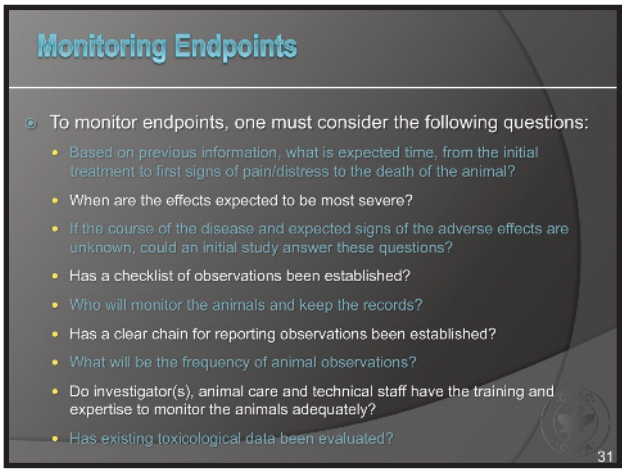
Slide 30 Challenges in Setting and Monitoring Endpoints

The challenges for principal investigators include setting the earliest scientific endpoint possible, defining limiting clinical signs, and using best technologies for obtaining necessary observations. The challenge for animal care committees is to balance the requirements for high quality science with the responsibility to minimize pain and distress. This includes assisting investigators to find earlier, data driven endpoints wherever possible. The challenges for veterinary, animal care and research staff include: ensuring careful, objective monitoring of all animals; documenting observations made; identifying animals nearing a pre-determined endpoint.



The validity and reliability of experimental results can benefit from improvements in animal welfare realized in the determination of endpoints.

Slide 31 Monitoring Endpoints



There may be some studies in which going beyond normally accepted endpoints could be scientifically justified, for example, cancer treatments or treatments for other serious diseases. For these to be acceptable from a welfare point of view, the animals need to be treated as if they were in intensive care, and provided with all possible measures to alleviate their pain and distress while allowing the study to proceed. The following questions may be helpful in assisting animal care committees and principal investigators to achieve optimal welfare when undertaking these types of studies.

What is the expected time course for the animals, from the initial treatment to first signs of pain/distress to the death of the animal, based on previous information with the specific model under study?

When this knowledge is not available from previous studies, a pilot study with a few animals might provide a means of assessing the time-course of events during a study, to predict the time at which the effects on the animals are the most severe, and the times at which the animals need the most careful monitoring. This information is needed to decide when the most intensive monitoring of the animals should take place so that the endpoint is reached when the relevant personnel are present and can terminate the experiment.

When are the effects to the animal expected to be the most severe?

Knowledge of the time course of the development of a tumour, or infectious disease can also assist in determining when the animals require the most attention. Conditions that are acute, i.e., that progress to severe dysfunction or death in a short period of time, are of particular concern.

Providing special care for animals whose condition is severely compromised would help ease the pain and distress they experience.

If the course of the disease and expected signs of the adverse effects are unknown, could an initial pilot study, under close observation by the investigator and/or laboratory animal veterinary staff answer these questions?

Observation by the investigator should ensure that the necessary scientific objectives are being reached, while the laboratory animal veterinary staff can provide the expertise with regard to clinical signs of pain and/or distress.

Has a checklist of observations, on which the endpoint will be based, been established?

If not, the pilot study affords the opportunity to compare observations of control animals with treated animals and to identify indicators that can be used to establish the earliest possible endpoint.

Who will monitor the animals (identify all responsible) and keep records?

All the persons involved in the care and monitoring of the animals should be identified at the outset, and must be skilled at recognizing signs of pain and distress.

Has a clear chain for reporting observations been established?

It is crucial that the individual(s) responsible for monitoring the animals have a clear reporting line so that the individual with responsibility for deciding on the termination of the experiment is informed promptly of changes in the animal that indicate the selected endpoint is imminent.

What will be the frequency of animal observations during the course of the study and during the critical times for the animals?

The animal care committee must be assured that the animals are going to be monitored with sufficient frequency to enable the staff responsible to identify any animals approaching the endpoint.

Do the investigator(s), animal care and technical staff have the training and expertise to monitor the animals adequately?

The animal care committee must also be assured that the individuals responsible for monitoring the animals have the training and experience to do the monitoring.

What provisions have been made to deal with any animals that show unexpectedly severe signs and symptoms?

Provision should always be made to deal with unanticipated pain and/or distress.

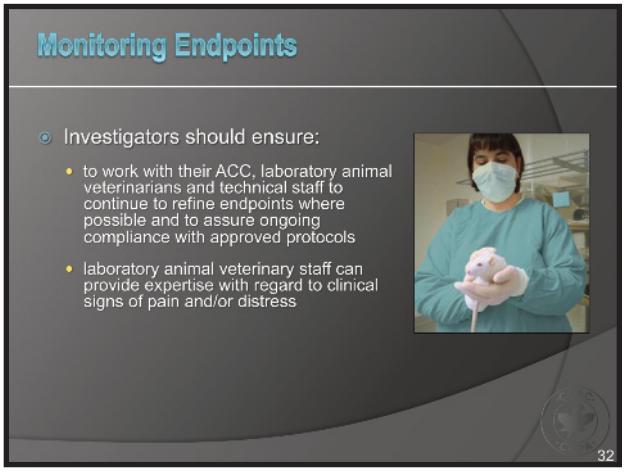
Has existing toxicological data been evaluated?

It may be possible to predict clinical signs from background data or databases for similar chemicals or substances. Information from human or veterinary clinical practice with similar substances may also be useful.

Slide 32 Monitoring Endpoints

Scientific justification for endpoints should not rest wholly on comparison with published data, as this does not permit refinement of endpoints. A pilot study might be used to compare a new scientifically justifiable endpoint with data from a previous study using an older, later endpoint.

After endpoints have been established and approved by animal care committees, investigators should continue to work with their committees, laboratory animal veterinary staff and technical staff to refine endpoints where possible. Searches of the scientific literature may yield information that could be used to refine and update endpoints. Investigators must also accept responsibility for assuring ongoing compliance with their endpoints as approved by their local animal care committees.



Slide 33 Summary

In deciding that an animal is in pain or distress, a single clinical sign may be sufficient. But more often we are required to evaluate multiple parameters in order to arrive at this decision. We have seen that careful assessment of the experimental intervention and circumstances of its application are instrumental in determining risk for pain and distress. With this informed approach, simple observation of changes in physical appearance and behaviour are often sufficient to recognize signs of pain or distress. These observations may be complimented with measurement of changes in physiology to arrive at a more definitive conclusion.

Where the risk for pain and distress is high, investigators must also consider how best to minimize the number of animals undergoing these procedures and/or how to maximize the amount of information obtained per animal.

As noted in the *CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing* (1998), in experiments involving animals, any actual or potential pain, distress, or discomfort should be prevented, minimized or alleviated by choosing the earliest endpoint that is compatible with the scientific objectives of the research. Investigators should strive to achieve their scientific goals in line with the best possible animal welfare standards.

By selecting appropriate endpoints that reflect a high standard of animal welfare, we can also achieve a higher standard of science.

Additional References and Resources:

CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing (1998). Visit the CCAC website at www.ccac.ca to access and consult this guidelines document.

Appendix I
Body Condition Scoring

BC 1	
	Mouse is emaciated • Skeletal structure extremely prominent; little or no flesh cover • Vertebrae distinctly segmented
BC 2	
	Mouse is underconditioned • Segmentatiom of vertebral column evident • Dorsal pelvic bones are readily palpable
BC 3	
	Mouse is well-conditioned • Vertebrae and dorsal pelvis not prominent; palpable with slight pressure
BC 4	
	Mouse is overconditioned • Spine is a continuous column • Vertebrae palpable only with firm pressure
BC 5	
	Mouse is obese • Mouse is smooth and bulky • Bone structure dissappears under flesh and subcutaneous fat
A "+" or "-" can be added to the body condition score if additional increments are necessary (i.e. … 2+, 2, 2-…)	

Appendix II
Example of a Checklist for the Determination of Endpoints

Parameter	Animal ID	Score	Date/Time	Date/Time
Appearance	Normal	0		
	General lack of grooming	1		
	Piloerection, fresh ocular and nasal discharges	2		
	Piloerection, hunched up	3		
	Above and eyes half closed	4		
Natural behaviour	Normal	0		
	Minor changes	1		
	Less mobile and isolated, but alert	2		
	Restless or very still, not alert	3		
Hydration status	Normal	0		
	Abnormal skin pinch test	5		
Clinical signs	Normal respiratory rate and pattern	0		
	Slight changes, increased rate only	1		
	Increased rate with abdominal breathing	2		
	Decreased rate with abdominal breathing	3		
	Marked abdominal breathing and cyanosis	4		
Provoked behaviour	Normal	0		
	Minor depression or exaggerated response	1		
	Moderate change in expected behaviour	2		
	Very weak and precomatose	3		
	Total	0-19		

Several parameters of physical appearance, behaviour and physiology have been assigned ordinal scales according to severity and compiled. Various planned interventions may be performed at predetermined total scores prior to reaching the endpoint.

From: Lloyd and Wolfensohn (1998) Humane Endpoints in Animal Experiments for Biomedical Research. In: *Humane Endpoints in Animal Experiments for Biomedical Research, Proceedings of the International Conference*, 22-25 November 1998, Zeist, The Netherlands. (Hendricksen C.F.M. and Morton D.S. eds). London UK: Royal Society of Medicine Press, pp.48-53.