



## Categories of Invasiveness in Animal Experiments

Investigators and teachers who consider it essential to use vertebrates or invertebrates in their research, teaching or testing in the laboratory or in the field, must adhere to humane principles, and take cognizance of the Canadian Council on Animal Care's (CCAC) *policy statement on: ethics of animal investigation* and other CCAC documentation in assigning a category. Protocols must be submitted to an appropriate review committee for all studies and courses which involve the use of vertebrates and some invertebrates in Categories B through E. Cephalopods and some other higher invertebrates have systems as well developed as in some vertebrates, and may therefore warrant inclusion in Category B, C, D, or E.

The following list of categories provides possible examples of experimental procedures which are considered to be representative of each category:

### A. Experiments on most invertebrates or on live isolates

**Possible examples:** the use of tissue culture and tissues obtained at necropsy or from the slaughterhouse; the use of eggs, protozoa or other single-celled organisms; experiments involving containment, incision or other invasive procedures on non-cephalopod invertebrates.

### B. Experiments which cause little or no discomfort or stress

**Possible examples:** domestic flocks or herds being maintained in simulated or

actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category C); acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose, or decapitation preceded by sedation or light anesthesia; short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

### C. Experiments which cause minor stress or pain of short duration

**Possible examples:** cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies, laparoscopy; short periods of restraint beyond that for simple observation or examination, but consistent with minimal distress; short periods of food and/or water deprivation which exceed periods of abstinence in nature; behavioral experiments on conscious animals that involve short-term, stressful restraint; exposure to non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters such as respiratory or cardiac rate, or fecal or urinary output, or in social responses.

During or after Category C studies, animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased recumbency or dormancy, increased vocalization, aggressive defensive behavior or demonstrate social withdrawal and self-isolation.

#### **D. Experiments which cause moderate to severe distress or discomfort**

**Possible examples:** major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of Freund's Complete Adjuvant (see CCAC *policy statement on: acceptable immunological procedures*).

Other examples include induction of anatomical and physiological abnormalities that will result in pain or distress; the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems.

Procedures used in Category D studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioral patterns or attitudes, the absence of grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.

#### **E. Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals**

This Category of Invasiveness is not necessarily confined to surgical procedures, but may include exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress; completely new biomedical experiments which have a high degree of invasiveness, behavioral studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the CCAC; any procedures (e.g., the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain tolerance threshold and cannot be relieved by analgesia (e.g., when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint).

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For more information on these and other policies contact:

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## CCAC CATEGORIES OF INVASIVENESS FOR WILDLIFE STUDIES

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The unedited information below was previously published in the 2003 *CCAC guidelines on: the care and use of wildlife*, a document which has been replaced by the [CCAC guidelines: Wildlife](#).

### Category of Invasiveness A

Methods used on most invertebrates or on live isolates

**Possible Examples:** The use of tissue culture and tissues obtained at necropsy; the use of eggs, protozoa or other single-celled organisms; experiments involving containment, incision or other invasive procedures on metazoa; and studies in which the animals are observed without any disturbance to them.

### Category of Invasiveness B

Methods used which cause little or no discomfort or stress

**Possible Examples:** Observational studies in which there is some disturbance to the animals, but not to the point that the same individuals are repeatedly observed so as to habituate or otherwise modify their behaviour; census or other surveys which disturb animals but which do not involve capture or marking individuals; noninvasive studies on animals that have been habituated to captivity; and short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

### Category of Invasiveness C

Methods which cause minor stress or pain of short duration

**Possible Examples:** Capture, using methods with little or no potential to cause injury and marking of animals for immediate release; long-term observational studies on free ranging animals where the behavior of individuals may be altered by repeated contact; brief restraint for blood or tissue sampling; short periods of restraint beyond that for simple observation or examination, but consistent with minimal distress; short periods of food and/or water deprivation which exceed periods of abstinence in nature; exposure to non-lethal levels of drugs or chemicals; low velocity darting and slow-injection darts with immobilization chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters (such as respiratory or cardiac rate, or fecal or urinary output), in social responses or inability to survive.

**Note:** During or after Category C studies, animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased recumbency or dormancy, increased vocalization, aggressive-defensive behavior, or demonstrate social withdrawal and self-isolation.

## Category of Invasiveness D

Methods which cause moderate to severe distress or discomfort

**Possible Examples:** Capture, using methods that have the potential to cause injury (e.g., high velocity darting and rapid-injection darts with immobilization chemicals, net gunning, etc.); maintenance of wild caught animals in captivity; translocation of wildlife to new habitats; major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation, aggression, predator-prey interactions; procedures which cause severe, persistent or irreversible disruption of sensorimotor organization.

Other examples in captive animals include: induction of anatomical and physiological abnormalities that will result in pain or distress; the exposure of an animal to noxious stimuli from which escape is impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems (**N.B. Experiments described in this paragraph would be Category E if performed on wildlife immediately prior to release**).

**Note:** Procedures used in Category D studies should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioral patterns or attitudes, the absence of grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.

## Category of Invasiveness E

Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized, conscious animals

**Possible Examples:** This Category of Invasiveness is not necessarily confined to surgical procedures, but may include exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress; behavioral studies about which the effects of the degree of distress are not known; environmental deprivation that has the potential to seriously jeopardize an animal's well-being; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the CCAC; any procedures (e.g., the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain tolerance threshold and cannot be relieved by analgesia (e.g., removal of teeth without analgesia, or when toxicity testing and experimentally-induced infectious disease studies have death as the endpoint); capture methods with a high potential of causing severe injury that could result in severe chronic pain and/or death (e.g., leghold traps).