Categories of invasiveness in SAMRC animal experiments

The five categories of invasiveness are defined as follows:

A. Most invertebrates or live tissue
B. Little or no discomfort or stress
C. Minor stress or pain of short duration
D. Moderate to severe distress or discomfort
E. Severe pain near, at, or above the pain tolerance threshold of unanaesthetized, conscious animals

In the tables below are examples of experimental procedures which are to be considered representative of the five categories of invasiveness.

Category A: Most invertebrates or live tissues

Description	Possible examples
Studies or experiments on most invertebrates, or on non-entire living tissues. ❖ Cephalopods and other higher invertebrates have nervous systems as well-developed as some vertebrates and in such cases Categories B-E should apply	• Use of tissues obtained at autopsy, necropsy or from the slaughterhouse • Use of vertebrate eggs (<50% development), protozoa and related single-celled organisms • Studies or experiments involving containment or other non-invasive action on metazoa

Category B: Little or no discomfort or stress

Description	Possible examples
Procedures with no significant impairment of the wellbeing or general condition of the animals.	• Domestic flocks or herds being maintained in simulated or actual commercial production management systems • Short periods of food and/or water-deprivation equivalent to periods of abstinence in nature • Injection of materials, not more than 3 times per week in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral, but NOT intrathoracic or intracardiac (these fall under Category D) • 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

Category C: Minor stress or pain of short duration

Description	Possible examples
Procedures on animals as a result of which the animals are likely to experience short term mild pain, suffering or distress. ❖ During or after Category C studies, animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased dormancy, increased vocalization, aggressive defensive behaviour or demonstrate social withdrawal and self-isolation.	• Studies involving short-term (< month) deprivation of social partners or solitary caging of adult rats or mice of sociable strains • Withdrawal of food for 8-12 hours in adult mice, 12 hours in adult rats and 12-16 hours for adult primates • Feeding of modified diets, that do not meet all of the animals' nutritional needs and are expected to cause mild clinical abnormality within the timescale of the study • Short term (<24h) restraint in metabolic cages • Evoking escape and avoidance reactions where the animal is unable to escape or avoid the stimulus and are expected to result in minor distress (depend on stimulus and species) • Non-invasive imaging of animals (e.g. MRI) with appropriate sedation or anesthesia • Application of external telemetry devices that cause only minor impairment to the animals or minor interference with normal activity and behaviour • Models which expose animals to noxious stimuli which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid • Administration of substances 4-14 times per week and not more than 3 times per day (for one day) depending on the route of administration (e.g. subcutaneous: 2x per day; intramuscular: 1x per day; intraperitoneal: 1x per day; gavage: 1x per day and intravenously via superficial blood vessels: 1x per day), where the substance has no more than mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal • Pharmacokinetic study where a single dose is administered, and a limited number of blood samples are taken (totaling <7.5% and < 10% of circulating volume over a period of 1 and 4 weeks respectively) and the substance is not expected to cause any detectable adverse effect • Superficial procedures, e.g. ear and tail snips in rats (acceptable size will depend on size of animal), non-surgical subcutaneous implantation of mini-pumps and transponders • Cannulation or catherization of blood vessels or body cavities under anesthesia, such as biopsies and laparoscopy • Induction of tumors, or spontaneous tumors, that cause no detectable clinical adverse effects (e.g. small, subcutaneous, non-invasive nodules) • Breeding of genetically altered animals which is expected to result in a phenotype with mild effects, e.g. db/db mice

Category D: Moderate to severe distress or discomfort

Description	Possible examples
Procedures on animals as a result of which the animals are likely to experience short term moderate pain, suffering or distress, or long-lasting mild pain, suffering or distress. Procedures that is likely to cause moderate impairment of the wellbeing or general condition of the animals. ❖ Procedures used in Category D studies should not cause prolonged or severe clinical distress as may be exhibited by several clinical signs, such as marked abnormalities in behavioral patterns or attitudes, the absence of grooming, prolonged anorexia, dehydration, abnormal vocalization, circulatory collapse, extreme lethargy or reluctance to move, and clinical signs of severe or advanced local or systemic infection.	• Isolation for prolonged periods of social species e.g. dogs and non-human primates, but including some contact with conspecifics • Withdrawal of food for 16 hours in adult mice and 24hours in adult rats and small primates, and 48hr in medium sized primates • Studies with modified diets that do not meet all of the animals' nutritional needs and are expected to cause moderate clinical abnormality within the timescale of the study • Use of metabolic cages involving moderate restriction of movement over a prolonged period (up to 5 days) • Frequent application of test substances which produce moderate clinical effects, and withdrawal of blood samples (>10% of circulating volume) in a conscious animal within a few days without volume replacement. Maximum blood collection allowed: 15% once off of circulating volume, or 20% if removed in multiple samples over 24 hours, after which a 4 week recovery period is needed • Administration of substances under anesthesia via intrathoracic or intracardiac routes • Acute dose-range finding studies, chronic toxicity / carcinogenicity tests, with non-lethal endpoints • Surgery under general anaesthesia and appropriate analgesia, associated with postsurgical pain, postoperative analgesia, suffering or impairment of general condition. Examples include thoracotomy, craniotomy, laparotomy, orchidectomy, lymphadenectomy, thyroidectomy, orthopaedic surgery with effective stabilization and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (e.g. telemetry transmitters, minipumps, etc.) • Models of induction of diseases (e.g. SHIV, SIV) and tumors, including spontaneous tumors, that are expected to cause moderate pain or distress or moderate interference with normal behaviour • Irradiation or chemotherapy with a sublethal dose, or with an otherwise lethal dose but with reconstitution of the immune system. Adverse effects would be expected to be mild or moderate and would be short-lived (<5 days) • Breeding of genetically altered animals which are expected to result in a phenotype with moderate effects • Creation of genetically altered animals through surgical procedures

Category E*: Severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals

Description	Possible examples
Procedures on animals as a result of which the animals are likely to experience severe pain, suffering or distress, or long-lasting moderate pain, suffering or distress. Procedures which are likely to cause severe impairment of the wellbeing or general condition of the animals.	• Complete isolation for prolonged periods of social species e.g. dogs and non-human primates • Use of metabolic cages involving severe restriction of movement over a prolonged period • Inescapable electric shock (e.g. to produce learned helplessness) • Forced swim or exercise tests with exhaustion as the end point • Testing of a device where failure may cause severe pain, distress or death of the animal (e.g. cardiac assist devices) • Toxicity testing where death is the endpoint, or fatalities are to be expected and severe pathophysiological states are induced. For example, single dose acute toxicity testing (see OECD testing guidelines) • Vaccine potency testing characterized by persistent impairment of the animal's condition, progressive disease leading to death, associated with long-lasting moderate pain, distress or suffering • Surgical and other interventions in animals under general anaesthesia which are expected to result in severe or persistent moderate postoperative pain, suffering or distress or severe and persistent impairment of the general condition of the animals. • Organ transplantation where organ rejection is likely to lead to severe distress or impairment of the general condition of the animals (e.g. xenotransplantation) • Production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure • Models with induction of tumors, or with spontaneous tumors, that are expected to cause progressive lethal disease associated with long-lasting moderate pain, distress or suffering. For example, tumors causing cachexia, invasive bone tumors, tumors resulting in metastatic spread, and tumors that are allowed to ulcerate • Immobilization stress to induce gastric ulcers or cardiac failure in rats Irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease • Breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, for example Huntington's disease, muscular dystrophy, chronic relapsing neuritis models

* ECRA will require a strong and acceptable justification for Category E studies to be performed at the SAMRC or collaborative institutions