

## CATEGORIES OF INVASIVENESS IN ANIMAL EXPERIMENTS

### 1. Category A

Experiments on most invertebrates or on live isolates.

Possible examples:

- Experiments involving containment, incision or other invasive procedures on Metazoa;
- The use of eggs, protozoa or other single-celled organisms;
- The use of tissue culture and tissues obtained at necropsy or from the slaughterhouse.

NOTE: *Animal Utilization Protocols are not required for projects involving A Categories of Invasiveness.*

### 2. Category B

Experiments/procedures which cause little or no discomfort or stress.

Possible examples:

#### EUTHANASIA/ANESTHESIA:

- 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.

#### FISH:

- Bleeding fish under anaesthetic;
- Dip-netting fish;
- IP injections of fish;
- Measuring fish (e.g. length, width) under anaesthetic;
- Weighing fish by mass.

#### HOUSING/HUSBANDRY:

- Domestic flocks or herds being maintained in simulated or actual commercial production management systems;
- Housing of piglets individually;
- Less than ~18 hour fasting period in rodents;

- Short periods of food and/or water deprivation equivalent to periods of abstinence in nature.

#### MISCELLANEOUS:

- Ear treatments/medication;
- ECG electrode wires under local anaesthesia;
- Leg banding;
- Treadmill for normal horses;
- Echocardiography.

#### RESTRAINT/INJECTION/SAMPLING TECHNIQUES:

- Blood sampling (venipuncture only, not cardiac);
- clipping < 1 mm. of tail of tadpoles;
- Digital retrieval of feces from calves, dogs, horses;
- Injection of material in amounts that will not cause adverse reactions by intravenous, subcutaneous, intramuscular, intraperitoneal or oral routes (excluding intradermal, intrathoracic or intracardiac and fish not under anaesthesia);
- Nasal, pharyngeal or rectal swabs;
- Short-term and skilful restraint of animals for purposes of observation or physical examination;
- Vaginal swab in bitches in heat.

#### RUMINANTS:

- Bottle feeding electrolytes;
- Cannulating teats of cows;
- Implantation of hormones e.g. cattle;
- Intramammary infusion of mastitis medications;
- Obtaining rumen fluid samples through rumen fistula of cows;
- Teaching demonstration with rumen fistula and small amounts of digesta removed by demonstration;
- PRID insertion and removal (for cows only);
- Rectal and AI for management purposes, demonstrated by trained personnel for students (as per normal management procedures); multiple rectal examinations, on cattle;
- Removing calves from cows at birth;
- Rumen fluid samples taken via manual pump once daily;
- Ultrasound (per rectum).

## **WILDLIFE**

- 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;
- Non-invasive studies on animals that have been habituated to captivity;
- Short periods of food and/or water deprivation equivalent to periods of abstinence in nature

### **3. Category C**

Experiments/procedures which cause minor stress or pain of short duration. 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.

Possible examples:

#### **EUTHANASIA/ANESTHESIA:**

- Any means of physical euthanasia without prior sedation or anaesthesia;
- Cervical dislocation (must be scientifically justified) of rodents, chickens, turtles without sedation (must be competent or must use sedation);
- Decapitation (must be required by experimental design or scientifically justified);
- Endotracheal intubation;
- Induction of general anaesthesia in horses;
- Minor surgical procedures under anaesthesia such as: biopsies, laparoscopy; short periods of restraint, beyond that for simple observation or examination but consistent with minimal distress.

#### **FISH:**

- Electroshocking fish;
- Measurement (length and width) of individual fish without anaesthetic;
- Swim mills for fish;
- Tagging fish under anaesthetic;
- Estivation in lungfish; inducing artificial torpor, hibernation in lungfish.

#### **HOUSING/HUSBANDRY:**

- >24 hour fast for large mammals;
- >18 hour fast for mice/rats;
- Metabolic caging if it is short term and animals are exercised regularly, do not show signs of distress and have olfactory, visual and auditory contact with conspecifics;
- Short periods of food and/or water deprivation which exceed periods of abstinence in nature.

#### IDENTIFICATION METHODS:

- Eartagging;
- Microchipping (<2mm diameter); PIT tags;
- Nose bars in birds;
- Tattooing;
- Wingbanding in birds.

#### MISCELLANEOUS:

- Delayed type hypersensitivity;
- Nylon bags incubated in rumen fistulated cattle;
- Pessaries or CIDR in sheep (intravaginally);
- Teaching demonstrations with rumen fistula and students palpating rumen.

#### MEDICAL/SURGICAL/MANAGEMENT PROCEDURES:

- Alzec (osmotic) pump implantation;
- Beak trimming;
- Castration;
- Dehorning calves with Lidocaine or other topical anesthetic;
- Gavage/orogastric tubing; stomach tubing; and pump mechanism/lavage PEG tube insertion (without gastropexy);
- Intravaginal examinations on bitches NOT in heat;
- Multiple transrectal ultrasound examinations (using rigid transducer);
- Teeth clipping (piglets);
- Vaginal impedometry (for estrus or parturition detection).

#### RESTRAINT/INJECTIONS/SAMPLING TECHNIQUES:

- Giesenauer probe
- Behavioural experiments on conscious animals that involve short-term, stressful restraint; exposure to non-lethal levels of drugs or chemicals;

- Catheterization of blood vessels or cannulation of body cavities under anaesthesia;
- FIA, FCA, RIBI, titremax, Quil A if the adjuvant/antigen combination has few deleterious effects; generation of immune spleen B-lymphocytes in mice using FIA IP (required in the generation of hybridoma cells);
- Intradermal injections (ID injections) without a significant inflammatory reaction;
- Lutalyse administration in horses -
- Periorbital bleeding in pigs without anaesthetic; periorbital bleeding in other species under anaesthetic;
- Vaginal swabs in non estrus animals.

## WILDLIFE

- Live capture of wild animals;
- 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 behaviour 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 behaviour or demonstrate social withdrawal and self-isolation.*

## 4. Category D

Experiments which cause moderate to severe distress or discomfort.

*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 behavioural 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.*

Possible examples:

HOUSING/HUSBANDRY:

- Metabolic caging of prolonged (several hours or more) duration or where animals are in isolation;
- Wire bottom caging for rodents

MEDICAL/SURGICAL PROCEDURES:

- Embryo transfer in ewes;
- Laparotomy e.g. ovariectomy;
- Major surgical procedures conducted under general anesthesia, with subsequent recovery;
- PEG Tube insertion (with gastropexy).
- Subcutaneous xenotransplantations;
- Surgical cannulation of organs under anaesthetic.

MISCELLANEOUS:

- Ascites production;
- Creation of transgenic animals before phenotype is known;
- Electros shocking of fish;
- Exposure to drugs or chemicals at levels that impair physiological systems;
- Induction of behavioural stresses such as maternal deprivation, aggression, predator-prey interactions;
- Induction of anatomical and physiological abnormalities that will result in pain or distress;
- Procedures which cause severe, persistent or irreversible disruption of sensorimotor organization;
- The exposure of an animal to noxious stimuli from which escape is impossible; The production of radiation sickness.

RESTRAINT/INJECTION/SAMPLING TECHNIQUES:

- FIA, RIBI, Quil A may be categorized as a AD@ until the effects on animal welfare can be recorded;
- Prolonged (several hours or more) periods of physical restraint;
- The use of Freund's complete adjuvant (FCA)

## WILDLIFE

- 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 wild caught animals in captivity;
- Translocation of wildlife to new habitats
- Major surgical procedures conducted under general anaesthesia, with subsequent recovery;
- Prolonged (several hours or more) periods of physical restraint;
- Induction of behavioural 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).

## 5. Category E

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

This Category of Invasiveness is not necessarily confined to surgical procedures, but may include:

- 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);
- Behavioural studies about which the effects of the degree of distress are not known;
- Burn or trauma infliction on unanesthetized animals;
- Completely new biomedical experiments which have a high degree of invasiveness;
- 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 severe pain, extreme distress or death;
- Gill netting of fish;
- Use of muscle relaxants or paralytic drugs for immobilisation without anaesthetics.

## WILDLIFE

- This Category of Invasiveness is not necessarily confined to surgical procedures, but may include exposure to noxious stimuli or agents whose effects are unknown;
- Behavioural 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;
- Capture methods with a high potential of causing severe injury that could result in severe chronic pain and/or death (e.g., leghold traps).