



# **LABORATORY ANIMAL THEORY**

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# Laboratory Animals

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## 1. The Animal Laboratory Facility

### 1.1 Tour and Orientation

A guided visit of the facility is mandatory before gaining access to the animal laboratory, and before starting a protocol involving animals. The visit will familiarize users with the facility, the risk factors present, and the laboratory rules.

Points discussed during the visit:

- limited authorization access to the animal laboratory;
- dress code;
- animal housing;
- circulation inside the lab;
- security code and symbols;
- location of various rooms.

### 1.2 Role of Personnel

Veterinarians, in collaboration with animal health technicians and animal care assistants respect and apply CCAC guidelines and current procedures. They assure the animals receive high quality care.

Personnel are trained to respond to problems related to animal care. All observations or problems must be relayed to them. The staff will conduct a follow-up.

#### Veterinarians

Adequate veterinary care as described by the Canadian Association for Laboratory Animal Medicine (CALAM) is crucial in implementing an animal care and use programme in accordance with CCAC standards. Veterinarians are also members of ACCs (Animal Care Committees). They assume the role of expert advisor on these committees, and assure that the three Rs tenet is respected. They also answer questions related to animal well-being, housing, care, and methods of use (analgesia, anesthesia, endpoints, etc.).

They regularly visit the animal facilities and follow-up on recommendations put forward by the different committees.

They contribute to improving veterinarian standards, animal care, guidelines, veterinarian techniques and techniques related to veterinary science.

They advise animal care staff and users, particularly concerning anesthesia, analgesia, antibiotics and other medical agents.

Veterinarians, in collaboration with the researcher and the ACC, define the appropriate endpoints specific to each study involving animals, particularly for invasive studies.

Finally, they assure the availability of veterinarian services at all times, during and outside of normal working hours.

### Animal Care Personnel

Animal care staff must carry out **all** care procedures other than those related to experimental protocols. Animal care technicians (ACTs) and veterinary assistants together, perform daily observations and provide basic care for the animals (changing cages, treatments, and health checks). ACTs assist veterinarians and lab users. They can also administer prescribed medication if health problems arise. They are trained in techniques performed on animals and can be asked to perform an experimental protocol. Animal care assistants have the solid role of maintaining a sound environment (maintenance, cleaning, disinfection, etc.).

### **1.3 Ordering, Acquisition and Identification of Animals for Research**

Before conducting any research using animals, an authorization form must be submitted. All forms are reviewed by the ACC.

The applicant must explain the purpose of the research, how the study will be carried out, the use of the three Rs tenet, the products used and their dosage, etc. The need for the use of living animals must be justified. No order can be placed, nor can a procedure commence without approval from the ACC.

Upon approval, an order for animals can be placed with the manager of the laboratory by filling out an order form. The number of animals ordered must be respected, which is indicated on the authorisation form.

Following animal acquisition, the animals are to be identified and files created for them. Here are the most frequent identification methods for each species:

Rodent	Tattoo, ear notching, tail marking with non-toxic marker
Rabbit	Ear tag
Ferret	Ear tag
Dog	Tattoo
Cat	Identification collar
Pig	Ear tag
Non- human primate	Tattoo

A file must be created for each animal. The file must contain the following information: **arrival date**, **sex**, **age** and **weight** estimate, **race** and **strain**, **color** and **marks**, any **physical abnormality** or other identifying characteristic, **project name or researcher**, **protocol number**, name of **supplier** and potential **method of disposal**.

The cage must be identified with a card containing the following mandatory information: **sex** and **number of animals** held in the cage, **name of researcher** in charge, and all **specific instructions** concerning care to be given.

The files must be kept for one year after euthanasia or after the departure of the animal, in order to track information. If the cage cards are also used as files (as is the case with rodents at Université Laval), they must be kept for a year after the departure of the animal.

## **2. Factors Influencing Research**

Many factors can influence research results such as:

- physical and environmental factors (temperature, ventilation, noise, etc.);
- animal-related factors (age, sex, disease, stress, etc.);
- basic care factors (feeding, water, litter, etc.);
- experimental protocol factors (transport, housing, care, etc.).

### **2.1 Physical and Environmental Factors**

The conditions of the environment in the animal facility are essential for an experiment to go well. The optimal environmental parameters differ according to the animal species and the research needs. This is why the housing conditions will differ in temperature, humidity, and lighting for each room according to the protocol and the species. The temperature must not vary by more than 2 degrees Celsius as to not modify the metabolism of the animals, which includes the metabolizing of medication administered. In addition, relative humidity can have an effect on thermoregulation, food consumption, reproduction performance and the capacity to combat disease. It is also important to maintain frequent air exchange at all times to reduce the amount of ammonia, and to let the heat and carbon dioxide produced by the animals escape. Light intensity, day-night cycle and wave length are to be considered in animal housing. Finally, noise can have an influence on many variables: feeding, reproduction, immune system, etc.

### **2.2 Animal-Related Factors**

Reactions to various stimuli can vary especially according to age, sex of the animal and genetic make-up. It is known that hormones can have a significant effect on results gathered. Furthermore, genetic alteration frequently results in a change in expected phenotype. Despite the purchase of pathogen-free animals, they are carriers of a normal microflora that can affect the protocol in certain cases. Animals' responses vary also according to the time of day

(circadian cycle). This is why it is important to administer medication at the same time of day for all animals. Finally, diseases, whether infectious or not, are one of the most important variables in research.

## **2.3 Basic Care Factors**

### **2.3.1 Housing**

#### Feeding

Feeding must be adapted to the species and must fulfill nutritional and hygiene criteria. Food must be fresh and free of contaminants, in order to avoid microbial growth that could sicken animals and interfere with experiment results. Food must be given regularly to animals and be accessible to them. Animals must have access to fresh water at all times. A stable diet is crucial.

#### Types of Shelters

The type of shelter varies from one protocol to another. It must be specified and described in the protocol. It must be adapted to the needs of the species and to the experiment and be consistent throughout the protocol. There are significant differences concerning temperature, humidity, air quality, noise, and vibrations of different cage types. Thus, it is imperative to use the same cages for all animals of a study.

Each species has specific characteristics and needs. Consequently, housing conditions must provide an environment which respects these characteristics and needs and which provides a quality of life essential to its mental and physical equilibrium. The housing must therefore take into account the following general factors and be adapted to the species utilized:

- the right to eat and drink in which a healthy diet is maintained, and there is direct access to fresh water;
- the right to live in comfort by providing an adequate environment, including a comfortable shelter and resting area;
- the right to live without pain, injuries or disease;
- the right to behave naturally by providing sufficient space, adequate facilities and other animal companions;
- the right to live without fear or distress by ensuring treatment and conditions which do not inflict mental suffering.

#### Acclimation

An acclimation period must be allotted for the animals upon arrival. This will allow the animals to adapt to housing conditions and will reduce stress hormones released during transport. The duration of this period varies depending on the species. To provide a stable environment for the animals, these rules must be followed:

- avoid changes in animal groups;
- avoid changes in diet;
- avoid location change in the room and/or in the laboratory;
- avoid abrupt noises or movement in animal presence.

### Environmental Enrichment

To enhance environmental enrichment and to encourage the natural behaviour of the animals, certain factors need to be kept in mind:

- occasions to socialise;
- activity options during waking hours;
- hiding possibilities;
- possibility and facilities for exercise.

Enrichment is not just the physical and social environment of animals. Our way of interacting with them is equally important. All enrichment of the environment would be useless if an animal fears a human next to its cage. However, human presence is not the only factor affecting animals. For example, smells and sounds associated with an experimental procedure can also have an effect. These environmental elements are also part of enrichment. It has been clearly proven that environmental enrichment improves the well-being of animals **and** research results.

### **2.3.2 Maintenance, Basic Care, and Handling**

As for all other factors, it is important to handle the animals in the same way. Animal handling must be done gently and consistently to reduce biases. The way of handling an animal may modify its behaviour and its physiological responses and in the end, modify the results.

Maintenance of cages or all other types of shelters must be carried out on a regular basis or in accordance with the protocol. As a general rule, maintenance must be done at least once a week, and the frequency is adapted to the requirements of the species.

### **2.4 Experimental Protocol Factors**

The correct moment and duration of experimental procedures must be standardized. Stress can be a major disruptive factor and have a considerable effect on experiment results. An acclimation to techniques used could also be required to familiarize animals with the procedures. In this way, stress related to experimental procedures will be reduced. In addition, un-treated pain significantly affects biological and physiological responses of the animal. This pain can vary from one animal to another and consequently modify its response, for example, its response to medication.

There are analgesic procedures that keep the animal from suffering following experimental procedures and/or surgeries. These procedures must be planned and described in the protocol

according to invasiveness and observed symptoms. The researcher has the responsibility to calculate the pain which could be felt by the animal and to stipulate the intervention plan to be executed while at the same time, respecting the requirements of the experiment. The ACC during examination of the protocols ensures that the intervention plans are well adapted and are practical.

### 3. Animal Health

The physical condition of the animal greatly influences the quality and the reproducibility of experimental results. It is also known that a sick or stressed animal does not respond to experimental procedures the same way as a perfectly healthy animal.

Therefore, it is important to do everything possible to limit the risk of disease in animal laboratories by implementing three measures: **prevention, detection** and **treatment**.

**Prevention** includes all procedures which reduce the risk of introducing a sick animal to the group or making an animal sick. The choice of the supplier, the quarantine and the shelter, as well as adhering to the dress code and hygiene regulations all play a role in prevention.

The selection of an animal supplier that guarantees pathogen free animals will maximize the chances of having healthy animals. If the supplier cannot guarantee pathogen free animals, a quarantine must be imposed upon arrival of the animals before starting a project. The animals are then housed in separate rooms, and hygiene and dress code regulations become much stricter.

All the animals need to be inspected at least once a day. Particular attention must be brought to the general state of the animal (alertness, weight loss, lethargic, hyperactivity, etc.), appetite, presence and type of feces, etc., and all abnormalities must be documented. The **detection** of health problems normally occurs during daily observations by care personnel or during handling included in the protocol. As soon as the first signs of change in condition are observed, the laboratory staff, researcher and veterinarian must be notified. The procedure planned in the protocol is then applied and the veterinarian assures medical follow-up.

When symptoms of sickness are present or when problems related to the protocol arise, the veterinarian is called in and then assures medical follow-up. The veterinarian also aides the follow-up of the researcher, by making medical recommendations and collaborating with him/her to ensure that the **treatment** plan does not interfere with the research, but that there is a continuous evolution between them.

### 4. Diseases

Diseases are one of the most important variables capable of compromising a research project. Among other factors, the effect of a disease depends on the animal (strain, age, sex, etc.), the

presence of stress, pain or distress, and on the virulence of the pathogenic agent, if such is the case. The presence of concomitant diseases can lead to difficulties in interpreting obtained results. In a research animal laboratory, it is important to do everything possible to prevent the spread of infectious diseases which can affect and/or invalidate experiment results. There are three modes of transmission of these diseases:

- direct contact between animals or with the contaminant: generally cutaneous diseases, but sometimes sexually transmitted diseases;
- indirect contact through the environment: the infectious agents can be inhaled, the diseases can be contracted from water contamination, from food or from litter;
- passive vector: inanimate objects can become disease carriers, for example like tools, contaminated cages, needles, etc.

There are four possible infection sources: the animal, the environment, humans, and the experimental procedure. This is why SOPs (standard operating procedures) on vermin control, restricted access, and on dress code need to be established and followed at all times. Respecting hygiene rules like hand washing and disinfecting working surfaces, and respecting the dress code are simple and efficient ways to prevent contaminating the animals. Housing certain species in ventilated cages also helps to prevent contamination.

It is important to put into practice all available methods to avoid contamination of the environment. Listed below are some rules to avoid diseases and infections:

- implement SOPs;
- clean and disinfect areas and material of common use;
- wear different protective clothing between animal handling or between different animal groups as needed;
- ensure that cleaning and sterilization material function properly and are up to standard;
- see to the creation of a sanitary follow-up programme (sentinel animals);
- circulate from the cleanest zones to the least clean ones;
- implement quarantines upon arrival for animals susceptible to infection.

Non infectious diseases are just as important, but cannot be transmitted from one animal to another. They can affect a variety of systems important in maintaining constant results: the immune system, the gastro-intestinal system, the nervous system, etc. These diseases can be physical, genetic, metabolic, or dietary. It is important to notify veterinary personnel right away of any abnormality observed concerning an animal.

## 5. Experimentation

### 5.1 Training and SOPs

Before executing a procedure from a protocol, the SOPs must be read and understood. Practical training in experimental procedures must be completed and passed before starting a protocol. Any procedure that deviates from the SOP in use must be justified in the authorization application and be approved by the ACC. SOPs are implemented to:

- standardize work methods et ensure that they do not interfere with results;
- assure the well-being of the animals;
- standardize methods used for animal experimentation;
- ensure ethical usage of animals.

Practical training is **mandatory** for anyone who will handle living animals. This training must be undergone and passed in order to gain access to the animal laboratory.

### 5.2 Analgesia

*“We have an obligation to reduce or abolish pain in animals whenever it occurs and particularly if it occurs in research, teaching or testing.”*

The word analgesia means absence of the feeling of pain. The administration of analgesics does not generally eliminate the sensation of pain, but reduces it and makes it tolerable. When creating a protocol, the researcher in consultation with the veterinarian, must estimate the pain which could be inflicted on the animals and plan for the use of analgesics. An emergency plan must also be discussed in order to be able to relieve pain from unexpected events, and in such a way as to not interfere with the study. In certain types of experimental activities, it is obvious that analgesia is necessary. Most surgical procedures cause pain, but the intensity varies according to the invasiveness of surgery, the organs involved, and even postoperative complications. The treatment is therefore adjusted to each protocol based on the degree of anticipated pain. **A procedure considered painful if carried out on a human, must be considered painful for an animal, and if there is doubt concerning the presence of pain following a procedure, the response must be in favour of the animal’s well-being.**

#### 5.2.1 Choosing an Analgesic

Many factors must be taken into consideration when choosing an analgesic. The cause and intensity of the pain will have an impact, as well as the duration of the analgesic required. The animal species used will influence the method of administration to be employed, and the amount required in some cases, might not be adequate. The possible side effects of the drug must also be studied, whether good or bad, along with their effect on the study results. The different types of molecules described below do not necessarily react in the same site on the pain pathway and each has its advantages and disadvantages. As no perfect drug exists, it can sometimes be advantageous to use several types of analgesics to obtain the desired effect. A

combination of drugs must therefore be proved beneficial and not result in a harmful medical reaction.

### Opioids

This analgesic family is made up of many fairly strong molecules which play a role in various brain receptors. Opioids generally give a moderate to intense level of analgesia; therefore, they are often preferable for more painful procedures. The effects on the different body systems vary greatly according to the given molecule, and according to the target species. While they have a sedative effect for most species, an energetic state can sometimes result (ex. a strong dose of morphine in cats, a strong dose of buprenorphine in rats). The effect on the cardiovascular and respiratory systems is generally depression, but hypothermia is also common due to dilation of blood vessels. It is interesting to note that antagonists exist and can be used when undesirable effects are too severe (the analgesia is then neutralised as well).

As opioids can lead to dependency, their use is regulated and each use of the products must be documented in a file created for this purpose. In the file, the reason for use and the quantity of the product used must be indicated. Inspectors from the Office of Controlled Substances of Health Canada hold the right to examine the file at any time.

### Nonsteroidal Anti-inflammatory Drugs (NSAID)

By reducing inflammation, NSAIDs reduce pain, provided it is light to moderate. They have the advantage of reducing fever, but are generally considered not very efficient for visceral pain. As with opioids, their mode of action can vary depending on the species. These substances have little effect on the cardiovascular and respiratory systems, but can lead to gastric ulcer formation, reduce platelet aggregation, and also affect the kidneys. A well hydrated state is then necessary with NSAID use.

### Local Anesthesia

These agents are efficient in combating pain by blocking painful stimulus before it reaches the central nervous system. While their use is limited to nervous structures (the local anesthetic must target a peripheral nerve easily accessible to the experimenter). This technique is quite useful when it is available. The pain is not only reduced, but prevented and the effects of the drugs only rarely have consequences at the systemic level. As physiological parameters are not modified, pain can be subdued without affecting the study results. Local anesthetics can be injected around a nerve, applied directly to the skin, or even administered as an epidural. Nonetheless, care must be taken to not surpass the toxic dosage, especially in small lab animals. A frequent example of this mode of analgesia is the application of EMLA™ cream on rabbit ears before blood work or an injection.

## Other Analgesic Agents

Some drugs used as anesthetics also have some analgesic properties, such as ketamine and xylazine.

Ketamine is a NMDA receptor antagonist and plays an important role in hypersensitivity development. As the analgesic effect is short and relatively average, postoperative use of the molecule is not recommended. On the other hand, use before the presence of a pain stimulus blocks hypersensitivity (wind-up effect). Ketamine should never be used alone to reduce pain. Its usage must be included in the **controlled substances registry** due to the high potential for dependency associated with it.

Among other substances,  $\alpha_2$ -adrenergic antagonists (xylazine, medetomidine, romifidine,) are quite efficient in counteracting visceral pain. However, they are much more useful for their sedative and tranquilizing properties. Given their affinity for  $\alpha_2$ -adrenergic receptors, many systematic effects are to be expected with use of these molecules. However, in the case of overdose or intolerance,  $\alpha_2$ -adrenergic antagonists are antagonisable.

### **5.2.2 Effects of Analgesics on Research Results**

It would be tempting to think that use of analgesics could have negative effects on study results. Yet, pain and distress cause unavoidable physiological and neuroendocrine phenomena that can further alter the results. To adequately manage pain without compromising the experimental model, the protocol should be attentively examined to correctly select the analgesic. It is clear that some research topics, primarily on pain, could require the absence of analgesia.

## **5.3 Anesthesia**

The main goal of anesthetic is temporary and reversible relief of the sensation of pain. It can target a limb or region of the body, or an entire organism. It can also be used as muscle relaxants to block reflexes or to induce unconsciousness, depending on the intervention requirements.

There are three possibilities for anesthetic protocol:

- volatile anesthetic : always first choice for invasive procedures (ex. isoflurane);
- injectable anesthetic : effects and duration difficult to predict (ex. ketamine, propofol); easier to control by continuous perfusion;
- local anesthetic: for minor procedures (ex. lidocaine, procaine).

A combination of these methods is also possible, and sometimes preferable.

Factors concerning the animal also must be considered as influences on anesthesia, as in species, strain, age, weight, sex, state of health, behaviour, as well as other previously

administered substances. Close observation of the anaesthetic must be done by recording data, from loss of consciousness to wake-up.

Once again, the veterinarian and the ACC, during review of the protocol, must ensure that the anesthetic protocol chosen is satisfactory. The veterinarian remains, nonetheless, a reference for protocol writing. Do not hesitate to contact him/her.

#### **5.4 Endpoints**

In research, there is always an obligation to prevent any unnecessary pain or distress to animals being used. The natural behaviour of animals is modified when they are in pain or in distress. This is why it is important to define the endpoints of the experiment and the action plan once the endpoint is reached.

An endpoint is the moment when pain and/or distress must be stopped, minimized or reduced by:

- euthanizing the animal according to the current SOP;
- stopping a painful surgery;
- administering medication to sooth pain or distress;
- maintaining basic conditions (ex. putting a mouse into group housing when isolation causes distress).

The endpoints must allow for early detection of pain/distress, and at the same time be compatible with scientific objectives. The researcher's decision of the endpoint must be developed in consultation with a veterinarian and the ACC.

In the protocol, the researcher must anticipate and specify the endpoints and the observation method, and the procedure to be applied once the endpoints are reached. The CCAC recommends at least two or three observations per day during critical periods of a protocol. The ACC must assure a correct balance between the requirements of a quality scientific study and the diminishing of pain and distress of animals.

The following link is the CCAC guidelines on recommended endpoints:

[http://www.ccac.ca/Documents/Standards/Guidelines/Appropriate\\_endpoint.pdf](http://www.ccac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf)

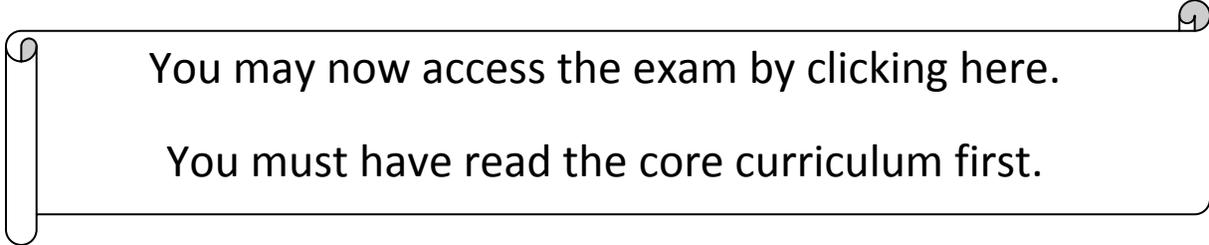
#### **5.5 Euthanasia**

An ethical putting to death of an animal must entail a rapid loss of consciousness, and not cause pain or distress. Each time euthanasia takes place, it must be carried out professionally and with respect by adequately trained staff. Good knowledge of pain and distress signs is necessary, as well as solid knowledge of restraint techniques and evaluation of the state of consciousness. This procedure should be done in an isolated and unoccupied room, in order to reduce stress on the animals. The method of euthanasia is chosen by the researcher and must be approved by the veterinarian and the ACC.

Once the euthanasia procedure is executed, the death of the animal must always be assured, and then the carcass and tissues are disposed of according to the current SOP. The animal will only be considered dead, when it is certain that the heart is no longer beating and therefore blood no longer reaches the brain; furthermore, all other movement including breathing and reflexes must be confirmed to have stopped. Consequently, in certain methods, two steps are necessary, the procedure that provokes initial loss of consciousness and the operation that confirms the animal will neither regain consciousness nor recover (exsanguination, opening of the thorax, section of major blood vessels, or cervical luxation after euthanasia by CO<sub>2</sub>).

The CCAC guidelines must be consulted to learn which euthanasia techniques are acceptable:

<http://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>



You may now access the exam by clicking here.

You must have read the core curriculum first.