UNIVERSITÉ LAVAL

LABORATORY ANIMAL THEORY

MAY 2012

Table of Contents

Laboratory Animals

1. Ethics in Animal Experimentation

1.1 Generalities

"…the use of animals in research is accepted insofar as it serves to help better understand fundamental biological principals or to help gain knowledge that can reasonably be expected to benefit human beings or animals…"

The use of animals in research is the source of genuine social debates. That is why researchers, Animal Care Committees and all those involved in the use of animals in research must make sure that they use animals in a responsible and respectful way. As soon as the matter of animal experimentation is raised, ethical issues as well as animal rights must be given due consideration. Always keep in mind that the use of animals in research is a privilege.

The review of protocols regarding animals by an Animal Care Committee (ACC) constitutes the cornerstone of any program concerning the care and use of animals. ACCs must always remain focused on the goal of reconciling public expectations and experimentation needs. Therefore, committees must address ethical issues in the context of research projects that involve animal testing. Therefore, a review based on Marshall Hall's principles is beneficial:

- No experiment should be carried out if the information searched for can be obtained through simple observation;
- No experiment should be carried out without a clearly defined, achievable goal;
- Unnecessary repetition of the experiment should be avoided especially if a renowned physiologist was in charge during the first experiment conducted;
- All experimentation should be carried out with the least possible infliction of suffering on the animal;
- All physiology experiments should be witnessed by peers to diminish the need for repetition.

The implementation of Marshall Hall's principles as well as the Three Rs tenet ensures an ethical and responsible use of animals. Université Laval and all affiliated research centres are committed to complying with this line of conduct.

1.2 Canadian Council on Animal Care

The Canadian Council on Animal Care (CCAC) is a national organization that relies on peer review. It is responsible for establishing and maintaining standards regarding the care and use of experimental animals (i.e., in research, teaching and testing) throughout Canada.

To accomplish this task, the CCAC:

- Establishes guidelines regarding the use of animals in research;
- Inspects institutions every three years to make sure they conform to these guidelines;
- Reviews the work accomplished by the care committee of every institution.

The CCAC then presents institutions with an evaluation report, so they may take corrective action within a given time frame.

The mission of the CCAC is to ensure that animals used in science are given optimal care, both physically and psychologically in accordance with acceptable scientific standards. Another task of the CCAC is to promote a high level of knowledge and sensitivity regarding ethics. In order to reach these goals, the CCAC offers the following programs:

- Assessment and Certification Program;
- Guideline Program;
- Education, Training and Communications Program;
- Three Rs Program.

For further information, visit: http://www.ccac.ca/en_/

1.3 Animal Care Committee

The mandate of the Animal Care Committee (ACC) is to ensure that CCAC guidelines and policy are observed. To fulfill this task, the committee must:

- Review and approve authorization requests and the protocols submitted by researchers;
- Annually review every protocol and of any modification made to the protocols;
- Ensure that a training program meeting the standards of the CCAC is available to users;
- Ensure that no animal is obtained or any protocol initiated without its authorization;
- Inspect the institutions under its jurisdiction at least once a year.

***No protocol can be initiated or any modification introduced**

without prior approval of the ACC *

The ACC is made up of:

- A president;
- Scientists and /or teachers whose research involves the use of animals;
- Veterinarian(s) having relevant experience in animal care and use;
- An institutional member whose regular activities do not involve the use of animals;
- At least one person representing public interest and concerns who is not involved with the institution or the use of animals in research, teaching or testing;
- A member of the technical staff involved in the care and use of animals;

- A student representative of the institution;
- The manager(s) of the animal supply facility;
- The coordinator of the ACC.

There are 2 ACC at Université Laval:

- Comité de protection des animaux de l'Université Laval (CPAUL) (animal care committee of Université Laval) that receives requests from researchers working for the following institutions:
    - University campus
    - Centre de recherche de l'institut universitaire de cardiologie et de pneumologie de Québec (CRIUCPQ)
    - Centre de recherche de l'institut universitaire en santé mentale de Québec (CRIUSMQ)
    - Laboratoire d'organogénèse expérimentale (LOEX)
    - Laboratoire de recherche en sciences aquatiques (LARSA)
- Comité de protection des animaux du CHUQ (CPAC) (animal care committee of the CHUQ) that receives requests from researchers working at:
    - Centre de recherche du CHUQ (CRCHUQ)

When a researcher issues an authorization request, it must meet the following criteria:

- Clearly identify the objective of the project;
- Justify the need for use of animals;
- Justify the number of animals used;
- Provide a detailed description of the manipulations carried out on animals and of anticipated effects;
- Describe measures taken to minimize the discomfort experienced by animals and describe limit humane endpoints.

Requests will be transferred to the ACC and reviewed during a committee meeting.

The ACC must ensure the following:

- The scientific merit of the project has been demonstrated previously through an independent peer review;
- Qualified personnel will be responsible for managing the animal supply facility and for the handling and care given to the animals;
- The animals will receive appropriate veterinary care;
- The chosen methods of euthanasia comply with CCAC standards;
- The Three Rs tenet is observed;
- The objective and procedures are thoroughly detailed.

Throughout the course of any research, the ACC is entitled, if necessary, to take the following actions:

- Put a term to any reprehensible procedure if unnecessary suffering or distress is found to be inflicted on an animal;
- Put a term to any procedure not authorized within the framework of the protocol;
- Have an animal euthanized if it is impossible to relieve the pain or distress experienced.

Personnel of the animal facility as well as users of animals must report any problem related to the care and use of animals to the veterinarian, who must contact the researcher when necessary or discuss the issue with team members.

Researcher and users of animals must comply with protocols, University policies and standard operating procedures at all time. In case of non-compliance, actions will be taken and may lead, after 3 offenses, to a suspension of funds and animal facility access.

1.4 Legislation and regulation

Different reference documents (*Legislative jurisdiction over animal used in research, teaching and testing*, 1998 and *The Protection of Animals Used for the Purpose of Xenotransplantation in Canada*, 2000) conclude that under the *Constitution Act 1867*, the federal government does not have jurisdiction to legislate in the animal experimentation field, which is subject to provincial jurisdiction. However, laws that were established by the government within three other fields can apply to animal experimentation:

- Sections 446 and 447 of the Criminal Code protect animals against cruelty, abuse and neglect;
- The Health of Animals Act protects livestock against infectious diseases that could be a threat to humans, other animals and Canadian international trade;
- The federal government has the jurisdiction—which is not strictly speaking legislative in nature—to award grants subject to various conditions. Therefore, grants awarded by the Canadian Institutes of Health Research (CIHR) or the Natural Sciences and Engineering Research Council (NSERC) are conditional to compliance by the institution with the CCAC policy. The CCAC establishes standards regarding the care and use of experimental animals in research, teaching and testing throughout Canada.

All Canadian provinces have legislated in the field of animal welfare, and in absence of more specific laws, animal welfare laws are to be observed when using animals in science. The following provinces have specifically legislated on the use of animals in research, teaching and testing: Alberta, Manitoba, Saskatchewan, Ontario, New Brunswick, Nova Scotia and Prince Edward Island.

1.5 The Three Rs tenet

The Three Rs tenet (replacement, reduction and refinement) serves as a guide for an ethical use of research animals. Université Laval has added a fourth R, which stands for **R**espect of the animal.

Numerous methods have been developed to progressively reduce the number of animals used in research. Nevertheless, these methods have limitations. Animals can be used when it has been determined that no replacement alternative is suitable to reach the objectives of a research project with previously established scientific merit.

Replacement: Using alternative methods such as computer programs, videos or dummies instead of experimenting on animals. It can also refer to the use of cells and tissues maintained in culture, invertebrates, or species more suitable for experimentation.

Reduction: Using the minimal number of animals required to obtain statistically valid experimental data without losing useful information.

Refinement: Modifying at least one aspect of the experimental procedure or the husbandry to minimize pain, distress or stress experienced by animals or to enhance their general welfare.

When presenting an authorization request for the use of live animals, researchers must demonstrate how they plan to respect and apply the Three Rs tenet.

Finally, at Université Laval, one guiding principle must be observed at all times. A fourth R was developed: **R**espect of the animal. In order to apply this principle, animal users must display compassion, sensitivity and empathy toward the animal, as well as concern for its physical and psychological welfare.

Above all, the researcher must demonstrate that his scientific objective cannot be achieved using alternative methods. There are many alternative methods, and they must be considered before opting for the use of animals.

Here are some useful links to inquire about available alternatives:

CCAC

The National Centre for the Replacement, Refinement and Reduction of Animals in Research

Before starting any experiment, the researcher must complete an authorization request for review by the ACC. In this request, among other things the researcher must present his research objective, the protocol to be followed, how he intends to apply the Three Rs tenet and the products to be used, as well as their dosage. No animal can be ordered priori to having obtained final approval of the protocol by the ACC. The number of animals ordered must be the same as the number indicated in the authorization request.

1.6 Standards and Procedures

Standard Operation Procedures (SOP) describes the procedure carried out on laboratory animals. They must be consulted prior to any intervention performed on animals and must be followed to the letter. If the procedure has to be modified, the modifications must be justified in the protocol and approved by the ACC.

When developing a protocol, researchers must consult pre-existing standards and policies. The SOP may be consulted on the web page of the Direction des services vétérinaires.

1.7 Confidentiality

Users of the animal supply facility as well as members of ACCs are subject to the CCAC confidentiality policy.

It is also forbidden to use cameras or video cameras in animal facilities without prior authorization from the ACC and the manager of the animal facility, and without first tabling a request in the authorization request for the use of live animals.

2. Prevention – occupational health and safety

2.1 Regulation

There are many laws and regulations concerning occupational safety and health (OHS):

- An Act Respecting Occupational Health and Safety → enforces and provides rules on prevention;
- An Act respecting industrial accidents and occupational diseases → indemnifies workers;
- Regulation respecting occupational health and safety → defines rules governing the work environment: workplace layout, protective personal equipment, storage, hazardous material, WHMIS, etc.;
- The civil code → deals with relations between people;
- Bill C-21 → deals with some rules of penal law concerning serious behavioural problems;
- The board of directors of the Université Laval (Resolution 2011-17) → enforces and structures the role of managers.

2.2 An Act Respecting Occupational Health and Safety

An Act Respecting Occupational Health and Safety entails some responsibilities for the employer and the worker. Among those responsibilities, the employer must ensure that management is adapted and provide appropriate methods and techniques. The employer must also monitor the work environment and provide information to workers on risks inherent in their job. As well, sufficient safety training for employees to work safely and minimal supervision are mandatory.

The worker also has responsibilities regarding health and safety at work: some measures should be taken by the worker to ensure safe work conditions for the worker as well as for the others. The worker must also take part in the accident risk evaluation process to help mitigate risks and submit to requisite medical examinations. It is necessary to collaborate with the health and safety committee.

2.3 Board of Directors of Université Laval resolution 2011-17

The obligations of Université Laval are the following:

- Providing a safe environment by removing, when possible, risks to health or safety;
- Promoting health, safety and the improvement of the work environment by observing laws, regulations, policies and procedures concerning these three themes;
- Establishing a means of communication to enable consultation between managers, employees and their union or association in order to identify job-associated risks and take measures to eliminate these risks;
- Defining the responsibilities of the authorities and various third parties.

2.4 Regulation respecting occupational health and safety: recommendations

2.4.1 Physical risks

Handling sharp objects

Sharp objects are used frequently in a protocol involving animals, such as needles used for injection or surgical tools. In such case, it is important to follow these rules:

- Do not replace the cap on a used needle; if you need to do so, hold it against a surface then use only one hand to replace the cap;
- Do not leave a needle without protection;
- Dispose of needles with used syringes in biohazard containers right after use;
- Sweep up broken glass and dispose of it in a container for breakable objects;
- Dispose of, or send for repair, slightly cracked glass or glass with a broken extremity.

Noise exposure

Exposure to noise should not exceed 85 adjusted decibels (dBA) during an 8-hour work shift for a 40-hour week.

Electricity

Accidents related to electric current may happen anywhere. In the presence of water and electricity exercise caution. Help prevent potential accidents by maintaining equipment, declaring deficiencies or equipment breakdown and following a safe work method.

Installing or repairing equipment must be done by a professional.

Radiation

There are two types of radiation:

- Ultraviolet radiation, which may cause burns; protection glasses are needed.
- Ionizing radiation (X-ray, computed tomography) must be controlled according to the standards issued by the local Radiation Protection Committee.

Using radiation requires a certificate issued by a Radiation Protection Committee.

The manager must be informed as soon as possible of any pregnancies among female staff.

Lasers

The use of lasers and others dangerous optical sources is supervised by the Comité de sécurité dans l'utilisation des lasers et des sources optiques dangereuses. The role of the latter is to counsel operators of lasers or apparatus working with lasers on safety aspects. Depending on the classification of the laser, operators must have received training and an eye examination, and the classification must be posted at the entrance of the laboratory.

2.4.2 Ergonomic risks

Carrying objects or heavy loads, handling carriages or cage pallets, repeated movements and repetitive movements are risks factors. A course on ergonomics at work and adapted tools may be offered.

When storing, there should be a sufficient space to move around and place objects or heavy boxes. Items should be stored efficiently as the material arrives in order to avoid overcrowding of the work environment.

2.4.3 Chemical risks

During experimental protocols, detergents/disinfectants, pesticides, anaesthetic gas and chemical products to preserve tissues are used during experimental protocols.

Each product used should have a data sheet visible in the work place. These data sheets are kept in a designated location and should be available at all times.

An Act Respecting Occupational Health and Safety states that all employers must provide WHMIS (Workplace Hazardous Materials Information System) training if workers must work with chemical products: training is **mandatory**.

Each product is labelled with a pictogram indicating the WHMIS category. Here are the main categories:

2.4.4 Bio-hazard

The use of experimental pathogens is legislated by *Laboratory Biosafety Guidelines* issued by Health Canada and the *Containment Standards for Veterinary Facilities* published by Agriculture and Agri-Food Canada, Appendix A. When research involves a bio-hazard, the candidates must receive a certificate from the Comité de gestion des risques biologiques de l'Université Laval to attest that containment requirements meet the safety standards corresponding to the containment level required by the project, or the research program.

The first rule for infection prevention is to maintain excellent personal hygiene. Washing the hands is to be performed before and after handling products or animals even if handling with gloves. Doing so helps to reduce the risks of contamination for both the researcher and the surrounding environment.

The main exposure channels to infectious agents are the following:

- Aerosols;
- Ingestion;
- Absorption by the skin, mucosa or wounds;
- Injection (accidentally during the research).

Protection requirements consider these channels of exposition; it is extremely important to respect these requirements.

Infectious agents are grouped in four levels of bio-safety depending of their contagion degree and the severity of the infection. Each level of bio-safety corresponds to a level of containment which should be respected for health reasons.

The Public Health Agency of Canada published pathogen safety date sheets (PSDS) which describes the characteristics of the pathogenic agents and also includes recommendations for working with these substances in the laboratory.

http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

2.4.5 Self-protection

Self-protection equipment

Some experiments require the use of personal protective equipment (PPE) which includes:

- Safety glasses;
- Frock;
- Gloves;
- Overshoes;
- Hat;
- Respiratory protection.

These measures must be posted at the entrance to offices, laboratories or any place where needed. Wearing sandals or open shoes is prohibited.

Even when wearing personal protective equipment, it is important to keep in mind that there are still risks. The material has to be maintained and used according to rules. Cleaning the workspace is also important and must be done frequently.

Laboratory hood and biological safety cabinet:

- **The fume hood:** Protects the handler when manipulating chemicals like paraformaldehyde. The fume hood MUST not be used when manipulating infectious agents.
- **Laminar flow positive pressure cabinet:** Air is filtered out of a HEPA (High Efficiency Particulate Air Filter) that removes fumes from the work space channelling them outside the hood. The material is protected, but the handler is not.
- **Biological safety cabinet:** The biological safety cabinet, when well maintained and used according to safety rules, makes primary containment efficient and is adapted to the handling of human pathogenic agents. The Laboratory Biosafety Guidelines are the reference to distinguish the different types of biological safety cabinet.

2.4.6 First aid

Event declaration: accident/incident

It is most important to notify the manager or another person in charge before leaving, if it is not possible, it should be done as soon as possible.

Spatter of chemical products

In the case of spatter, call the emergency number of the establishment and rinse with water for 15 minutes. Thereafter, the person must go to the hospital and bring the data sheet when possible. Eyewash fountains or eyewash bottles are located near the sinks. Do not put any product on the surface of the eyes or put any fat substance on the burns.

Bite or abrasion

In the case of bite, abrasion or contact with the fluids of an animal, immediately bleed the wound, clean with water and soft soap for 15 minutes then rinse with water. If the eyes or mucosa are involved in the accident, rinse with tepid water for 15 minutes. After, go to the hospital with the protocol explanation sheet. Any accident or incident involving contact with an animal or its excretions must be reported. There is a specific procedure for injuries involving a primate.

2.4.7 Vaccination

Vaccination, when available, helps reduce the risk involved in handling infectious agents. An evaluation prior to hiring determines which employees are more likely to get infected and provides the information on adequate preventive vaccination according to the handling the employee will be involved in and depending on the availability of the vaccines.

According to the protocols, an additional vaccination may be offered later.

2.4.8 Zoonosis

Zoonosis is a disease transmitted from animals to humans or from humans to animals. There are different types of zoonosis for each species. When the animal comes from a recognized supplier that certifies the health status of the animal, it is rare that the animal is infected with this type of disease.

Since there are risks that cannot be completely eliminated, it is essential for health concerns to:

- Respect the dress code established according to the species;
- Report any accident or incident involving contact with an animal or its excretions;
- Receive the necessary vaccines according to the species the handler is exposed to;
- Quarantine animals recently arrived.

If primates are used, there is a specific procedure in case of accident or incident since the risk of zoonosis is higher with primates.

In order to learn more about existing zoonosis, read Appendix VII in the Guide to the Care and Use of Experimental Animals of the CCAC:

http://www.ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf

2.4.9 Allergies

Developing allergies as a result of handling laboratory animals is an increasingly frequent phenomenon.

Most common allergens are:

- Rat urine and saliva;
- Mouse urine;
- Rabbit hair;
- Saliva, skin and hair of cats and dogs;
- Latex gloves.

Personal protective equipment helps prevent the development of allergies. Proper wearing of gloves, respiratory protection and compliance with the dress code help to manage the risk.

2.4.10 Psychosocial risks

Every individual may experience personal or professional problems and require support. A confidential professional counselling service is offered when someone has problems that may affect health, personal life or the ability to work.

http://www.santepsy.ulaval.ca/webdav/site/cspt/shared/pdf/Depliant_PAP_06-2010.pdf

3. The Animal Laboratory Facility

3.1 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.

3.2 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.).

3.3 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.

4. 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.).

4.1 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.

4.2 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.

4.3 Basic Care Factors

4.3.1 Housing

<u>Feeding</u>

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.

<u>Types of Shelters</u>

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.

4.3.2 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.

4.4 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.

5. 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.

6. 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.

7. Experimentation

7.1 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.

7.2 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**.

7.2.1 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.

<u>Opioids</u>

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, α_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 α_2-adrenergic receptors, many systematic effects are to be expected with use of these molecules. However, in the case of overdose or intolerance, α_2-adrenergic antagonists are antagonisable.

7.2.2 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.

7.3 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.

7.4 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

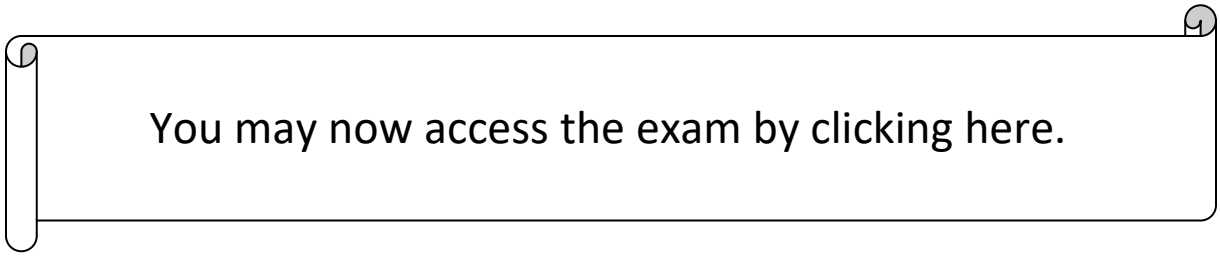
7.5 Euthanasia

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_2).

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

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



References

CCPA (CCAC)

http://3rs.ccac.ca/en/

Centre for Alternatives to Animal Testing (CAAT)

http://caat.jhsph.edu/

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

http://iccvam.niehs.nih.gov/

The National Centre for the Replacement, Refinement and Reduction of Animals in Research

http://www.nc3rs.org.uk/