Contents of the Animal User Training Program

1. Ethics

The use of animals in research is a privilege granted with the understanding and expectation that such research will be conducted according to the highest ethical and legal standards. The basic principles governing animal research are found in the Public Health Service Policy on Humane Care and Use of Laboratory Animals (PHS Policy) and the Guide for the Care and Use of Laboratory Animals (Guide).

These principles include the following imperatives:

Animal experiments should be undertaken only after due consideration of their relevance for human or animal health and the advancement of biological knowledge.

- Use of appropriate species, quality, and number of animals to achieve scientific objectives.
- Use the least sentient species that will permit the attainment of research objectives.
- Use of appropriate sedation, analgesia, or anesthesia.
- Use the least painful or distressful procedures needed to meet research objectives should be used, and all reasonable measures to minimize pain and distress.
- Establish experimental end points.
- Consider always the principles of replacement, reduction, and refinement (3 R’s) when planning and conducting studies.
- Procedures that would be considered painful in humans should be considered to be painful in animals.
- Maintain the best possible living conditions for animals kept for research, training, or testing purposes. Animal care should be supervised by a veterinarian. Housing should ensure that the general health of animals is safeguarded and that undue stress is avoided, with appropriate attention paid to environmental factors such as temperature, ventilation, and humidity.

2. Intuitional Animal Care and Use Committee (IACUC): Aims, Structure and Functions

The AUB Faculty of Medicine has established in 2003 an “Institutional Animal Care and Use Committee” (IACUC).

2.1 The aim of the IACUC is to make sure that laboratory animals used in research, teaching, testing and training at AUB are treated in accordance with the “US Government
Principles for the Utilization and Care of Vertebrate Animals used in Testing, Research and Training”.

2.2 **IACUC Structure**

The IACUC must be composed of **at least 5 members, appointed by the Dean of the Faculty of Medicine**.

The members must be qualified through experience and expertise to provide oversight for the institution’s animal programs, facilities, and procedures.

The mandatory members include the following:

- A veterinarian with direct or delegated program responsibility for animal-related activities at the institution

- A practicing scientist experienced in animal research

- A person whose primary concerns are in non-scientific areas (community representative), a person who is unaffiliated with the institution.

2.3 **IACUC Functions:**

- The IACUC must review all activities involving the care and use of animals in research or teaching. *(The USDA Animal Welfare Act requires that the IACUC perform the same duties for all activities involving animals covered by the Act, regardless of funding source.)*

- All new proposals for animal use and significant changes to existing proposals for animal use must be reviewed and approved by the IACUC before animal use can begin.

- No animals may be used for experimental procedures, including field studies, pilot studies, breeding, or euthanasia for sample collection without receiving prior IACUC approval.

- Review and approve, require modification in, or withhold approval to, proposals for research and teaching that require the use of animals.

- Review and approve, require modification in, or withhold approval to, changes regarding the use of animals in ongoing, previously approved, activities.

- Suspend any activity that is not in compliance with the policies and guidelines that govern the use and care of animals at AUB-FM.

- Inspect the institutional animal care facilities at least once every 6 months.
- Review the Animal Care Program for the humane care and use of animals at AUB-FM at least once every 6 months.

- Submit reports to the Dean or designated institutional official on the review and inspection processes at least once every six months.

- Make written recommendations relating to the Animal Care Program, animal care facilities and related personnel or programs.

- Review concerns involving the care and use of animals at the AUB-FM.

- The IACUC must have oversight of all areas where animal procedures are performed and may determine the appropriate interval for inspection of procedural spaces:
  - This includes spaces in research laboratories where routine procedures are performed
  - This includes any non-surgical procedures, such as weighing, blood collection, and euthanasia.

- The IACUC must inspect surgical records, drug records, and experimental records as appropriate to monitor for protocol adherence.

2.4 **Review Methods of Research Projects**

There are two valid methods of animal proposal review by the IACUC

- Full committee review by a convened quorum of the IACUC. This can be an in person meeting, or a real time meeting by teleconference, video conference, etc.
- Designated review by one or more members of the committee only after all voting members have been provided the opportunity to review the proposal and call for full committee review.

After review of animal proposals, the IACUC may take one of the following actions

- Approve as written

- Require modifications to protocol to secure approval

- Disapprove the proposal.
3. Design, organization and management of an animal experiment

Animal experiments should be designed and executed so that the results are as informative as possible. It is wrong to use too few animals as the experiment may lack the power to detect some biologically meaningful effects. Such experiments will be a waste of time and resources, and an unnecessary sacrifice of animals. Likewise, it is wrong to use more animals than are required to detect a treatment effect. In order to prevent the use of either too few or too many animals, it is essential to consider, prior of the experiment, the experimental design and the number of animals needed.

Steps of design and execution of an animal experiment:

<table>
<thead>
<tr>
<th>DESIGN</th>
<th>ORGANIZATION</th>
<th>MANAGEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Formulate hypothesis</td>
<td>- assign tasks: who, what, when</td>
<td>This includes all activities necessary to ensure that</td>
</tr>
<tr>
<td>- Condense to working hypothesis (WH)</td>
<td>- check regulatory requirements</td>
<td>– personnel, animals, equipment and space is used efficiently</td>
</tr>
<tr>
<td>- choose test object (human, research animal: “in vivo”, “in vitro”)</td>
<td>- establish time table</td>
<td>- the quality of the experiment according to the experimental design is guaranteed</td>
</tr>
<tr>
<td>- is method appropriate to test WH</td>
<td>- assign personnel according to qualifications</td>
<td>important notes:</td>
</tr>
<tr>
<td>- prepare outline of experiment</td>
<td>- supervise proper execution of design</td>
<td>- literature search/study as thorough as possible</td>
</tr>
<tr>
<td>- re-evaluate degree of pain and distress</td>
<td>- document results in report or publication</td>
<td>- evaluate all alternative methods</td>
</tr>
<tr>
<td>- is experiment selective enough to test WH</td>
<td></td>
<td>- review experiment from ethical perspective</td>
</tr>
<tr>
<td>- is experiment “valid” and “reproducible”</td>
<td></td>
<td>- integrate regulatory requirements (animal use methods and techniques)</td>
</tr>
<tr>
<td>- ensure means of data collection, data processing and data analysis</td>
<td></td>
<td>- personnel must be trained appropriately</td>
</tr>
<tr>
<td>- ensure resources (personnel, space, costs)</td>
<td></td>
<td>- submit animal permit application in time (animal order, possible quarantine, condition of animals, pilot study, etc.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- consider (all) factors, which could influence animal experiment (genetic quality, colony of origin, age, weight, gender, health, nutrition, transportation, housing [cage + animal room], acclimatization to environment, experimental conditions, handling by caretaker and/or investigator, standardization of methods, duration of procedures, application of substances, reproducibility of procedures and applications, pain and distress degree, anesthesia, analgesia, euthanasia or re-use of animals)</td>
</tr>
</tbody>
</table>
4. The Laboratory Animal

4.1 The Animal Model: An animal model is a living, non-human animal used during the research and investigation of human disease, for the purpose of better understanding the disease process without the added risk of harming an actual human. It is required that animals in such models should:

- Be Handy, easy to care for and handle, and are inexpensive.
- Be small in size which means they can be housed in large numbers.
- Have short generation time and high reproductive potential which contribute to their usefulness in genetics research and to the economy of their production.
- Be amenable to germfree and pathogen-free production techniques.

4.2 Scientists can create animal models, by multiple methods including, the transfer of new genes into them.

Examples of animal models include:

- Induction of cancer in a mouse to simulate cancer in a human
- Studying a genetic disease in a pure-bred dog that is similar to a human disease
- Transplanting an organ between 2 pigs ...etc...

4.3 It is required by law that scientists first seek alternatives before using animals in experiments.

To illustrate the extensive use of animal alternatives in science today, compare the numbers of animals used in research now with the numbers used 30 years ago, in the 1970's. That number has not changed very much despite an enormous growth in medical research and in our population. The additional research is being done on something other than animals.

Alternatives cannot always answer all of the questions scientists have, they resort to animals.

A computer model is only as good as a person can make it. Therefore, it is limited by what is already known.

Cell culture systems are also limited in that they represent tissues taken out of the body. They may not behave normally when removed from the blood, immune system, nervous system and neighboring tissues that help make them what they are. In addition, even cells for culture must come from an animal initially.
Studying patients is also not ideal.

Usually patients with disease have many other problems at the same time. It is harder to control an experiment using a patient, and conclusions about the experiment can be less clear.

The natural course of a disease in a patient such as a human or a dog may take many years. This makes it extremely difficult to study it, compared to using a mouse, for example, in which the disease may progress over a few weeks or month.

But animal alternatives have their limitations, as do animal models. Sometimes an animal model is too complex and a cell culture system or computer model allows study of a single aspect of the disease. In addition, an animal model can't be created until patients are studied and the disease is described and defined. All of these models are used together to arrive at an answer.

4.4 Nutrition for Laboratory animals

Laboratory diets are standardized and balanced diets. Different diets are available like:

- non-purified diets (from unrefined ingredients), or purified diets (from refined ingredients),
  - chemically defined diets (from purified ingredients),
  - formulated diets (for special purposes like nutritional or toxicological studies),
  - autoclavable diets (excess level of thermo-labile nutrients).

**Note that:**

For Rats

- the daily feed intake is 5-6 g / 100g Bodyweight
- the daily water intake is 10-12 ml / 100 g Bodyweight.

For Mice

- the daily feed intake is 4-5 g / mouse
- the daily water intake is 6-7 ml / mouse

It is advisable for laboratory animals to have food and water ad libitum (except where experimental procedures dictate otherwise).
4.5 Housing:

Rats should be housed in spacious, ventilated, and dry cages constructed of either non-chewable plastic or stainless steel. The Bedding should be changed frequently (2 times weekly, diabetic animals daily) to minimize odor and to reduce the possibility of disease in the colony. Humidity within the colony room should be maintained between 40 and 60 percent whereas the temperature should be kept between 22-24°C. Lighting should be diffuse throughout the colony and of intensity sufficient to allow laboratory procedures to be carried out. Light schedules should be diurnal (i.e. 12 hr/12 hr day/night schedule) because continuous lighting schedules may produce partial retinal degeneration in rats.

The following parameters influence housing:

- **Temperature:** High tiredness, food consumption, fecundity, and growth rate

- **Humidity:**
  - High humidity reduces food consumption, impairs body temperature regulation
  - Decreases activity and enhances disease transmission, increases NH3 production (from increased bacteria development)

- **Lighting:**
  - Should be 12 hr – 12 hr day/night schedule
  - Effect of constant light: cessation of cycling, excessive ovarian follicles, enhanced tumor growth and metabolism. Re-final degeneration

- **Change in Circadian rhythm:** Has effects on physical parameters;
  - Affects research results and test values; it is important to test always at same time of day!

- **Noise:**
  - Alters water consumption,
  - Leads to changes in blood pressure
  - Leads to changes in adrenal function
  - Influences the immune system

- **Caging:**

Conventional caging for rodents consists of a plastic “shoebox” – available in different sizes with bedding, water bottle, and feed lid. This unit is then placed on a rack with many other cages. Variations on this type of housing are available. Some examples are: micro-isolator lids to prevent gross contamination and wire bottom cages to prevent access to bedding material, feces and urine.
- Housing density:

Maximum number of animals per cage

<table>
<thead>
<tr>
<th>Species</th>
<th>Cage Type I</th>
<th>Cage Type II</th>
<th>Cage Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 cm²</td>
<td>360 cm²</td>
<td>810 cm²</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30g</td>
<td>4</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>&gt; 30g</td>
<td>2</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 100 g</td>
<td>-</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>100 – 250 g</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>250 – 500 g</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 500 g</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

5. Normal Behavior, Health Assessment, Adaptation to changed environmental Conditions

Becoming familiar with the appearance, behavior, and physiology of the normal animal is of primary importance in assessing its well-being. It is also important to be aware of the variation in appearance, behavior and physiology depending on the age, strain, sex and time of day.

In addition to the normal behavior patterns of the individual animal (posture, grooming, feeding, sleeping, urinating, defecating, etc.) its interactions with cage mates and its awareness of the surrounding environment should be noted. Included in the animal's normal behavior is the normal response to being handled and examined.

The normal, healthy laboratory animals have a smooth, clean, well-groomed hair coat. Lack of grooming is usually a reliable indicator of dysfunction. Body condition can be assessed from observing the shape and posture of the animals.

An understanding of the normal food and water consumption, and the nature and amount of urine and feces produced daily are important aspects of the knowledge of the normal healthy animal. The behavior of animals when eating or drinking should also be noted.

In general, most healthy small laboratory mammals are active, alert and inquisitive when they are
approached. Normally any disturbance of the animals should produce a response. When handled, the animals should feel warm to the touch.
For a more detailed physical examination, small rodents and rabbits will have to be handled and restrained. The normal reaction of these animals to handling should be understood, so that unusual or abnormal reactions are noted.

For researchers it is important to know that the following stressors may influence animal experiments and their results:

- Transportation
- Changes in husbandry (cage, feeding, bedding, handling!!)
- Formation of new groups (disruption of established social structures)
- Environmental changes (temperature, microbiological status)

Adaptation to changed environmental conditions:

<table>
<thead>
<tr>
<th>Conditioning time</th>
<th>Stressor</th>
<th>Controlled parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Approx. one week</td>
<td>Formation of a new social group</td>
<td>corticosteroids</td>
</tr>
<tr>
<td></td>
<td>transportation</td>
<td>Body weight</td>
</tr>
<tr>
<td></td>
<td>Handling</td>
<td>Red blood cell count</td>
</tr>
<tr>
<td></td>
<td>Loss of 10% of blood volume</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>One week to one month</td>
<td>Light cycle</td>
<td>Motor activity</td>
</tr>
<tr>
<td></td>
<td>Change in temperature</td>
<td>Food consumption</td>
</tr>
<tr>
<td></td>
<td>Long Transportation (rat)</td>
<td>Serum proteins</td>
</tr>
<tr>
<td></td>
<td>Transportation (pig)</td>
<td>Plasma testosterone</td>
</tr>
<tr>
<td>Longer than one month</td>
<td>Fat (cholesterol in feed)</td>
<td>Cholesterol concentration in liver</td>
</tr>
</tbody>
</table>
6. Pain Recognition and Management:

The American Academy of Pain Medicine defines pain as:

“An unpleasant sensation (that can range from mild, localized discomfort to agony) and emotional response to that sensation”.

<table>
<thead>
<tr>
<th>Pain Category</th>
<th>Type of Experiments / Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Experiments / Procedures causing little discomfort or stress</td>
</tr>
<tr>
<td>D</td>
<td>Experiments / Procedures causing moderate to severe distress or pain using anesthesia and / or painkiller</td>
</tr>
<tr>
<td>E</td>
<td>Experiments / Procedures causing prolonged or severe clinical distress or pain without the use of anesthesia and / or painkiller</td>
</tr>
</tbody>
</table>

6.1. Category C

Experiments causing minor stress of short duration

Level C procedures should not cause significant changes in the animal’s appearance

Category C procedures include:

- Injection of material in amounts that will not cause adverse reactions
- Acute non-survival studies in which the animals are completely anaesthetized and do not regain consciousness
- Approved methods of euthanasia following rapid unconsciousness
- Short periods of food and/or water deprivation equivalent to periods of abstinence in nature
- Canulation or catheterization of blood vessels or body cavities under anesthesia
- Minor surgical procedures under anesthesia- biopsies, laparoscopy
- Short periods of skillful restraint beyond that for simple observation or examination
- Blood sampling
6.2 Category D

Experiments causing moderate to severe distress or pain using anesthesia and / or painkiller

Level D procedures should not cause prolonged or severe clinical distress

Category D procedures include:

- Major surgical procedures conducted under anesthesia with subsequent recovery
- Prolonged (several hours or more) periods of physical restraint
- Induction of behavioral stress - like maternal deprivation, aggression, predator-prey interactions
- Procedures causing severe, persistent or irreversible disruption of sensorimotor organization

6.3 Category E procedures include:

Experiments causing prolonged or severe clinical distress and / or pain without the use anesthesia and / or painkiller

- Procedures inflicting severe pain near, at, or above the pain tolerance threshold of not anesthetized, conscious animals
- Not confined to surgical procedures, but may include exposure to noxious agents or those having unknown effects
- Exposure to drugs or chemicals at levels that may impair physiological systems and cause death, severe pain, or extreme distress
- New biomedical experiments having a high degree of invasiveness
- Behavioral studies having unknown degree of distress
- Muscle relaxant or paralytic drug use without anesthetics
- Burn or trauma infliction on not anesthetized animals
- Toxicity testing and experimentally-induced infectious disease studies that have death as the endpoint

One of the major problems in recognizing the signs of pain and/or distress in the laboratory rodents is the small size of the animals. Some of the clinical observations made in larger animals, to assess their health and well-being (e.g., temperature, pulse and respiration) are not easily accomplished in
laboratory rodents. Thus it becomes even more important to assess the behavior of these animals for any deviations from normal behavior that may be signs of pain or discomfort. To do this both the individual animal and its behavior in the group environment must be considered.

There is tremendous variation in the behavioral and physiological responses to pain and/or distress in animals, from species to species, and within a species there are individual, sex, and age differences. The signs suggestive of acute pain, and those of chronic pain, should be understood by all persons responsible for monitoring the animals during the research. Signs of chronic pain or distress are often more insidious and careful observation is required to detect the changes in an animal's appearance and behavior which indicate deterioration in the condition of the animal. Observation of the animals should be done frequently, since signs of pain and discomfort are not constantly present.

Depending on the organ system(s) affected, there are some specific behavioral and physiological signs that can be used to assess the condition of the animal. For example, if the respiratory system is affected, the rate and nature of the respiration will change. Change in the nature and amount of feces (e.g., diarrhea) may indicate an intestinal effect. For a detailed listing of the potential signs of disease, pain and/or distress in relation to body systems affected, the reader is referred to the appendix. The presence or absence of these specific signs should be recorded.

The recognition of pain, pain-induced distress, and non-pain-induced distress in animals is necessary for proper clinical management of laboratory animals to ensure compliance with current guidelines and regulations.

Well-being in animals is associated with normal species-typical behavior. A well animal will interact with its cage mate, exhibit curiosity through exploration of its surroundings, keep itself well-groomed, grow normally and have normal reproduction.

An animal in severe pain may not like to move around. Wounded or painful areas may be wet from excessive licking or chewing and be red and swollen. The animal may look repeatedly at the painful area. The animal may vocalize when in pain. Not all vocalization is associated with pain as restraining an animal may elicit vocalization.

Chronic or persistent pain is different from acute pain and its clinical manifestation can be harder to recognize. Chronic pain is more likely to lead to distress and maladaptive behavior. Signs of chronic pain include decreased appetite, weight loss, reduced activity, irritability and decreased reproductive performance. Some chronic conditions can lead to self-injurious behavior such as chewing the skin to the point of creating an open wound.

Pain thresholds are similar among all species and breeds of animals; however, the perceived intensity and tolerance of pain is highly variable among individuals. There are notable differences between animals and humans related to the site of pain. Abdominal surgery is thought to be less painful in four-legged animals than in humans because humans use their abdominal muscles to a greater extent in
maintaining posture and for walking. A median sternotomy produces low to moderate pain in humans but much pain in animals because four-legged animals use their forelimbs for walking. A lateral thoracotomy is less painful in animals than humans because animals rely more on abdominal respiration and humans more on thoracic respiration.

Species-specific Behavioral Signs of Pain as Criteria for Administering Analgesics

<table>
<thead>
<tr>
<th>Species</th>
<th>Behavioral Signs</th>
</tr>
</thead>
</table>
| Rodents | Decreased activity  
Rapid, shallow respiration  
“Red tears” in albino rats  
Vocalize, may be above human hearing  
Aggressive  
Feed and/or water refusal  
Weight loss |
| Rabbits | Appear apprehensive, anxious, dull, or inactive  
Squeal or cry  
Teeth grinding  
Tonic immobility (play dead)  
Feed and/or water refusal  
Weight loss |
| Pigs    | Dull, depressed, head held low  
Rapid, shallow respiration  
Grunting or grinding teeth  
Reluctance to move  
Loud, persistent vocalization |
In general, analgesics should always be administered after any type of surgical procedures unless there is a scientific justification for not doing so; that justification extends to both opioid and non-steroidal anti-inflammatory drugs and the justification has to be approved in the animal care and use protocol by the IACUC.

7. Anesthesia

When anesthesia, analgesia, or chemical restraints are used, it may be advisable to ascertain any distortion of results by anesthetics through limited trials. Check the literature and package inserts for the effect of the agent on the systems being experimentally evaluated. These changes need to be taken into consideration when evaluating the effect of an experimental manipulation. Choose the agent which has the least effects on the systems under investigation. General anesthetics often depress the cardiovascular and respiratory systems, alter blood gases, lower metabolism, decrease body temperature, and alter tissue perfusion. Anesthetics can also produce histopathologic changes.

7.2 Utilization of anesthetics and analgesics in laboratory animals

<table>
<thead>
<tr>
<th>Local Anesthesia</th>
<th>General Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition:</strong></td>
<td>Reversible block in central nervous system with</td>
</tr>
<tr>
<td></td>
<td>- loss of consciousness</td>
</tr>
<tr>
<td></td>
<td>- analgesia</td>
</tr>
<tr>
<td></td>
<td>- Suppression of reflex activity</td>
</tr>
<tr>
<td></td>
<td>- Muscle relaxation</td>
</tr>
<tr>
<td><strong>Methods:</strong></td>
<td>Injection Anesthesia</td>
</tr>
<tr>
<td></td>
<td>Inhalation Anesthesia</td>
</tr>
<tr>
<td>- Topical</td>
<td>- Local infiltration</td>
</tr>
<tr>
<td>- Nerve block</td>
<td>- Epidural</td>
</tr>
<tr>
<td>- Epidural</td>
<td></td>
</tr>
</tbody>
</table>

Anesthesia is a state of controllable, reversible insensibility in which sensory perception and motor responses are both markedly depressed.
General Anesthetics

Mice, Rats

7.2 Anesthesia of Small Animals

Over the years, the types of anesthesia used have changed. Inhalation anesthetics, such as isoflurane and methoxyflurane have replaced ethyl ether and chloroform.

Avoid using ethyl ether. The use of ethyl ether in the Animal Care Facility needs the permission of the IACUC.

7.2.2 Inhalation Anesthesia:

Methoxyflurane or isoflurane are administered in bell jar, by nose cone, by tube or mask (use hood).

7.2.3 Injectable Anesthesia – recommended dose of some anesthetics:

Mice and Rats: Ketamin 70-100 mg/kg with Xylazine 7-10 mg/kg IP or IM

7.3 Anesthesia Guidelines

Choose the right anesthetic by paying attention to:

- Type of procedure (laparotomy; blood collection; examination)
- Duration of procedure
- Species

7.4 Maintenance:

- Monitoring (Depth of anesthesia and analgesia)
- Hydration (Loss of fluid to due reduction of intake, evaporation from surgical site and blood loss)
- protection of cornea
- body temperature (loss of thermoregulation, body mass to surface ratio)

7.5 Monitoring:
- Respiratory system (Resp. rate, quality of resp.)
- Cardiovascular system (Pulse oxymeter, Blood pressure, Heart rate)
- Temperature
- Analgesia (check depth of anesthesia)

7.6 Complications:
- Vomiting (not in rodents or rabbits, large animals fast overnight, Atropine)
- Respiratory arrest (mechanical ventilation)
- Cardiac arrest
- Bleeding

7.7 Post operative care:
- Thermoregulation
- Prevention of bedding inhalation
- Analgesia
- Hydration (IV, IP or subcutaneous fluids)
- Monitoring (general condition, wound healing)

7.8 Recommended analgesics

7.8.1 for rodents are:
- tramal, carprofen (moderate pain)
- aspirin (moderate pain)
- buprenorphine (strong pain)

7.8.2 For large animals, e.g. rabbits:
- tramal, carprofen (moderate pain)
fentanyl (strong pain)
buprenorphine (strong pain)
marcaine (long acting local)

Always keep in mind that based on a short term observation it is not possible to form an accurate opinion on the behavior of the species, especially in regards to pain and suffering. We should assume than any procedure that would be expected to cause pain in a human will cause pain in an animal as well.

8. Euthanasia

Animals must be euthanized by humane methods as described in the 2000 Report of the AVMA Panel on Euthanasia and the Guidelines for Euthanasia, IACUC - AUB. The veterinarian has the authority to use professional judgment to request or perform euthanasia when he encounters conditions that have placed research animals under severe, unanticipated pain or distress that has not or cannot be relieved in a timely manner by alternative methods.

Investigators may choose to have Animal Care Facility personnel perform this procedure by marking the cage with XXXXX on the orange sticky notes that are available in all animal holding rooms.

Every animal must have access to food and water and be housed within cage density guidelines until they are euthanized.

Common acceptable euthanasia methods:

Rodents:

1. CO2 100% inhalation. Neonates have resistance to hypoxia and should be kept longer in CO2 chamber or use a physical method such as decapitation following CO2 inhalation.

2. Anesthesia over dose, 300 mg/kg Ketamine and 30 mg/ kg xylazine.

3. Under deep general anesthesia removal of a vital organ, cervical dislocation or exsanguination (drawing a large amount of blood)

Death must be verified by cessation of breathing, cardiac function and loss of reflexes.
9. **Alternative Methods to Animal Experimentation, Teaching and Testing**

Pain, suffering or injury shall only be inflicted on an animal when the purpose of the experiment can be achieved in no other manner.

*Experiments with animals which cause the animal pain, suffering, injury, intensive fear or significantly disturb their general conditions, must be limited to the indispensable extend.*

The concept of alternatives was first enunciated in 1959 by two British scientists W.M.S. Russell and R.L. Burch in *The Principles of Humane Experimental Technique*—who argued that animal researchers should always follow the principle of the "Three R's"—*reduction* of animal numbers, *refined* procedures to minimize or avoid pain, and *replacement* of animals with non-animal models.

**Replacement** refers to situations where non-animal techniques may be substituted for techniques using research animals. There are a number of examples of such replacement in the diagnosis of disease and in the testing and standardization of biological therapeutic agents. Rabbits are no longer used in pregnancy tests. Using mice to test the potency of batches of yellow-fever vaccine was long ago replaced by a cell culture test. We may be close to eliminating the use of mice in insulin-standardization procedures as a result of a variety of technical advances.

**Reduction** refers to cases where the number of animals required for a particular activity or project can be reduced. One example of recent progress comes from the field of acute toxicity testing. Most toxicologists now agree that it is not necessary to use from 60 to 200 rodents to generate the statistically precise lethal dose. Today one can obtain perfectly adequate lethal-dose data using no more than 10 to 20 animals.

**Refinement** is an often neglected aspect of the alternatives concept. It refers to the modification of a technique to reduce the pain and distress experienced by research animals. For example, various jacket and tether systems have been developed to protect catheters inserted into research animals which then allow an investigator to administer doses of test chemicals and take blood samples from an animal without having to restrain it. Capture and restraint often cause significant distress to an animal, so the use of the jacket and tether constitutes a real refinement.

According to the Animal Welfare Act (AWA) regulations (9 CFR 2.31(d)), "the IACUC shall determine that the principal investigator has considered alternatives to procedures that may cause more than slight pain or distress to the animals, and has provided a written narrative of the methods and sources used to determine that alternatives were not available."

10. **Health and Safety**

The key to preventing injuries is proper training and meticulous attention to proper work practices.

- Use appropriate techniques for animal handling and restraint.
- Avoid recapping needles and dispose in approved Yellow Containers.
- Dispose sharps in the approved Yellow Containers (biohazard “sharps” container)
• Wear recommended personal protective equipment such as a lab coat, gloves, and eye protection, if required.
• Work in a clean, well-ventilated environment
• To reduce the risk of needle stick injuries, consider sedating or anesthetizing animals if hazardous materials will be used, or if manual restraint is problematic

The proper use of hazardous biological, chemical, and physical agents depends on careful planning, proper training, and careful attention to prescribed work practices. Signs should be posted indicating the nature of the hazard, necessary precautions, and emergency contact information. The personal protective equipment needed depends on the agent in use, but in all cases gloves should be worn and hands should be washed after handling potentially contaminated materials. A biological safety cabinet should be used when handling infectious materials, especially if there is a potential for generation of aerosols, and a fume hood should be used when handling toxic chemicals or radioactive materials.

Reference:

- The Guide for Care and Use of Laboratory Animals 1996, National Research Council