Education and Training for Animal Experiments in Kyushu University

Institutional Animal Care and Use Committee (IACUC)
Kyushu University
Education and training for Animal Experiments in Kyushu University

The fundamentals of Laboratory Animal Science
What is Proper Animal Experimentation?

Your research---

● Should have Universality and Clearness in the purpose and meaning.
● Should be Scientifically operated.
● Should have Reproducibility: one of the main principles of the Scientific data.

You---

● Should concern the Ethical Treatment & Welfare for Laboratory Animals.
● Should Compliance with Legal Restrictions.
What is Proper Animal Experimentation?

- Universality and Clearness of purpose and meaning
- Scientifically operated researches
- Reproducibility: one of the main Principles of the Scientific data
- Ethical Treatment & Welfare for Laboratory Animals
- Compliance with Legal Restrictions
Reproducibility: One of the main principles on Scientific Research

*In vitro* Experiments (Chemistry Expert)

- Pure Reagents

*In vivo* Experiments (Medical or Life Science Expert)

- Complex & Rough Reaction System in Animal bodies
- Less Reproducible Data With Large Dispersion

Simple & Rigid Reaction System in Test Tubes

Precise Measure Instruments

Reproducible Data with Small Dispersion
Reproducibility of Animal Experiments

Uniform Reaction In Animals

Genetic Control

Environmental Control

Prevention of Infectious Disease

Restricted environment in animal room

Inbred Strain

SPF Animals
How to produce an Inbred Strain

Over 20 Generations With Sib mating

1#Generation

2#Generation

20th Generation Inbred Strain

21th Generation Inbred Strain

Homo zygotes cromosome and 99.99% genes identical
Environmental Control
Keeping Constant Environment in Animal Facility

Temperature: 20~26°C
Humidity: 30~70%

Number of Ventilation: 10~15 times/hour
Current of Air: 13~18cm/second
Bad Smell: <20ppm of Ammonia

Brightness: 150~300 lux
3Rs Principle for Ethical Treatment on Animal Experimentation

This is an international principle for ethical treatment on animal experimentation.

Russell & Burch (1969) proposed it as ethical standard for the investigators using animal experimentation.

**Replacement:** Apply the alternative methods that do not require the use of animals or use animals of the lower orders, such as fishes, reptiles instead of mammals

**Reduction:** Use as few animals as possible

**Refinement:** Use skillful methods and apply the methods that do not distress the animals or subject them to pain
What are "alternatives?"

What is meant by the use of the term "alternatives?"

The word alternative clearly implies whole or partial substitution for some existing technique or approach.
Types of “alternatives”

There are many different types of alternatives that can be introduced into research and testing.

1. Inanimate systems such as computer simulations, life-like models, or video presentations to replace animals or reduce the number used.

2. In vitro techniques such as cell or organ cultures likewise can replace or reduce the number of animals used.

3. The use of nontraditional animals or plants lower on the phylogenetic scale may be considered a refinement in animal use.
Japanese Regulatory System on Laboratory Animal Welfare
Japanese Regulatory System on Laboratory Animal Welfare

- Law for the Humane Treatment and Management of Animals
- Standard Relating to the Care, Management and Alleviation of Pain and Distress of Laboratory Animals
- Various Guidelines
  - Ministry of Education, Culture, Sports, Science and Technology
    “the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions”
  - Ministry of Health, Labor and Welfare
  - Ministry of Environment
  - Ministry of Agriculture and Fisheries
  - The Science Council of Japan
The SCJ has submitted recommendations and reports on how proper animal tests should be since 1980. As a result, the 3R principle (Replacement, Reduction and Refinement) on animal tests was stipulated in the partial revision of the “Law Concerning Protection and Management of Animals” in 2005. The Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor and Welfare asked the SCJ on November 22, 2005, and on March 9, 2006, respectively, to prepare common guidelines as a model regulation on animal experiments and related activities by research institutes. In response to the requests, the SCJ submitted the “Guidelines for Proper Conduct of Animal Experiments” for both ministries and publication on June 1.


(Fundamental Principle)

Article 2

Because animals are living beings, all people are required not only to avoid purposeless killing, and mistreatment of animals, but also to care for respecting animals properly, while the relationship shared people and animals, taking the natural habits of animals into account.
(Methods When Animals are Used for Scientific Purposes and Subsequent Disposal of Such Animals)

Article 41

1. **Replacement**: the application of alternative methods that do not require the use of animals within limits that allow scientific objectives to be achieved,

**Reduction**: the use of as few animals as possible within limits that allow scientific objectives to be achieved, and

2. **Refinement**: the application of methods that do not distress the animals or subject them to pain within limits required for use.
Summary of 3Rs

- Consider using alternative methods when carrying out an animal experiment.
- Consider reducing the number of the animals used for an animal experiment.
- Conduct an animal experiment by the method with the least pain and distress to the animal.
- Euthanize laboratory animals by the method with the least pain and distress to the animal.
- 3Rs mean Reduction, Replacement and Refinement.
Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain

- Usage of animals for scientific purpose is necessary and indispensable for the advancement of biomedical science and the development of medical technology.
- However the 3Rs should be considered when animals are used for scientific purposes.
Summary of the Standard

- Holding of laboratory animals cannot be conducted outside the approved facility.
- Ask for treatment, when the animals have sustained an injury or contracted an illness.
- The laboratory animal administrator does need to perform quarantine and isolating housing of laboratory animals when introducing animals.
- Provide appropriate food and hydration.
- Only introduce laboratory animals from proper contractors (regulation etc).
Education and training for Animal Experiments in Kyushu University

Regulation for Animal Experiments at Kyushu University
(Basis)

Article 1

This Regulation covers the proper and safe performance of Animal Experiments and Related Activities in Kyushu University (the “University”) from the standpoints of science, animal welfare, environmental conservation. It is based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1973), the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notice No. 88 of Ministry of Environment, 2006), the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Notice No. 71 of the Ministry of Education, Culture, Sports, Science and Technology, 2006) and other related laws and regulations.
(The President)

Article 3

The President of the University unifies the proper and safe performance of Animal Experiments and Related Activities in the University.
Article 4

1. The Institutional Animal Care and Use Committee (the “Committee”) defined in the regulations of Kyushu University Deans and Directors Meeting (Regulation No. 14, 2004) deliberates the matters of Animal Experiments and Related Activities.

2. Membership of Committee, Committee Meeting and relevant particulars were based on the rules of Kyushu University Institutional Animal Care and Use Committee (Rule No. 195, 2004; the “Committee Rules”).
(Education and Training)

Article 9

The President provides education and training for the Animal Experiment Researcher, etc. before starting the Animal Experiments and Related Activities.
(Registration of the Animal Experiment Researcher)

Article 10

1. The Animal Experiment Researcher, etc. shall apply for the registration of the Animal Experiment Researcher to the Dean of the Faculty.

2. The Dean of the Faculty shall register the applicant as the Animal Experiment Researcher after confirming that the applicant attending the education and training in the previous paragraph.

3. The Dean of the Faculty shall report the names of registrants to the President.
Article 12

1. When conducting Animal Experiments and Related Activities, the Principal Investigator shall draft and submit an Animal Experiment Protocol to the President through the Dean of the Faculty, and receive approval before beginning the Animal Experiment and Related Activities.

2. In cases where changes are made to an approved Animal Experiment Protocol in the previous paragraph, the Principal Investigator shall submit an application form for the alteration of the protocol to the President through the Dean of the Faculty, and receive approval.

3. When submitting the applications in the previous two paragraphs, the Dean of the Faculty shall request that the Faculty Committee pre-reviews the protocol and report the decision to the President.

4. When accepting the report in the previous paragraph, the President shall request that the Committee reviews the protocol and then decide whether or not approve.
Regulation for Animal Experiments at Kyushu University
Regulation No. 14, 2005

(Responsibility of the Animal Experiment Researcher)

Article 14

1. The Animal Experiment Researcher shall take the items below into consideration for drafting and conducting the Animal Experiments and Relative Activities.

(1) When drafting the Animal Experiments and Relative Activities, the Animal Experiment Researcher shall consider the application of alternative methods that do not require the use of Laboratory Animals within limits that allow scientific objectives to be achieved.

(2) Consideration of the selection of Laboratory Animals species appropriate for the purpose of Animal Experiments and Related Activities and the use of as few Laboratory Animals as possible within limits that allow scientific objectives to be achieved, and the genetic and microbiological quality.

(3) Consideration of the use of appropriate anesthetics and analgesics and the application of methods that do not distress the Laboratory Animals or subject them to pain within limits required for use.
(4) Selecting proper procedure of euthanasia to cause as little pain as possible, when completing the experiment.

(5) Taking proper measures with carcasses of Laboratory Animals related waste, so as not to have any adverse influence on the environment.

(6) Consideration of the prevention of the safety of the surrounding humans or animals as well as the Animal Experiment Researcher, and any adverse influence on the environment, in cases of animal experiments as may require special attention to safety management (those involving materials that may pose a physical or chemical risk or that involve pathogens).

(7) Conducting Animal Experiments and Related Activities using the facility and laboratory including equipments maintained appropriately.

2. The Animal Experiment Researcher shall request animal technicians to conduct the previous items, as required.
(Responsibility of the Principal Investigator)

Article 15

The Principal Investigator shall be a teaching staff in the University and ensure that the Animal Experiment Researcher perform the responsibility based on the previous Article 14.
Article 16

1. The Principal Investigator shall report the results of Animal Experiments and Related Activities to the President through the Dean in the Faculty after the completion or cancellation of Animal Experiments and Related Activities.

2. The President shall report the results to the Committee.

3. The Committee shall advise to the reports as required.
Article 17

The Animal Experiment Researcher, etc. shall supply the Laboratory Animals with food and water, as appropriate to the physiology, ecology, and behavior of the animals, endeavor to preserve the health and safety of Laboratory Animals and provide them with appropriate treatment as required.
(Application and approval for establishing, changing or closing the Facility for Care and Management and the Laboratory)

Article 18

1. The Dean shall submit an application for an establishment and shall request the approval of the President for establishing a Facility for the Care and Management or Laboratory (the “Facilities”).

2. The Dean shall submit an application for a changing the Facilities and shall request the approval of the President for the changing.

3. The President shall ask the Committee to review the application and then the President shall approve or deny the establishment or change of the Facilities concerned.

4. In the event that Facilities are closed, the Dean shall notice the closing to the President.
(Person in charge of the Facilities)

Article 18-2

1. A Person in charge of the Facilities shall be appointed.

2. A Person in charge of establishing Facilities shall be responsible for management and maintenance of the Facilities.
(Retention and report of records)

Article 18-3

1. The Principal Investigator shall prepare and retain record books related to Laboratory Animal sources, rearing history, history of disease, and rearing environment.

2. The Person in charge of the Facilities shall submit a report annually about the species of the Laboratory Animals that cared and managed, and their numbers, etc. to the Faculty Committee.

3. The Faculty Committee shall collect the reports and submit a report annually about the species of the Laboratory Animals that cared and managed in the Faculty, and their numbers, etc. to the Committee.
(Measure to Accidents)

Article 19

1. In cases in which the infection, the environment pollution or other accident is occurred in the Animal Experiments and Related Activities, the Animal Experiment Researcher etc. shall inform the Dean as soon as possible.

2. If the Dean receives such a notification, the Dean shall take necessary measures, promptly report the details and handling of the matter to the President.
(Self-inspection, Assessment, and Verification)

Article 20

1. The President requires that the Committee conducts inspections and assessments to determine whether the situation regarding the Animal Experiments and Related Activities in the University complies with related ordinances and regulations etc. of the University.

2. The Committee shall implement self-inspections and assessments and shall report its findings to the President.

3. The Committee requires that the Faculty Committee conducts self-inspections and assessments to determine whether the situation regarding the Animal Experiments and Related Activities in the Faculty and shall report its findings to the Committee.

4. The President shall endeavor to have the results of self-inspections and assessments verified by persons or agencies outside the University.
(Public Disclosure of Information)

Article 21

The President shall publicly disclose information on the conduct of Animal Experiments and Related Activities at the University every academic year.
- The President is responsible for animal experiments.
- The IACUC is organized to report the result of judgment on the protocol.
- The President approves the animal experiment, not the IACUC.
- Education and training for animal experiment is prerequisite for everybody conducting animal experiment.
Categories of Pain and Distress
Categories of Biomedical Experiments Based on Increasing Ethical Concerns for Non-human Species

Scientists Center for Animal Welfare (SCAW) (Laboratory Animal Science. Special Issue : 11-13, 1987)

Category A
Category B
Category C
Category D
Category E
Category A

Experiments involving either no living materials or use of plants, bacteria, protozoa, or invertebrate animal species.

(Examples and Comments)

Biochemical, botanical, bacteriological, microbiological, or invertebrate animal studies, tissue cultures, studies on tissues obtained from autopsy or from slaughterhouse, studies on embryonated eggs. Invertebrate animals have nervous systems and respond to noxious stimuli, and therefore must also be treated humanely.
Category B

Experiments on vertebrate animal species that are expected to produce little or no discomfort.

(Examples and Comments)

Mere holding of animals captive for experimental purposes; simple procedures such as injections of relatively harmless substances and blood sampling; physical examinations; experiments on completely anesthetized animals which do not regain consciousness; food/water deprivation for short periods (a few hours); standard methods of euthanasia that induce rapid unconsciousness, such as anesthetic overdose or decapitation preceded by sedation or light anesthesia.
Category C

Experiments that involve some minor stress or pain (short-duration pain) to vertebrate animal species.

(Examples and Comments)

Exposure of blood vessels or implantation of chronic catheters with anesthesia; behavioral experiments on awake animals that involve short-term stressful restraint; maternal deprivation with substitution of punitive surrogates; immunization employing Freund's incomplete adjuvant; noxious stimuli from which escape is possible; surgical procedures under anesthesia that may result in some minor post-surgical discomfort. Category C procedures incur additional concern in proportion to the degree and duration of unavoidable stress or discomfort.
Category D

Experiments that involve significant but unavoidable stress or pain to vertebrate animal species.

(Comments)

Category D experiments present an explicit responsibility on the investigator to explore alternative designs to ensure that animal distress is minimized or eliminated.
Categories of Biomedical Experiments Based on Increasing Ethical Concerns for Non-human Species

(Examples)

Deliberate induction of behavioral stress in order to test its effect; major surgical procedures under anesthesia that result in significant post-operative discomfort; induction of an anatomical or physiological deficit that will result in pain or distress; application of noxious stimuli from which escape is impossible; prolonged periods (up to several hours or more) of physical restraint; induction of aggressive behavior leading to self-mutilation or intra-species aggression; procedures that produce pain in which anesthetics are not used, such as toxicity testing with death as an end point; production of radiation sickness, certain injections, and stress and shock research that would result in pain approaching the pain tolerance threshold, i.e. the point at which intense emotional reactions occur.
Category E

Procedures that involve inflicting severe pain near, at, or above the pain tolerance threshold of unanesthetized, conscious animals.

(Examples)

Use of muscle relaxants or paralytic drugs such as succinylcholine or other curariform drugs used alone for surgical restraint without the use of anesthetics; severe burn or trauma infliction on unanesthetized animals; attempts to induce psychotic-like behavior; killing by use of microwave ovens designed for domestic kitchens or by strychnine; inescapably severe stress or terminal stress.
Category E

(Comments)

Category E experiments are considered highly questionable or unacceptable irrespective of the significance of anticipated results.

Many of these procedures are specifically prohibited in national policies and therefore may result in withdrawal of federal funds and/or institutional USDA registration.
Education and training for Animal Experiments in Kyushu University

Anesthesia for Laboratory animals
Eliminating or reducing pain

Animal experiments and related activities are regulated by the provisions of the law for the Human Treatment and Management of Animals (Law No.105,1973) and other standards, etc.

Experiments in animals can result in pain and distress and, these should be reduced to a minimum or even completely be eliminated for both ethical and scientific reasons. The scientific reasons become apparent when it is appreciated that pain and discomfort evoke a range of physiological responses affecting a large number of organ systems. Eliminating or reducing pain can reduce the magnitude of these effects, and improve the validity of an animal model.
Criteria for selecting anesthesia

When trying to determine the most appropriate anesthetic for use on rodents, several criteria should be considered:

First, what is the purpose of the anesthesia? The purpose will directly influence the duration of anesthesia--whether for short term restraint in order to perform a physical exam or radiograph or for long-term general anesthesia for a complicated protocol.

Second, what is the type of experiment or procedure? Is this a survival procedure or a non-survival procedure? With a survival procedure, it is important that the anesthesia has minimal adverse effects, thereby minimizing its influence on the data being collected. For example, for a respiratory study, opioids which are known to cause respiratory depression should be avoided.

Third, is a postoperative analgesia required? Some anesthetics provide better postoperative analgesia than others. Consider also if the appropriate equipment is available. For example, inhalation anesthetics require equipment to administer and an adequate method of scavenging the waste gas so personnel are protected from exposure. In addition, consider both the skill and experience of the anesthetist, because the margin of safety varies among different types of anesthetics. In general, more technical skill is required to administer inhalants compared with injectables. Regulation of the agent should be taken into account since some anesthetics are controlled substances and require a DEA license for purchase.

The final criteria to consider are the costs of the agent and the delivery equipment.
Patient characteristics

Characteristics of the individual patient that need to be considered when selecting anesthetics include:

• Species and strain
• Age, size, and sex
• Physical condition
• Temperament
• Health (preexisting diseases)
• Previous administration of other drugs

Be aware of the health status and physical condition of the patient.
Methods of Delivery

This section discusses methods of anesthetic delivery and applicable equipment. In general, anesthesia is administered to rodents by either the parenteral route or the inhalation route.

**Parenteral route**
- intraperitoneal (IP)
- intramuscular (IM)
- intravenous (IV)
- subcutaneous (SC)

**Inhalation route**
- open drop delivery
- precision vaporizer
Disadvantages of Injectables

The disadvantages to using injectable anesthetics:

• Once a given dose is administered, the anesthetic cannot be changed.
• The duration of anesthetic effect is patient-dependent.
• There is individual variability for a given dose.
• Some injectable agents are irritating and measures need to be taken to minimize this effect.
Prior to administering injectable anesthetics, consider the drug volume, the site, and any irritant properties of the drug. Because concentrated agents are delivered to very small animals, the actual drug volume may be quite small. Many agents can be diluted with physiologic saline to at least a one to one solution (1:1) and, in some cases, one to ten (1:10).

Dilutions

• decrease the chance of overdosing,
• facilitate drug absorption,
• minimize irritation, and
• increase the accuracy of volume measurement by making sure that a significant percentage of the compound is not left within the "dead space" of needle and syringe.
IM route

- IM injections should be administered in a large muscle mass, and the two most common sites used in rodents are the thigh muscles and the epaxial muscles located along the spine.
- Small gauge needles, 26 to 30 gauge, are generally recommended.
- Large volume doses can be administered in several IM sites and dilutions are recommended if a compound may have irritating properties.
The intraperitoneal route is probably the most common for administering injectable agents in rodents.
The proper site for IP injections is in the lower abdomen with the animal's head in a down position which allows gravity to move abdominal viscera away from the injection site.
Depending on the size of the animal, a 25 to 30 gauge needle is recommended.
Intravenous administration of anesthetics is not commonly used in rodents, because access to peripheral vessels is limited, and the techniques are difficult.

- In rats, mice, and gerbils, the lateral tail vein is the peripheral vessel most easily accessed.
- To facilitate access, the vein can be vasodilated by heating, for example, in warm water.
- In guinea pigs and hamsters, the ear vein, dorsal metatarsal vein, and the pubic or penile vein are the only accessible peripheral vessels.
Inhalation anesthesia involves delivery of a volatile anesthetic agent to the patient via the respiratory tract. Advantages of using inhalants:

• Increased control over depth as well as duration of anesthesia.
• Increased safety and survivability.
• Minimal metabolism, biotransformation and excretion compared with injectables, which is desirable in a toxicology studies.
• Animals generally wake up faster from inhalant administration requiring less patient support during the postoperative recovery phase.
Most of the disadvantages associated with inhalants involve the required technical skills and the expense of specialty equipment. In addition, an appropriate scavenging system is needed to protect.
Methods of inhalation anesthesia

Open drop delivery  Precision vaporizer
Hypothermia has been described as an adequate anesthetic for short procedures in neonatal rodents. The neonate is encased in a latex sleeve and immersed up to the shoulders in an ice-water slurry for 3 to 4 minutes. After the procedure, the neonate is rewarmed in an incubator at about 33 degrees.
Steps for anesthesia

1. Health check of the animal (Can this animal tolerate anesthesia?)
2. Pre-anesthetic preparation (administration of anticholinergics and/or sedatives)
3. Administration of analgesics and antibiotics
4. Induction of anesthesia
5. Maintenance of anesthesia (endotracheal intubation and artificial ventilation as necessary)
6. Arousal from anesthesia
7. Post-operative care (administration of analgesics and antibiotics)

For small animals (mice and rats) you can skip some steps, but for larger animals (rabbits, dogs and pigs) you should take all steps of anesthesia.

When using an anesthetic for the first time, we should anesthetize only one animal to ensure that an appropriate depth of anesthesia is attained and recovery is uneventful, because the response of different inbred strains of rodents and rabbits to anesthetic agents varies considerably.
Regulation concerning anesthetics

- Most of anesthetics are dangerous drugs.
- **Ketamine** is a narcotic and **pentobarbital** is a psychotropic. These anesthetics are the most widely used, but induce psychiatric problem in human. Therefore they are strictly regulated by the **LAW** of Narcotic and Psychotropic Drug Control.
- When you use anesthetics, you should ask the anesthetics manager in your lab about How to use.
General anesthesia for mice

Inhalation anesthesia
Using an induction box only or for maintenance using calibrated vaporizer and mask.
- Isoflurane, Sevoflurane (5% for induction and 3% for maintenance)

Injection anesthesia
intraperitoneal injection (i.p.) of these agent will give the animal the surgical anesthesia for 30~60 minutes.
- Medetomidine Hydrochloride 0.3mg/kg + Midazoram 4mg/kg + Butorphanol tartrate 5mg/kg i.p.
- Pentobaribital 30~50mg/kg i.p.
  Pain sensation is only decreased at surgical planes of unconsciousness, and may even be heightened (hyperalgesia) at subanesthetic doses.
- Ketamine 80~100mg/kg + Xylazine 10mg/kg i.p.
General anesthesia for rats

Inhalation anesthesia

Using an induction box only or for maintenance using calibrated vaporizer and mask.
- Isoflurane, Sevoflurane (5% for induction and 3% for maintenance)

Injection anesthesia

intraperitoneal injection (i.p.) of these agent will produce the surgical anesthesia for 30~60 minutes.
- Medetomidine Hydrochloride 0.15mg/kg + Midazolam 2mg/kg + Butorphanol tartrate 2.5mg/kg (Just half of Mice’s) i.p.
- Pentobaribital 30~40mg/kb i.p.
  Pain sensation is only decreased at surgical planes of unconsciousness, and may even be heightened (hyperalgesia) at subanesthetic doses.
- Ketamine 90mg/kg + Xylazine 10mg/kg i.p.
General anesthesia for rabbits

Because rabbits are very sensitive animals, you should administrate sedatives (Ketamine 25mg/kg i.m.) before anesthesia.

Inhalation anesthesia
- Isoflurane, Sevoflurane (recommended)
  Use a calibrated vaporizer and mask.

Injection anesthesia
- Ketamine 10mg/kg + Xylazine 3mg/kg i.v.
- Ketamine 35mg/kg + Xylazine 5mg/kg i.m.

We should not use Pentobarbital because of the cardiac and respiratory depression effects.
Monitoring anesthetic depth

Throughout the course of anesthesia, animals should be observed for pink mucous membrane color, a general indication of adequate oxygenation. Respiratory rate should remain even. Movement or change in respiratory rate or depth in response to manipulation may indicate insufficient depth of anesthesia. Confirm the animal's lack of response to stimulation, such as withdrawal from a paw pinch, every 15 minutes or so throughout a surgical procedure.
Assessment of depth of anesthesia

To determine whether the animal has sufficient anesthesia, the following reflex responses can be assessed.

1. **The righting reflex**: the animal normally attempts to turn over to the sternal position after being placed on its back. Under anesthesia the animal remains on its back.

2. **The palpebral reflex**: blinking when inner or outer canthus of the eye is touched. The reflex is abolished during surgical anesthesia.

3. **The pedal reflex**: withdrawal and flexion of a leg when a digit (finger) or the interdigital skin is pinched. This reflex disappears during anesthesia.

4. **The swallowing reflex**: pulling the tongue or pressing the throat results in swallowing without anesthesia.

5. **The tail pinch reflex**: pinching the tail with finger-nails or haemostat results in a flick of the tail and occasionally in vocalization, when the animal is not deeply anesthetized.

6. **The ear pinch reflex**: pinching the ear in rabbits and guinea pig produces a head shake response when awake.
Assessment of pain

Pain in rodents is most commonly assessed on the basis of subjective measures and observations combined with professional judgment. Clinical assessments of pain include behavioral changes such as immobility, unkempt appearance, lack of appetite, abnormal vocalization, and abnormal posture.

Animals experiencing pain may appear

- unresponsive
- lethargic
- anxious
- apprehensive
- hypersensitive
- aggressive
Supportive care

Intra- and post-surgical supportive care includes ocular lubrication, topical antibiotics, nutritional considerations, and minimizing heat loss. Rodents typically are not fasted or water restricted prior to anesthesia, as is done with larger species.
Humane Endpoint
Humane Endpoint

- The humane endpoint refers to **the timing of termination of an experiment** (in other words, the timing of the application of euthanasia procedures) to release a laboratory animal from severe pain and suffering.
- It is a term used *in contrast to “death” as an endpoint* that is used in protocols of animal experiments where the experiment continues until the animal’s death.
Reference should be made to pertinent international guidelines for details concerning determination of the humane endpoint.

When conducting animal experiments in which the degree of pain and suffering is high, such as lethal toxicity studies, infection experiments and radiation experiments, the principal investigator should examine setting of the humane point in the planning stage of the animal experiment.
Humane Endpoint

- As a rule, euthanasia procedures should be available for termination of animal experiments.
- At the final stage of an animal experiment or when analgesics, sedatives or other agents do not provide relief, euthanasia procedures should be performed to release the laboratory animal from pain and suffering (one pain relief method).
Indications of when humane endpoint is applicable include:

- food and water intake difficulties,
- moribund symptoms (self-injurious behavior, abnormal posture, respiratory disorders, vocalization, etc.),
- abnormal appearance over a prolonged period with no visible indications of recovery (diarrhea, bleeding, soiled genital area, etc.),
- weight loss (20% or more over several days), and
- marked increase in tumor size (10% or more of body weight).
CCAC guidelines on Euthanasia of animals used in science

Canadian Council on Animal Care, 2010
Guideline 1:
Whenever an animal’s life is to be taken, the animal must be treated with the highest degree of respect.
Guideline 2: When performing euthanasia, the intention should be to make the animal’s death as distress-free and painless as possible. Therefore, the method likely to cause the least distress and pain to the animal should be selected, consistent with the nature of the experimental protocol.
Guideline 3: Euthanasia should result in rapid loss of consciousness, followed by respiratory and cardiac arrest and ultimate loss of all brain function.
Guideline

4: Euthanasia should aim to minimize any pain and distress experienced by the animal prior to loss of consciousness. When appropriate, restraint should be used in such a manner that pain and distress associated with the entire process are minimized.
Guideline 5:
Methods used for euthanasia must be appropriate for the species, age and health status of the animal.
Guideline 6: Death must be verified following euthanasia and prior to disposal of the animal.
Guideline 7:
Personnel responsible for carrying out the euthanasia must be trained to carry it out in the most effective and humane manner; recognize signs of pain and distress in relevant species; and recognize and confirm unconsciousness, and subsequently death, in relevant species.
Guideline 8:
Human psychological responses to euthanasia should be taken into consideration when selecting the method of euthanasia, but should not take precedence over animal welfare considerations.
Guideline 9: Animal care committees are responsible for approval of the method of euthanasia for any study involving the use of animals. This includes euthanasia as part of the experimental protocol, as well as euthanasia for animals found to be experiencing unrelievable pain and distress or approaching previously agreed endpoints.
Guideline 10:
A veterinarian experienced with the species in question should be consulted when selecting the method of euthanasia, particularly when little research has been done on euthanasia of that species.
When disposing of laboratory animals on completion of the experiment in accordance with the animal experiment protocol or due to the laboratory animals being subjected to severe pain and suffering during the course of the experiment when anesthetics and analgesics can not be used in the research, the researcher(s) should conduct **euthanasia**.
Selection of the agent and method used for the euthanasia procedure

- Depends on the animal species and the objective of the experiment.
- Chemical methods
  - overdose of a barbiturate anesthetic,
  - administration of a non-explosive inhalation of anesthetic or carbon dioxide gas
- Physical methods
  - cervical dislocation,
  - decapitation,
  - exsanguination under anesthesia, etc.
- From the standpoint of animal welfare, the principal investigator should seek the advice and guidance of a laboratory animal specialist as required since there are slight international differences on what are judged to be appropriate methods of euthanasia for laboratory animals.
* Euthanasia procedures refer to procedures resulting in the rapid loss of consciousness and then death of a laboratory animal not associated with pain or suffering. In addition to Guidelines on Methods of Sacrificing Animals (Notice No. 40 of the Prime Minister’s Office, July 4, 1995), international guidelines should be taken into consideration.

* Euthanasia should be performed by methods that do not cause distress to other animals in the laboratory. This requires careful attention because until animals lose consciousness they can vocalize and release pheromones.
* A person who has acquired the skills required for handling a particular animal species should conduct euthanasia procedures, and the death of the animal should always be verified.

* Confirmation of death is very important. A mistake of dose is occasionally occurred.
Chemical methods

*Overdose administration of pentobarbiturate (100-120mg/kg) is applicable to many species of animals such as mice, rats, rabbits, dogs, pigs, monkey so on.

*Administration of a non-explosive inhalation of anesthetic gas can be carried out with halothane, enflurane, isoflurane or sevoflurane. In case of carbon dioxide, we can pick up two methods, a combination of CO₂/O₂ method or a 100 % CO₂ method. The former is the use of a combination of CO₂/O₂ (6:4) and a humidifier. After the animal has lost consciousness, the concentration of CO₂ is raised to 100%. The latter is the use of a 100 % CO₂ at a flow rate of 20 % of the chamber volume per minute. The chamber become a 100 % CO₂ for five minutes. In both case, animals must remain in 100% CO₂ for at least 10 minutes to ensure that they are dead.
Physical methods

*Cervical dislocation is carried out by stretching the animal and rotating the neck. The spinal cord is disrupted and nerve impulses to the vital organs such as the respiratory system and the heart are no longer transmitted. This method is applicable in mice, rats, hamsters, gerbils, kittens and small birds, but not in larger animals. If it is done quickly and expertly it is a painless method.

*Decapitation can be performed by using scissors or a guillotine.

*Exsanguination from a cut through the carotid arteries under anesthesia is applicable to large animal such as dogs and pigs.
Infectious Disease in Experimental Animals
Surveillance concepts

First, infection and disease are not synonymous terms. Infection is the invasion and multiplication of an agent in the body. Disease is a clinically manifest or symptomatic condition that impairs normal physiologic functioning. Disease results from infection. Infection, not disease, is the primary concern in a rodent health surveillance program.
The second concept to keep in mind is prevalence. Prevalence is defined as the number of cases that test positive for a specific condition in a specified population at a stated time. For example, in this image, 2 rodents test positive in a colony or population of 4; therefore, the prevalence is 50%.
A viral surveillance program is the testing of selected animals to determine the infection status of a population. The goal is to detect the presence of infectious viruses. It is not meant to determine the prevalence of a virus, or a virus' epizootiology within a colony. For example, in this image, out of the total population, four animals are selected and tested for the presence of virus(s). One animal tests positive. Therefore, the presence of virus(s) within the colony is confirmed. This prevalence of infection in tested animals is 25%, but the prevalence of infection in the entire population is 50% at the time of testing.
Mouse hepatitis virus (MHV)

An epizootic can produce nonspecific clinical signs in naive, juvenile mice, such as runting, as shown here, or failure to thrive. The disease state varies with the viral strain's virulence and organotropism and on the host's immune status, genotype, age, pathogen status and experimental history. Infection is usually subclinical. However, virulent enterotrophic MHV strains can produce diarrhea with mortality up to 100%.
MHV infection has been shown to confound tumor-growth studies. It can induce alpha-fetoprotein production, anemia, thrombocytopenia, and leukopenia. It can activate NK cells, alter interferon responses, and alter IgM and IgG responses to sheep red blood cells. It can also increase an animal's susceptibility to other viral infections by interfering with interleukin-2 (IL-2) secretion.
Sialodacryoadenitis virus (SDAV), another coronavirus, is one of the most common viruses found in laboratory rats. It is highly contagious, and is spread by direct contact with infected animals or by respiratory aerosol. Lewis, WAG/Rij, and spontaneously hypertensive rats (SHR) are very susceptible to SDAV; while Wistar, Sprague-Dawley, Long-Evans and Fischer-344 rats are less susceptible.
Effects on research

SDAV infection can complicate ocular and respiratory system studies. By depleting epidermal growth factor in submaxillary salivary glands, it affects carcinogenesis studies. It reduces alveolar macrophage interleukin-1 (IL-1) production, and exacerbates Mycoplasma pulmonis infection. Indirectly, SDAV infection reduces food consumption, which leads to weight loss and reduced breeding performance.
Sendai virus

Sendai virus is an RNA virus of the family Paramyxoviridae. Its natural hosts include mice, rats, hamsters, and possibly guinea pigs. It is a prevalent, adventitious viral infection in rodent colonies. Sendai is extremely contagious and is spread by infective nasal secretions.

Sendai infections are usually asymptomatic in neonates, weanlings, and adults. In mice, common clinical signs include chattering, mild respiratory distress, prolonged gestations, poor growth in weanlings, and neonatal deaths. In rats, Sendai infections are usually asymptomatic, but can cause reductions in litter size and growth rates.
Sendai infection has significant implications for research. Infected mice and rats can have lifelong lymphocyte abnormalities, increased NK cell-mediated cytotoxicity, and altered host responses to transplantable tumors. This interference is unpredictable. Therefore, it is best to not conduct research with Sendai-infected rodents.
Mycoplasma pulmonis is the cause of murine respiratory mycoplasmosis, or MRM. M. pulmonis colonizes the surface of the epithelium of the respiratory and genital systems. In the respiratory tract, M. pulmonis preferentially colonizes the nasal passages and middle ears. Strains of the organism vary widely in virulence for both mice and rats.
Pasteurellosis is by far the most common and most significant disease of laboratory rabbits.

Mucopurulent discharge

Signs -- red eye

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Two other zoonotic viruses associated with rats and mice are Hantaan virus and lymphocytic choriomeningitis virus (LCMV). The genus Hantavirus, family Hantaviridae, includes several RNA viruses associated with various human syndromes worldwide.
Hantaan virus is the prototypic virus of the genus Hantavirus and is the cause of Korean hemorrhagic fever (KHF). Healthy laboratory rats can be natural carriers, transmitting the virus to research personnel via respiratory, salivary, and urinary excretions and aerosols. Human mortality can occur, but recovery is the usual course. Hantaan virus has the reputation of being one of the most significant zoonotic pathogens associated with laboratory.
Lymphocytic choriomeningitis virus (LCMV) is a member of the family Arenaviridae. It is another RNA virus recognized as an important zoonotic agent. Unlike Hantaan virus, it is an indigenous rodent pathogen.

In humans, the usual clinical manifestations are fever, headache, myalgia, nausea, vomiting, sore throat, and photophobia. A differential diagnosis includes influenza, mononucleosis, herpes encephalitis, and, perhaps, tuberculous meningitis.
Wild mice are the principal reservoir hosts, but laboratory mice and Syrian hamsters serve as important natural transmitters to several species, including humans. The virus is shed in urine, saliva, and milk during chronic, asymptomatic infections. In infected mice, vertical transmission, the direct transmission of the virus from parent to offspring, is considered 100% efficient.
Herpesvirus simiae (B virus) is the most serious of the zoonotic viruses. In monkeys, it may be subclinical or cause mouth lesions resembling cold sores. But in humans it can result in fatal encephalitis. Over thirty cases have been reported in the last forty years in persons who have had contact with macaque monkeys, usually following an incident of a bite or scratch, as the virus is shed in saliva and lacrimal secretions. Cases have also been reported following exposure to monkey tissues. Each animal care facility should have on-call medical care personnel who are knowledgeable about the hazards of Herpesvirus simiae, as well as its symptoms and treatment.
Strong Motivation for Animal Experiments

Alternatives to animal experiment
Animal welfare consideration
Good veterinary care
Anesthesia and analgesia
Aseptic surgery
Comparison Between Animal Suffering and Scientific and Social Benefit