ANIMAL MODELS IN CANCER RESEARCH

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Outline

- What is an animal model?
- Principles of model selection
- The process of using animals for research, testing or teaching at RPCI

What is an Animal Model?

- Introduction- Types of models
  - *in vitro* assays
  - Computer simulations
  - Mathematical models
  - Animal models

Animals may model **analogous** processes (relating one structure or process to another) or **homologous** processes (reflecting counterpart genetic sequences).

Genetically Engineered Mice

- The primary driver of homologous modeling is the Genetically Engineered or Manipulated mouse.
- The rapid advancement of genomic sequencing and genomic manipulation improved the animal model selection based on phenotypic analogs of human processes as previously done.

Modeling Concepts

- One-to-one modeling vs many-to-many modeling.
  - **One-to-one** – A model is pursued that generally demonstrates a similar phenotype to that which is being modeled.
    - Infectious disease
    - Spontaneous or induced monogenetic disease
  - **Many-to-many**- Results from analysis of a process in an organism in which each component of that process is evaluated at several levels.
    - System
    - Organ
    - Tissue
    - Cell

Many-to-many-modeling is more common

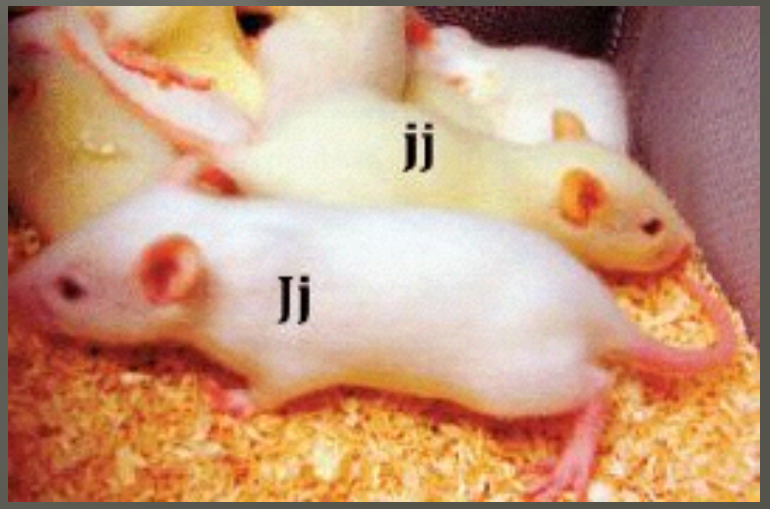
- Many of the most common diseases such as cancer are complex, often polygenic, with multiple interactive environmental influences.
- The advent of high-throughput techniques such as sequencing, proteomics and transcriptomics has facilitated this process.
- Comparative genomics demonstrates the impressive degree of genetic conservation between common research species and humans.

Animal Model Classification Spontaneous or Induced

- Spontaneous models – normal animals with phenotypic similarity to those of humans or by abnormal members of a species that arise through spontaneous mutations(s).
- Induced models- Animals submitted to surgical, genetic, chemical or other manipulation resulting in an alteration to their normal physiologic state.

Examples of Spontaneous Mutations

- Gunn rat- (Hereditary Hyperbilirubinemia) These rats were jaundiced and the defect (a lack of the enzyme uridine diphosphate glucuronyltransferase) was transmitted as an autosomal recessive characteristic.



Spontaneous models
Type 1 Diabetes mellitus

- Non obese diabetic mouse

- BB Wistar rats

Spontaneous models

- SCID (Severe combined Immune deficient mouse)

- Nude mouse – Disruption of the FOX N1 gene

Other Spontaneous models

- Watanabe rabbit- hypercholesterolemia
- Brattleboro rats – Diabetes insipidus
- Obese chickens- Autoimmune thyroiditis
- Spontaneous Hypertensive Rats
- Dogs and mice with Duchenne X-linked Muscular dystrophy
- Dogs with hemophilia A and B

Induced models

- Helped unravel important concepts in physiology and medicine
  - Surgical models-
    - Organ transplantation
    - Coronary bypass
    - Balloon angioplasty
    - Replacement of heart valves
    - Development of cardiac pacemakers
    - Discovery of insulin
    - Surgical resection of the intestines including techniques of colostomy

Induced models continued…..

- Models induced by diet or administration of drugs and chemicals.
  - Alloxan and Streptozotocin- To induce diabetes as these drugs destroy the Beta cells of the islets of Langerhans.
  - Chemical mutagenesis approaches in mice and zebrafish.
  - Diet induced models – discovery of vitamins, trace minerals needs and pathogenesis of many diseases.

More recently…humanized mice

- Already immune deficient mice (SCIDs) are exposed to myeloablation irradiation and then reconstituted with human stem cells (generally hCD34+ human hematopoietic stem cells) these mice can then be used to study a variety of infectious and immunological diseases.
- A promising new model has been developed for personalized cancer treatment – Transplanting tissue biopsies from patients with tumors into a variety of immune deficient mice and then testing various treatment modalities to determine most efficacious treatment for that patient's specific tumor.

The process of using animals for research testing and teaching

Animals in Research

- Laboratory animal science professionals accept responsibilities of caring for the animals, supporting quality research, and complying with a variety of regulatory requirements.

- The use of animals in research is a privilege and the animals must be treated respectfully, carefully and responsibly.

The IACUC
Institutional Animal Care and Use Committee

- By law, an institutional committee is responsible for assessment and oversight of the institution's animal care and use program and facilities.
- This committee is most commonly referred to as the "Institutional Animal Care and Use Committee", or IACUC.

IACUC Basic Functions

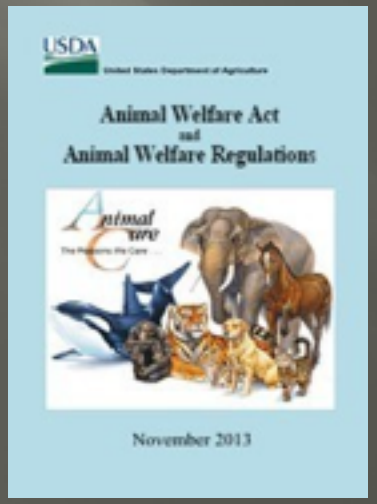
- The IACUC has many jobs. Some of them include:
- **Reviewing** and approving animal use protocols submitted by investigators.
- **Monitoring** the animal care and use program by conducting thorough reviews of the program and inspections of the animal facilities semiannually

The Privilege of Conducting Animal Research

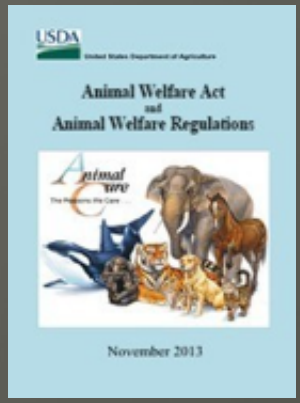
- An effective IACUC protects both the individual investigator and the institution, while inspiring confidence in the general public that animal research is being performed in an ethical manner.
- Research utilizing animals is a privilege, not a right.
- A single incident of serious noncompliance with animal welfare regulation or guidelines can jeopardize the entire institution's privilege of conducting animal research.

Basic laws and guidelines that govern animal research in the United States

- The first important agency regulating animal research is the United States Department of Agriculture (USDA) endorses the Animal Welfare Act.



Who is covered by USDA?



- The Animal Welfare Act regulates any institution that fits the following criteria:

1. Purchases or transports live animals in commerce

or

2. Receives funds under a grant, award, loan, or contract from a department, or agency of the United States for the purpose of carrying out research, tests, or experiments.

To what animals do the Animal Welfare Regulations apply?

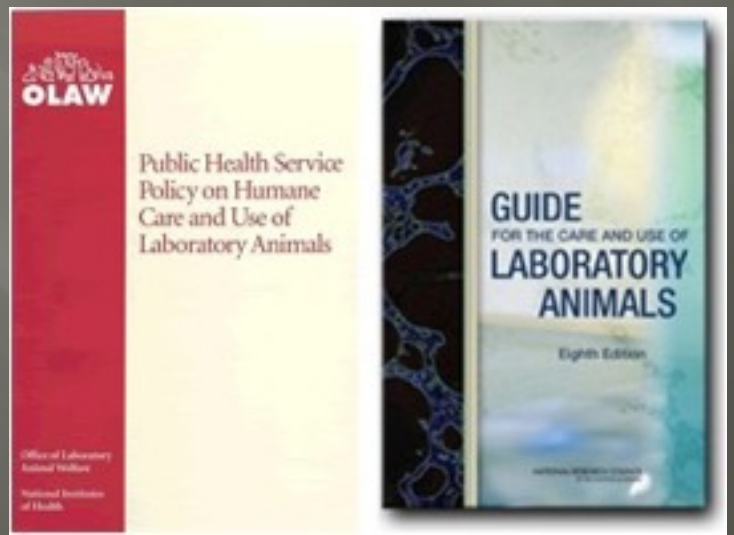
- "Animal means any live or dead dog, cat, nonhuman primate, guinea pig, hamster, rabbit, or any other warm blooded animal, which is being used, or is intended for use for research, teaching, testing, experimentation, or exhibition purposes, or as a pet".
- Excludes mice, rats, birds, horses not used for research and other farm animals.

The Public Health Service (PHS)

- The second important agency involved in regulating animal use is the Department of Health and Human Services, which is the home of the Public Health Service (PHS).

The Office of Laboratory Animal Welfare (OLAW)

- OLAW is responsible for monitoring institutional compliance with PHS policy and guidelines. OLAW relies primarily on two documents for judging compliance, both of which are very important to animal research.



Application of PHS Policy

- PHS Policy covers all vertebrate species used for research, teaching, and testing.
- "Animal- Any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes.“ (includes mice and rats).

Association for Assessment and Accreditation of Laboratory Animal Care

- AAALAC is a nonprofit organization that accredits animal facilities.
- If an institution meets all applicable standards, then it is awarded AAALAC accreditation.
- In general AAALAC accreditation is considered to be a symbol of a commitment to excellent laboratory animal care and use.

Checking your Knowledge

- Which organization is responsible for monitoring institutional compliance with PHS Policy?
  1. Centers for Disease Control and Prevention
  2. Animal and Plant Health Inspection Service
  3. Office of Laboratory Animal Welfare
  4. Fish and Wildlife Service

- Office of Laboratory Animal Welfare

Checking your Knowledge

- Mice of the genus *mus* and rats of the genus *rattus* used in research are covered by?

1. The Animal Welfare Act
2. The PHS policy
3. USDA

The PHS policy

Getting Started

- Explaining Why the Use of Animals in Research is Important
- Some items on an animal protocol form such as
  - "How will the proposed use of animals improve the health of people or animals?"
  - "What is the experimental design of the animal studies planned?"

In general, there must be a compelling potential for benefit to human or animal health to warrant the use of animals

- Points to consider:
- If you are studying a human or animal disease or health concern.
- Because one of the IACUC members is a non-scientist try to use language that a high school student would understand.
- Make sure you explain medical terms, and define abbreviations the first time they are used.

Experimental Design

"What is the experimental design of the animal studies planned?"

- Keep in mind that the IACUC needs to understand the **proposed use of animals**.
- For more complex experiments it is very helpful to provide a flow chart to make the experimental design clear.
- The description of the animal procedures should stand by itself. Once again, define all abbreviations the first time they are used to facilitate comprehension.
- Try not to use technical language that only specialists in your field would understand.

Selecting the Species

The hierarchy of sentient "aware" species:

- Apes (chimps, orangutans, gorillas)
- Monkeys (baboons, macaques, marmosets, tamarins)
- Larger animals such as pigs and goats commonly used as farm animals
- Larger animals commonly kept as pets such as dogs and cats
- Rabbits
- Rodents (guinea pigs, hamsters, rats, mice)

- Non-mammalian vertebrates (poultry, amphibians, reptiles, fish)
- Invertebrates (crustaceans, slugs)
- Smaller life forms (insects, arachnids, worms)
- Single cell organisms (yeast, bacteria, etc)

Justifying the Species

- The presence of previous work in the biomedical literature that validates the use of a particular species in an animal model of a human disease.
- The existence of a large body of previous laboratory data that would have to be repeated if another species was used instead.
- Characteristics of the species that render it uniquely suited to the proposed research.
- Size, availability and cost.
- Availability of reagents or research tools unique to that species.
- **Cost savings alone is not an adequate justification for using a particular species! The justification should be based on sound scientific reasoning.**

Justifying the Number of Animals Requested

Some important points:

- A statistical analysis should be used to justify animal numbers. The goal is not to minimize the number of animals used but to determine the right number of animals for obtaining valid results.
- It is acceptable to ask for animals that will be used to perfect surgical or other techniques prior to initiating planned experiments.
- Studies on cadavers from other approved protocols in advance of any procedure on a live animal are strongly encouraged. By doing this, techniques can be perfected as much as possible before any live animals are used.
- It is also acceptable to ask for animals that will be used in pilot experiments in addition to animals requested for more robust experiments.

Description of the Animal Procedures

- Your descriptions must include:
- Nonsurgical methods, such as injections, administrations, sample collections, and food or water restriction. Routes and volumes of injections, etc., should be included.

- Surgical methods, to include aseptic technique, the surgical approach, suturing, perioperative care and monitoring, and postoperative analgesia.

- Anesthesia; requirement for and duration of pre-anesthetic fasting, drug agents used, routes of administration, duration of anesthesia, methods of anesthetic monitoring, and care during anesthetic recovery.

Testing

- Which of the following is helpful to the IACUC when reviewing an animal protocol?
  1. Frequent use of abbreviations and jargon to make responses shorter
  2. The use of highly technical language that proves complete scientific familiarity with the subject matter
  3. A description of proposed procedures on the animal protocol form that require the reviewer to refer back to other documents
  4. The use of a flowchart to illustrate complex experimental designs

1. The use of a flowchart to illustrate complex experimental designs

Testing

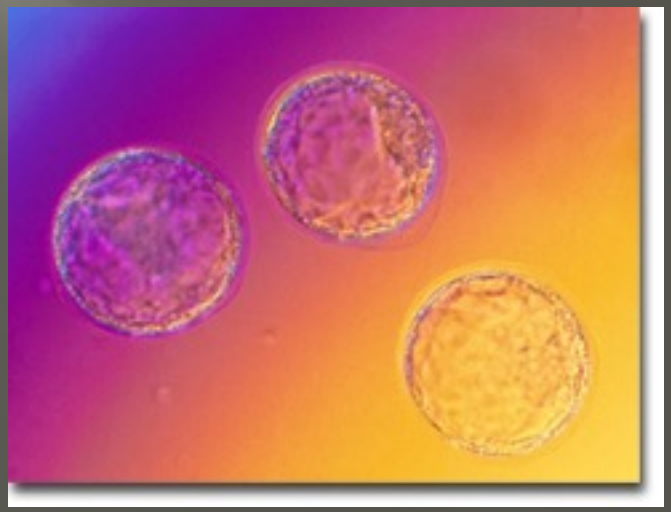
- Which of the following statements concerning selection of species for research is FALSE?
  1. Vertebrate species should be used instead of invertebrates whenever possible.
  2. Dogs are higher on the species hierarchy than rabbits.
  3. The least sentient species that can provide the needed data should be considered for use.
  4. Apes are higher in the species hierarchy than rodents.
- Vertebrate species should be used instead of invertebrates whenever possible.

Alternatives

- They described three important concepts now known widely as the "three R's":
- The purpose of these concepts is to minimize animal use and pain or distress while still achieving the critical scientific objectives that lead to advances in health and medicine.

The first "R" is replacement

- Replacement is simply replacing the use of animals with non-animal techniques.
- - Computer models.
- - Cell culture or tissue culture systems.
- - In vitro assays.



Practical examples of “Replacement" include:

- Use of cell culture techniques to replace animals as incubators for cell lines
- Use of immunologic bench assays to replace bioassays involving animals
- Use of computer software to model the pharmacokinetics of drugs in place of animal studies.

The second "R" is reduction

Reduction is simply reducing the number of animals used.

- Using appropriate group sizes to obtain statistically significant data.
- Performing multiple experiments simultaneously so that the same control group can be used for all the experiments.
- Sharing tissues with other investigators so that additional animals are not needed.
- Designing experiments so that animals serve as their own controls, when scientifically appropriate.
- Using newer instrumentation that improves precision and reduces the number of animals needed per data point.

The last "R" is refinement

- Refinement refers to changing experiments or procedures to reduce pain or distress in those animals that must be used.
- Examples of refinements include:
- - New anesthetics that allow rapid induction and reduced recovery times.
- - New analgesics that provide more extended pain relief postoperatively with less frequent administration.
- - New bleeding and injection techniques that cause less tissue damage or distress.
- - Improved surgical techniques that minimize trauma and the length of anesthesia.
- Check with the literature and your veterinarian to see if better techniques have evolved that reduce pain or distress on the animals.

Consider Alternatives

- The **Animal Welfare Regulations** require the IACUC to do two things regarding alternatives:
- Ensure that the principal investigator has considered alternatives if painful or distressing procedures are proposed.
- Evaluate a written narrative provided by the principal investigator that describes which source or sources were used to determine that alternatives were not available.

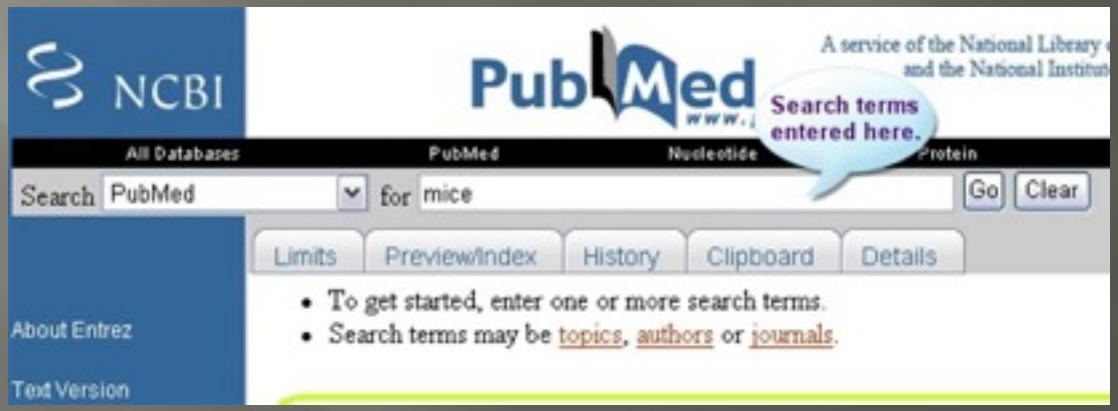
Organizations

There are a number of organizations that have active research programs into alternatives to animal use.

- They include the Johns Hopkins Center for Alternatives to Animal Testing (CAAT)
- Institute for In Vitro Sciences.

Avoiding Unnecessary Duplication

- You will be asked to document that your proposed work is not unnecessarily duplicative.
- Acceptance of new ideas in science is often dependent upon the ability of other scientists to duplicate published reports. The IACUC can allow duplication of previous work if convinced that it is important scientifically to do so.



USDA Pain/Distress Categories

- Even if you use non-USDA covered species (such as mice or rats) you will be required to place your animals into pain/distress categories.

- A simple yet useful definition of a painful or distressful procedure on an animal is this:

"A procedure that would cause pain or distress in a human."

Endpoint Criteria

- The **criteria used for intervention** in research studies to prevent unnecessary pain and distress are called "endpoint criteria" because they describe when it is time to:
- Euthanize an animal to prevent suffering.
- Discontinue a painful procedure.
- Remove an animal from a study.

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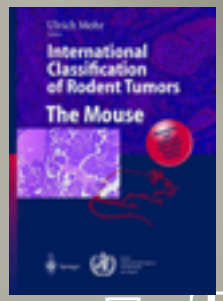
Common Examples of Endpoint Criteria

- Limit on weight loss as a percentage of body weight
- Sudden pain or distress that cannot be controlled with analgesics, sedatives or tranquilizers.
- Severe medical conditions that cannot be controlled with appropriate therapy (e.g. severe systemic infections, kidney or liver failure, heart disease).



At RPCI

- Tumor size as an endpoint often include maximum tumor volumes or tumor weight as a percentage of body weight, skin ulceration over the tumor, interference with normal gait or movement, and interference with normal feeding and drinking behaviors.

Surgery

- Surgery will be addressed in detail in your animal use protocol.
  - Sterile or aseptic technique
  - **General anesthesia**- a state of unconsciousness characterized by a complete lack of pain and sensory perception.
  - **Regional (or local) anesthesia** refers to preventing pain and sensory perception in one small part or a region of the body

Survival and Non-Survival Surgery

- **Survival surgery** is surgery in which the animal regains consciousness after anesthesia.
- If an animal undergoes survival surgery, **aseptic (sterile) technique must be used** to prevent postoperative infections, "no matter what vertebrate species is involved."
- The **incision site** must be properly prepared prior to the incision. The hair/feathers must be clipped and the skin must be disinfected.

Survival and Non-Survival Surgery

- **Non-survival surgery** is surgery in which the animal is euthanized while under anesthesia, and does not regain consciousness. If an animal undergoes non-survival surgery, **sterile technique may not be required**.
- Even though the animal will not survive beyond the end of surgery, at a minimum these procedures should be followed:
- The surgeon should wear gloves.
- The surgical site should be clipped.
- The instruments and work area should be clean.

Major vs. Minor Surgery

- **Major surgery** is defined as:
- Surgery that penetrates and exposes a body cavity such as the chest or abdomen, or
- Surgery that produces substantial physical or physiological impairment.
  - Laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation.
- Minor surgery is less invasive surgery that does not meet the criteria for major surgery above.

Location

- The rooms that can be used for surgery vary depending on:
  1. The species
  2. Whether a surgery is major or minor
  3. Whether the surgery is survival or non-survival

Anesthesia and Analgesia

- Pre-anesthesia: A pre-anesthetic regimen may incorporate agents that will provide analgesia during the postoperative period. This is known as preemptive analgesia, since it provides analgesia before a painful stimulus (i.e. the initial incision) is applied.
- Anesthesia: The anesthetic regimen should provide a duration of anesthesia that matches the duration of the surgical procedure.

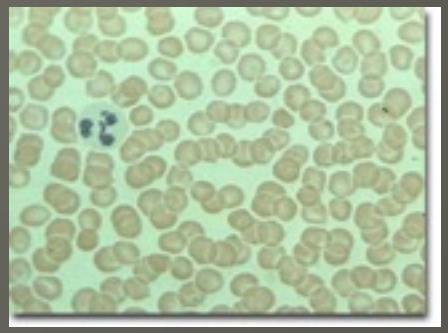
Postoperative Analgesia

- Plan which postoperative analgesics will be used at the time when the anesthetic regimen is established. The agent, dose, route, frequency, and duration of treatment should be discussed with and approved by a veterinarian.
- The Animal Welfare Regulations and PHS Policy stress the importance of using postoperative analgesics.

Postoperative Care for Survival Surgeries

- The animal should be monitored to make sure it is recovering properly
- **Documentation:**
  - For animals larger than rodents, individual health care records are usually maintained, with records of daily observations and treatments during the postoperative care period.
  - For smaller animals like rodents, group records instead of individual records are usually kept.

Blood Collection Information



- When collecting blood samples, the volume and frequency of collection must be carefully limited so that neither shock nor anemia result.

- One simple guideline is to collect no more than 1% of the body weight of blood at one time.
  - If a mouse weighs 20 grams, 0.2 ml (0.01 x 20= 0.2 ml) could be safely collected.

Blood Collection in Rodents

- Blood collection from rodents can be challenging.
- The following locations do not require anesthesia for blood collection:
- Lateral tail vein
- Facial (submandibular) vein
- Jugular vein
- Lateral saphenous vein

Personnel Training and Experience

- State your experience and training in performing the proposed procedures.
- Although academic degrees are useful indicators of educational experience, they are not often useful by themselves in evaluating an individual's experience in animal research.

Using Hazardous and Toxic Agents in Animals

- If your animal work requires the use of hazardous or toxic agents, there are many important considerations.
- **Infectious diseases**
- **Toxic chemicals** - including carcinogens, mutagens, biological toxins, and organic chemicals
- **Radioactive substances**
- **Recombinant DNA**

Considerations when using such agents in animals

- The risk of accidental human infection or exposure is usually reduced if animals are anesthetized or sedated before they are injected with agents using a hypodermic needle.
- If using a toxic agent, know in advance what antidote or action to take if accidental exposure through an injection, spill, or break in the skin occurs.
- Consult with the appropriate safety officer in advance of experiments.
- Before beginning any animal studies involving infectious agents, both research staff and personnel in the animal facility must understand how to safely conduct the study in the animal facility.

Housing Social Animals

- **Social animals** should be housed in pairs or groups whenever possible unless scientific, health, or behavioral considerations prevent it.
- Some justifications for single housing include:
- **Behavioral problems.** For instance, adult male C57BL/6 and BALB/c mice will fight if not raised together from birth.
- **Health problems.**
- **Scientific reasons.** If single housing must be used to achieve scientific objectives, then the IACUC may approve single housing.

Euthanasia

- Euthanasia literally means a "good death". A more appropriate simple definition is a "gentle death".
- Euthanasia techniques should result in a rapid loss of consciousness followed by cardiac or respiratory arrest and finally, the loss of brain function.
- Because it is necessary to euthanize most animals as part of experimental protocols, it is very important to use appropriate euthanasia techniques.

Euthanasia Training

- Personnel must be trained to properly and humanely perform euthanasia.

- Proper training for euthanasia is an area of emphasis because of the increased potential for harm to animals.

Methods of Euthanasia

- Euthanasia methods can be broadly separated into physical and nonphysical methods.
- **Physical methods** include cervical dislocation, decapitation, and exsanguinations. Usually under deep anesthesia.
- **Non-physical or pharmacologic methods** rely on drugs to cause loss of consciousness and death.

CO_2 Euthanasia Advantages

- CO_2 provides a rapid depression and anesthesia (narcosis).
- CO_2 is non-flammable and non-explosive.
- CO_2 does not introduce chemical residues into tissues.
- CO_2 does not result in distortion of cellular architecture.

IACUC Approval

- Whether you are performing research or testing on animals, or using animals for teaching, you must receive IACUC approval before any use of animals begins.

Making Changes after You Receive Approval

- Some changes often considered significant are:

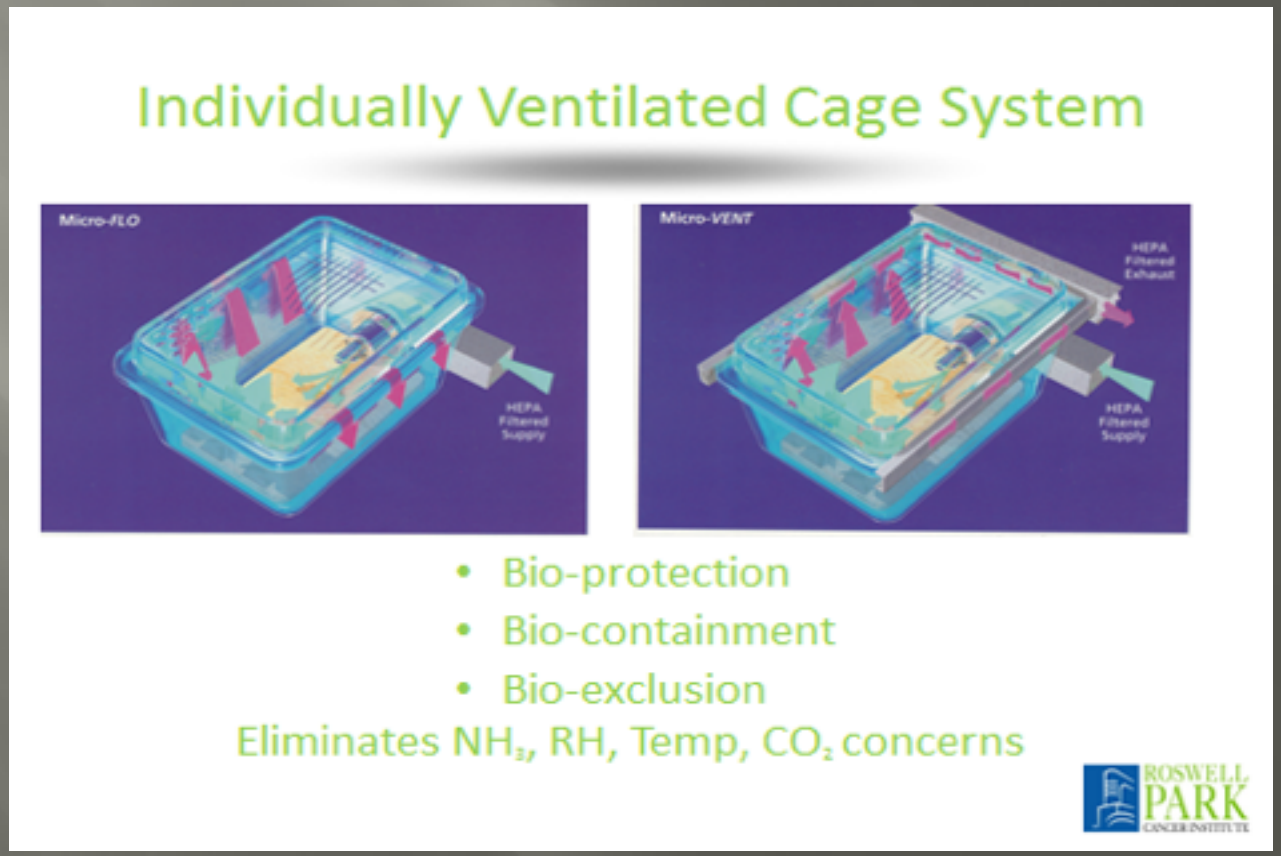
1. Drug dosage changes
2. Increasing the number of animals used
3. Addition of new drugs/agents
4. Performing an additional procedure
5. Changing procedures in any way that might increase the pain/distress category in which the animals are placed
6. Using animals approved for use on one of your protocols for use on another of your IACUC-approved protocols.

Services in the Laboratory Animal Shared Resource at RPCI

- Training in animal care and use
- Animal care
- Health Surveillance Program

Housing

- Our institution use micro-isolation cages, which are made of hard plastic and have a filter top to contain allergens and to protect animals from potential pathogens in the environment.



Laboratory Animal Resources (LAR)

Our Facilities

MRC Building

Main Animal Facility
45,000nsf

- ✓ 41 Animal Holding Rooms
- ✓ 13 Procedure rooms
- ✓ Bio-Bubble room
- ✓ Cage Processing Areas

CCC Building

Additional 5,000nsf

- ✓ 22 Animal holding rooms
- ✓ Pre-Clinical MRI, GFP

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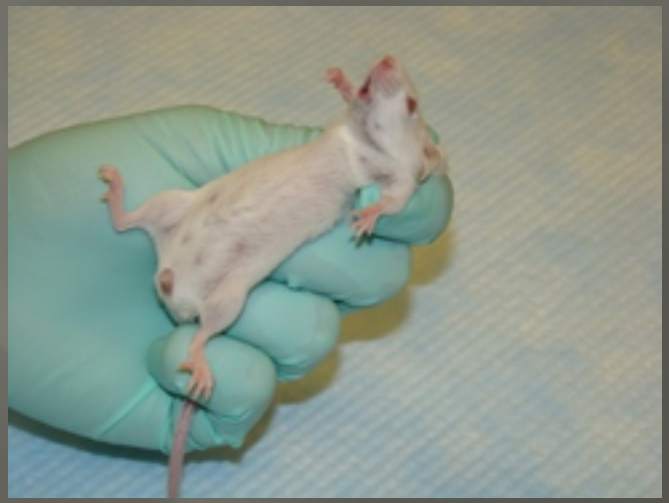
Additional training in handling and restrain

- Mice are easy to restrain for the purpose of examination, injection and other administrations, and blood collection
- Pick up a mouse by the tail (away from the tail tip) and lift the mouse directly to the wire lid.

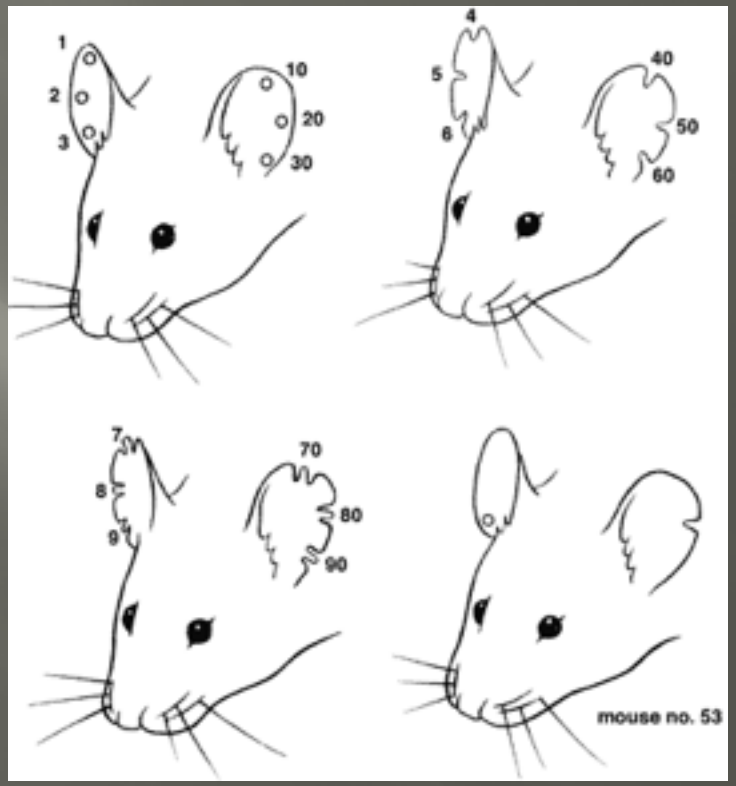


Determining Sex and Age

- Mice are sexed on the basis of the anogenital distance, which is the distance between the anus and genital papilla.
- The anogenital distance is greater in the male than the female for all ages – adults, juveniles, and newborns.

Animal identification

- Rodents can be identified with the numbers 1 through 99 by putting a hole, a notch, a double notch, or any combination of these three marks in one or both ears.



Common Routes of Drug administration in mice

- Sub cutaneous
- Intraperitoneal
- Intravenous
- Oral Gavage

WOODCHUCK COLONY AT RPCI

Animal model for liver cancer

Bedding and Enrichment

Woodchips (aspen) bedding should be provided.

Woodchucks will defecate in one corner and cover their feces. Woodchucks often move all of their bedding into their nest box.

Stainless Steele "Mailboxes"
(Notice handle)

Significance of animal care

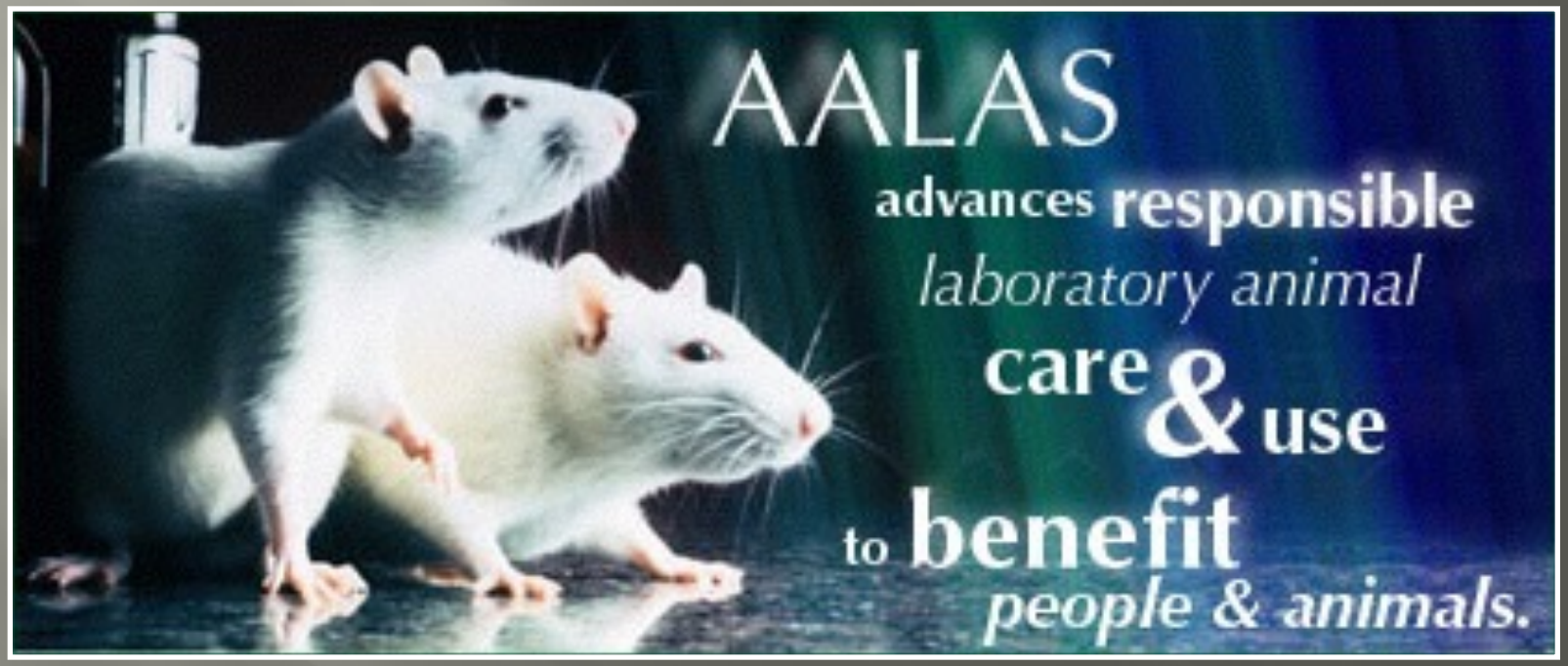
- Reliable Research.

- Animal Welfare

Final Comments

- Animal Research is Important
- By understanding more about animal research, you help your IACUC and the research community assure the American public that animal research is conducted according to the highest standards.
- Our society needs animal research and the accompanying medical advances that have reduced suffering and increased the quality of our lives.

Great Animal Care = Great Science



THANKS!