CCAC training module on: laboratory animals used in biomedical research

Companion Notes

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Slide 1 CCAC training module on: laboratory animals used in biomedical research

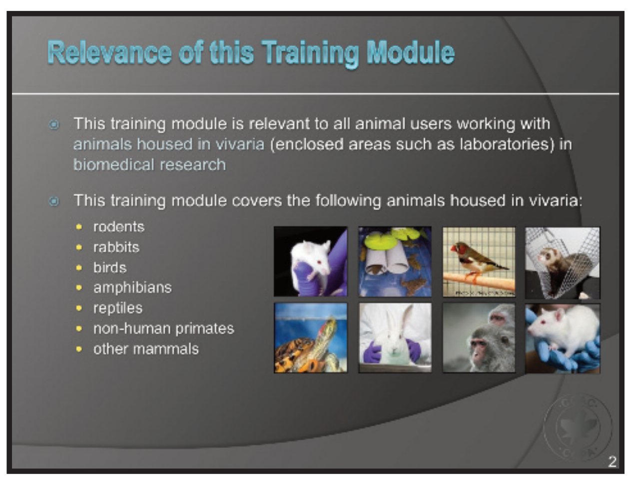
This module provides an introductory overview of the factors and welfare issues that relate to the use of animals housed in vivaria in biomedical research. The intention is that this overview be utilized in conjunction with hands-on training. Before any research is carried out, all personnel involved must receive practical instruction, and be able to demonstrate competence in all techniques required within the protocol.

Slide 2 Relevance of this Training Module

This training module is relevant to all animal users working with **animals housed in vivaria** (enclosed areas such as laboratories) in **biomedical research**.

This training module covers rodents, rabbits, birds, amphibians, reptiles, non-human primates and other mammals housed in vivaria.

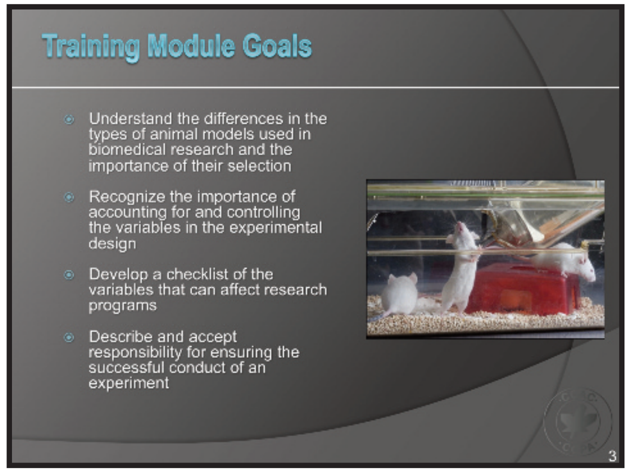
Note: This module does not cover fish; training materials relevant to fish users are available in the Fish Stream. This module does not cover farm animals; training modules relevant to farm animal users are available in the Farm Animal Stream.



Slide 3 Training Module Goals

At the end of this training module, the reader should:

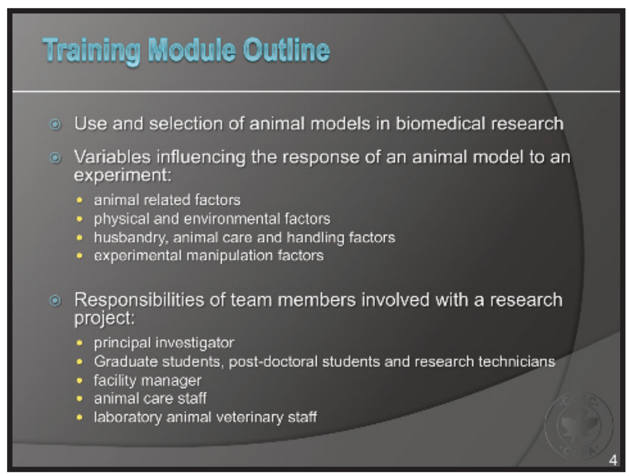
- understand the differences in the types of animal models used in biomedical research and the importance of their selection
- recognize the importance of accounting for and controlling the variables in the experimental design
- develop a checklist of the variables that can affect research programs
- describe and accept responsibility for ensuring the successful conduct of an experiment



Slide 4 Training Module Outline

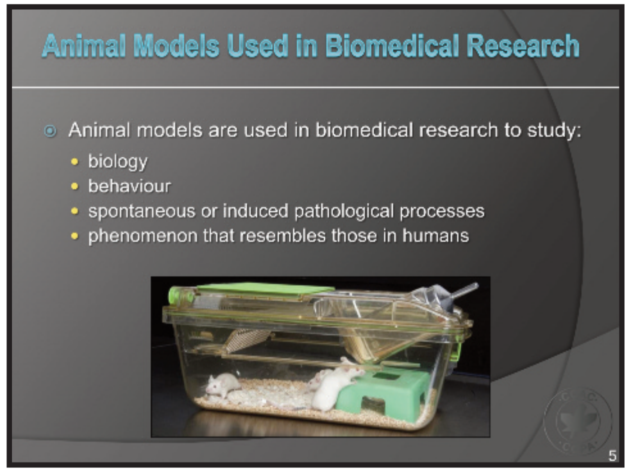
This training module will provide an overview of the:

- use and selection of animal models for biomedical research
- many variables that influence the way the selected animal model will respond in an experiment
- importance of controlling all the non-experimental variables, including the many protocol variables, and of monitoring and recording the variables
- respective responsibilities of each team member involved with a research project (principal investigators, graduate students, post-doctoral students, research technical staff, facility managers, animal care facility staff and laboratory animal veterinary staff) in controlling the variables that may alter the animal model's response



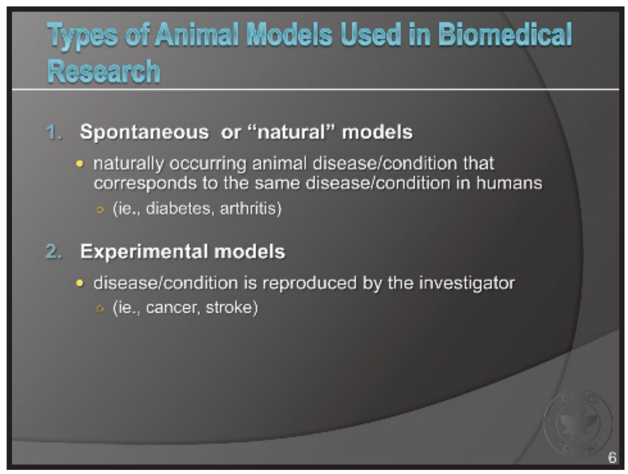
Slide 5 Animal Models Used in Biomedical Research

Although the term "animal model" is commonly used, a definition may help clarify the context of the term. The American National Research Council Committee on Animal Models for Research and Aging drafted the following definition: "An animal model for biomedical research is one in which normative biology or behaviour can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animals."



Slide 6 Types of Animal Models Used in Biomedical Research

Animal models used in biomedical research, particularly those used in the study of diseases and other conditions in humans, can be grouped as follows:



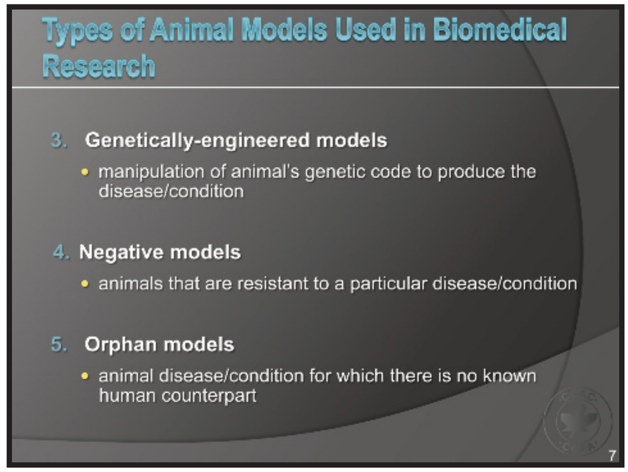
Spontaneous models or "natural" models

These include naturally occurring animal diseases or conditions that correspond to the same diseases or conditions in humans. Diabetes, hypertension, arthritis, immune deficiencies are just a few examples. Many hundreds of animal strains with inherited conditions have been characterized and conserved.

Experimental models

Experimental models are models in which a condition or disease is experimentally reproduced by the scientist. Examples include producing diabetes using the chemical streptozotocin to damage the insulin producing cells in the pancreas; using a chemical carcinogen to produce a certain type of cancer; producing a stroke model through surgery.

Slide 7 Types of Animal Models Used in Biomedical Research



Genetically-engineered models

Genetically-engineered models are a special group of induced animal models, involving manipulation of the animal's genetic code to produce the condition that the scientist wants to study. Genetically modified animals may carry inserted foreign DNA in their genome, or have genes replaced or removed ("knock-out" models). These models can help scientists study the genetic basis of disease, susceptibility and resistance, etc.

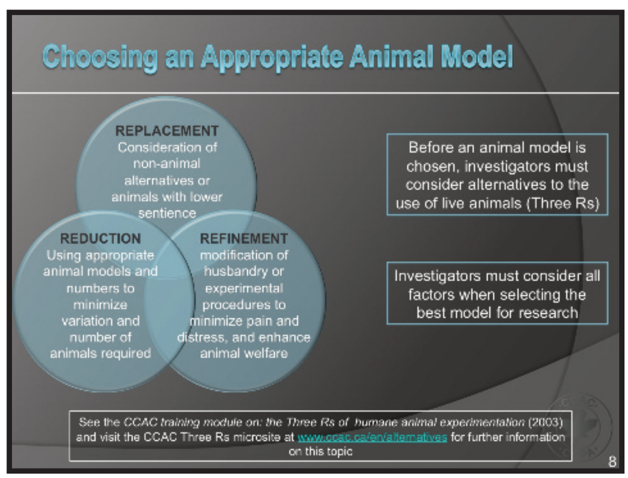
Negative models

Some animals are resistant to a particular condition or disease. Examining why this is the case may provide answers to questions about disease resistance and its physiological basis. For instance, certain strains of mice are resistant to some infectious agents while others are susceptible.

Orphan models

Orphan models are conditions appearing naturally in an animal, for which there is no known human counterpart. Historically scrapie in sheep was such a model, but now is useful as a model for the human spongiform encephalopathies that are of so much concern (e.g., BSE, "mad cow disease" and Chronic wasting disease in deer).

Slide 8 Choosing an Appropriate Animal Model



Before an animal model is chosen the principal investigator must consider alternatives to the use of live animals in his/her experiment (alternatives of Replacement, Reduction and Refinement).

For more detailed information on the Three Rs:

- CCAC Three Rs microsite: www.ccac.ca/en/alternatives
- *CCAC training module on: the Three Rs of humane animal experimentation* (2003). Visit the CCAC website at www.ccac.ca to access and consult this training module.

Replacement and species selection

In line with the *CCAC guidelines on: animal use protocol review* (1997), protocol forms should include a declaration that before making the decision to use animals in his/her research, the principal investigator has considered non-animal alternatives, or using species that current scientific evidence indicates have a significantly lower potential for pain perception, such as some invertebrates.

Reduction and species selection

The appropriate choice of an animal model can be considered a Reduction alternative, as it should minimize variation in the experiment and hence the numbers of animals that are required.

Using an appropriate model can be considered a Reduction alternative when:

- the use of isogenic, or genetically identical, strains (inbred strains and F1 hybrids) results in less experimental variability than would occur when outbred strains are used
- the model has been validated and shown to meet the objectives of the research and/or be predictive of the endpoint of interest
- the model has been fully characterized so that all aspects of the animal's life cycle and their potential impact on the experiment are understood (for example, to ensure the animal will survive long enough to complete the experiment, or whether the rapid growth of a young animal is appropriate to model an adult human)
- the model is being continuously improved to decrease variability and improve it's predictivity

Refinement and species selection

Using an appropriate model can be considered a Refinement alternative when:

- the research facility ensures it is able to properly house and care for the species, including accommodation of the species' behavioural and companionship needs
- competent welfare assessments of the species can be made, including knowledge of pain and pain management in the species
- purpose-bred animals are used rather than those caught in the wild

The most obvious choice of animal species and model for a specific research program may be the same model used by other researchers for the same research. However, with ever increasing numbers of animal models available including new spontaneous mutations, and genetically modified animals constantly being developed, the investigator must consider all factors when selecting the best model for his/her research.

Some of the factors that will influence which animal model the investigator selects are:

- appropriateness of the model or organ system for the proposed study
- genetic aspects of the model
- natural vs. experimentally produced models - both natural models and induced models of disease are useful depending on the objectives of the study
- responses of the animal to procedures
- environmental aspects important to that particular animal model
- background information available on the animal and specific model
- species availability
- numbers needed, according to statistically appropriate design

- age and sex needed
- life span of the animal model
- size of the animal model
- cost of both the animal model and its ongoing care
- facilities required to house the chosen model appropriately
- husbandry expertise - some models require not only special housing, but also special care

Among these factors are scientific considerations as well as purely practical ones. All are important to the success of the research program.

Additional References and Resources:

CCAC guidelines on: animal use protocol review (1997) Visit the CCAC website at www.ccac.ca to access and consult this guidelines document.

Dell R.B., Hollerhan S., and Ramakrishnan A. (2002) Sample Size Determination. *Institute for Laboratory Animal Research Journal* 43(4): 207-213.

Festing M.F. (2010) Improving toxicity screening and drug development by using genetically defined strains. *Methods in Molecular Biology* 602: 1-21.

Festing M.F.W. and Altman D.G. (2002) Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals. *Institute for Laboratory Animal Research Journal* 43(4): 244-258.

Festing M.F.W. (2006) *Animal models in research*. Isogenic.info, http://www.isogenic.info/html/animal_models_in_research.html

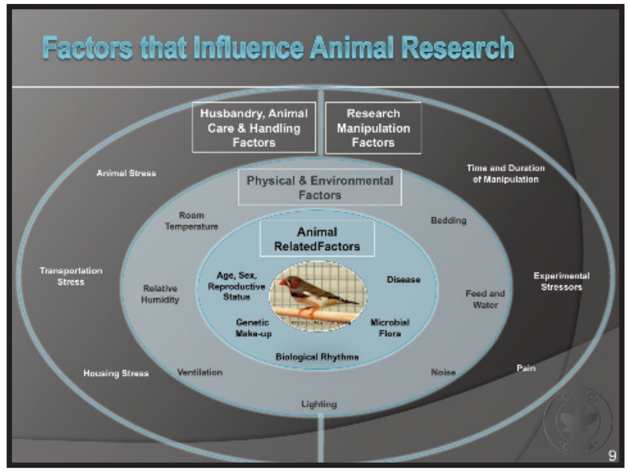
Johnson P.D. and Besselsen D.G. (2002) Practical Aspects of Experimental Design in Animal Research. *Institute for Laboratory Animal Research Journal* 43(4): 202-206.

Shaw R., Festing M.F.W., Peers I. and Furlong L. (2002) Use of factorial designs to optimize animal experiments and reduce animal use. *Institute for Laboratory Animal Research Journal* 43(4): 223-232.

Wood M.W. and Hart L.A. (2008) Selecting appropriate animal models and strains: making the best use of research information and outreach. *Alternatives to Animal Testing and Experimentation* 14:303-306.

Slide 9 Factors that Influence Animal Research

When conducting research, investigators want experimental and control groups to be exactly the same, except for the variable they are interested in. However, laboratory animals are influenced by many non-experimental factors that might potentially affect the outcome of the study. Those factors should be identified and accounted for at all stages to ensure they do not increase the variability or adversely affect the outcome of the experiment.



Additional References and Resources:

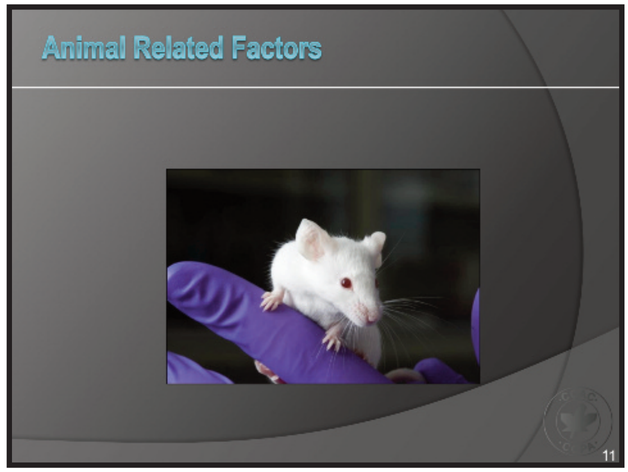
Lipman N.S. and Perkins S.E. (2002) Factors that May Influence Animal Research. In: *Laboratory Animal Medicine*, 2nd ed. (Fox J.G., Anderson L.C., Loew F.M. and Quimby F.W., eds) Orlando FL: Academic Press, Chapter 29. pp. 1143-1184.

Slide 10 Non-Experimental Factors that Influence Animal Research

The many non-experimental factors that can influence the response of the animal model in an experiment can be grouped as:

- animal related factors
- physical / environmental factors
- husbandry, animal care, and handling factors
- research manipulation factors

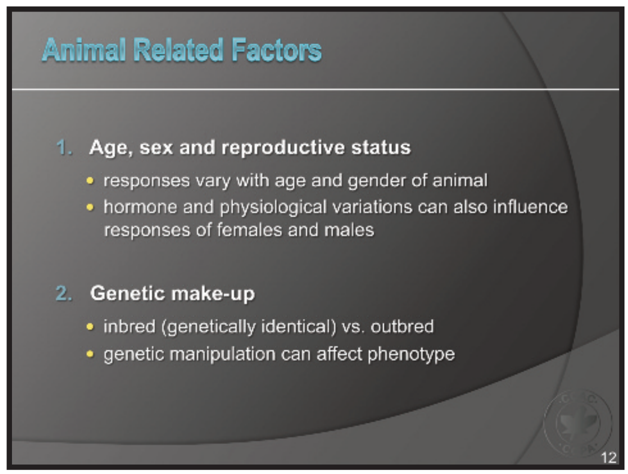
Slide 11 Animal Related Factors



Some of the factors affecting research results are related to the animals themselves. These factors include:

- age, sex and reproductive status
- genetic make-up
- microbial flora
- biological (circadian) rhythms
- disease

Slide 12 Animal Related Factors



1. Age, sex and reproductive status

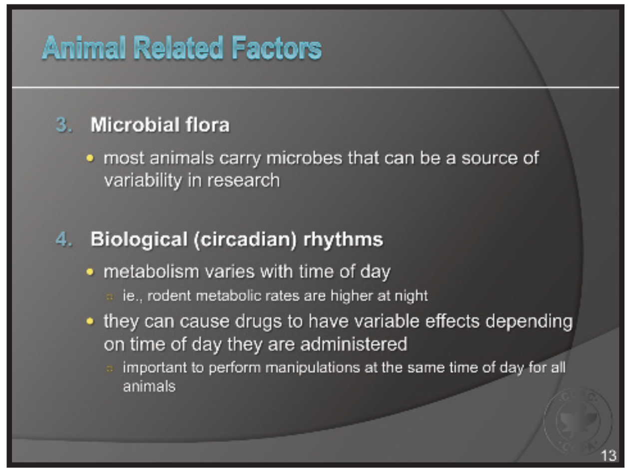
The age of the research animals is an important variable to control because younger animals can have different responses than older animals. For example, white blood cell counts, immune responses, and enzyme activity can vary with the age of the animal. Gender can also be a variable. For example, liver biotransformation of certain chemicals tends to be slower in females than in males, which means that the toxicity of some compounds may be different between the sexes, and different doses of anesthetics or therapeutic drugs may be required. Reproductive status is another variable, since the physiology of females that are pregnant or lactating is obviously different from those that are not. Likewise, hormonal variations that occur during different stages of the estrus cycle can influence responses of females.

2. Genetic make-up

The genetic make-up of the animals is another important consideration. Are the animals inbred or outbred? All individuals in an inbred strain are essentially genetically identical, although it is important to realize that there may be subtle differences between animals of the same inbred strain obtained from different commercial suppliers. In most cases, inbred strains of research animals are preferred to outbred stocks, since this reduced genetic diversity means that smaller numbers of animals can be used to obtain statistically significant results. But inbred animals tend to be smaller and less robust than their outbred counterparts.

Are the animals genetically modified? Genetic manipulation can affect more than just the gene of interest, and may alter the phenotype of the animal in unexpected ways that can affect research results.

Slide 13 Animal Related Factors



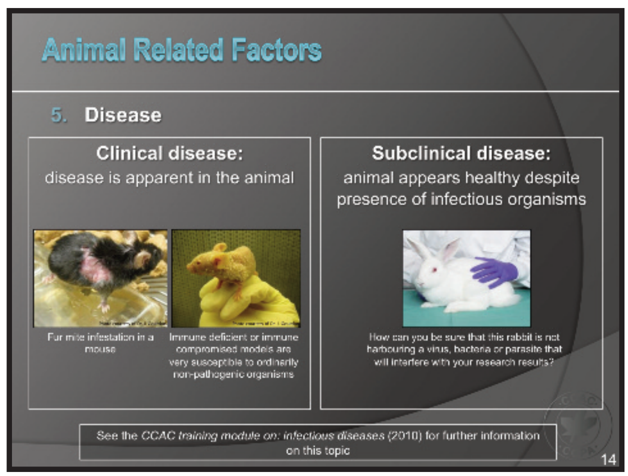
3. Microbial flora

Most research animals carry a mixed microbial population that depends in part on housing conditions, diet, exposure to microbes, and other factors. These microbes may be a source of variability between animals housed in different facilities, and can influence parameters such as nutritional requirements and drug metabolism. In addition, the microbial flora can influence the phenotype of genetically modified animals.

4. Biological (circadian) rhythms

Biological or circadian rhythms can also influence research results. The animal's metabolism varies with the time of day, according to a circadian rhythm that is entrained by the light cycle. Nocturnal animals such as rodents are more active during the dark phase, and their metabolic rate is increased at this time as well. This means that drugs, such as anesthetics for example, can have a variable effect depending on the time of day they are administered. Thus it is very important to perform research manipulations at the same time of day for all animals.

Slide 14 Animal Related Factors



5. Disease

Clinical disease

Disease is one of the most important variables that interfere with research. Disease in research animals can be clinical, meaning that it is readily apparent, and the animals actually look sick. These sick animals are clearly not good research subjects, since their responses will be influenced by the presence of disease.

Example 1: Infection causing disease

Fur mites (*Myocoptes musculinus, Myobia musculi, Radfordia affinis*) infestation in mice is an infectious disease of the skin which causes alopecia, pruritus and ulcerative dermatitis. Interference with research: Animals with mites and severe clinical signs such as ulcerative dermatitis are not suitable for use in research. For example, mice may have an increased IgE response, an increase in the formation of secondary amyloid, hypoalbuminemia, and a decreased mean hemoglobin concentration.

Example 2: Ordinary bacteria causing disease in immune deficient models

Immune deficient or immune compromised models (e.g., nude or severe combined immunodeficiency (SCID) mice) are very susceptible to ordinarily non-pathogenic organisms such as *Corynebacterium spp*. Special caging and care procedures are vital to minimizing such infections. Inadvertent infections in these special models can not only cause serious disease, but they will also interfere with the animal's immune response during research.

Subclinical disease

More commonly however, laboratory animal disease can be subclinical or silent, meaning that the animals may appear outwardly healthy despite the presence of infectious organisms. These subclinical infections can nonetheless interfere with research results by causing changes in immune response, drug metabolism, behaviour, and reproduction. Two examples of silent infections affecting research results are presented here:

Example 1:

Mouse parvovirus (MPV) is a clinically silent infection. In mice with normal immune systems the infection lasts up to 6 months, while in immunodeficient or immunosuppressed mice, infection and shedding of the virus may be lifelong. MPV persistently infects the lymphoid tissue and causes changes in immune system responses. Infected T cells may not grow properly when cultured in vitro. Parvoviruses may also interfere with cancer research; T cells from infected mice may have diminished cytolytic capacity.

Example 2:

Pasteurellosis is a disease of rabbits caused by the bacteria, *Pasteurella multocida*. Many infected rabbits show clinical signs such as nasal discharge, conjunctivitis or abscesses. However, in other cases healthy rabbits can harbor the bacteria in their upper respiratory tract without any outward signs. These unapparent carriers can shed the pathogen and infect other rabbits. When infected rabbits are subjected to stress, as from experimental manipulation, they may suddenly become sick or even die unexpectedly. These unanticipated animal losses cost researchers time and money, and also have a high ethical cost. The use of specific pathogen free rabbits that do not carry this disease is an example of Reduction.

From these four examples, it should be obvious that infections, even subclinical ones causing no overt disease signs, can significantly affect the results obtained in a study. The efforts of many people, at many levels, to eliminate such infections from the laboratory animal models, have contributed to minimizing this unwanted variable in biomedical research that uses animal models.

Knowing the health status of the animals and documenting it are a very important part of the records that must be kept by the principal investigator, as part of defining the animal model in the publications arising from the research.

Additional References and Resources:

CCAC training module on: infectious diseases (2010). Visit the CCAC website at www.ccca.ca to access and consult this training module.

Charles River Laboratories. (2009) *Technical sheet. Fur, Skin, and Ear mites*. http://www.criver.com/SiteCollection Documents/rm_ld_r_mites.pdf

Slide 15 Physical & Environmental Factors

In addition to the animal-related factors, there are many factors in the environment that can influence an animal's response to experimental manipulations. These physical and environmental factors include:

- room temperature
- relative humidity
- ventilation
- lighting
- noise
- feed and water
- bedding

For more detailed information on physical and environment factors:

Canadian Council on Animal Care (1993) The Environment. In: *CCAC Guide to the Care and Use of Experimental Animals*, vol. 1, 2nd ed. Ottawa ON: CCAC, Chapter III. pp. 52-65. Visit the CCAC website at www.ccac.ca to access and consult this guide.

Additional References and Resources:

Castelhano-Carlos M.J. and Baumans V. (2009) The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals* 43(4):311-27.

Gonder J.C. and Laber K. (2007) A renewed look at laboratory rodent housing and management. *Institute for Laboratory Animal Research Journal* 48 (1):29-36.

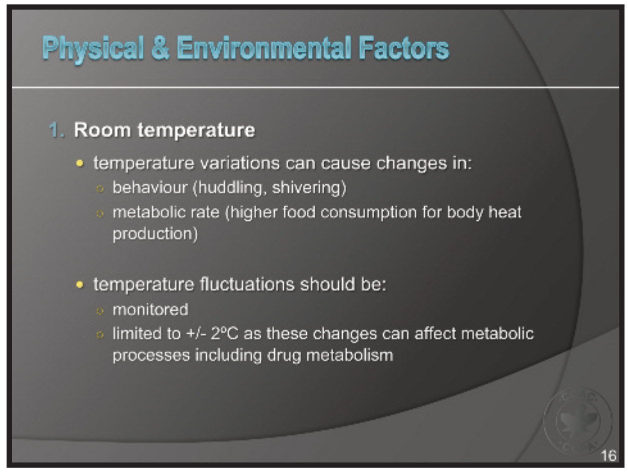
Hessler J.R. and Hoglund U. (2003) Laboratory Animal Facilities and Equipment for Conventional, Barrier, and Containment Housing Systems. In: *Handbook of Laboratory Animal Science*, Vol. 1, 2nd ed. (Hau J. and Van Hoosier G.L. Jr., eds.) Boca Raton FL: CRC Press, Chapter 8. pp. 127-172.

Johnson N.A. and Nevalainen T. (2003) Impact of the Biotic and Abiotic Environment on Animal Experiments. In: *Handbook of Laboratory Animal Science*, Vol. 1, 2nd ed. (Hau J. and Van Hoosier G.L. Jr., eds.) Boca Raton FL: CRC Press, Chapter 13. pp. 311-326.

Lipman N.S. (2007) Design and Management of Research Facilities for Mice. In: *The Mouse in Biomedical Research*, Vol. 3, 2nd ed. (Fox J.G., Barthold S.W., Davisson M.T., Newcomer C.E., Quimby F.W. and Smith A.L., eds.) New York NY: Academic Press, Chapter 9. pp. 271-320.

Slide 16 Physical & Environmental Factors

1. Room temperature

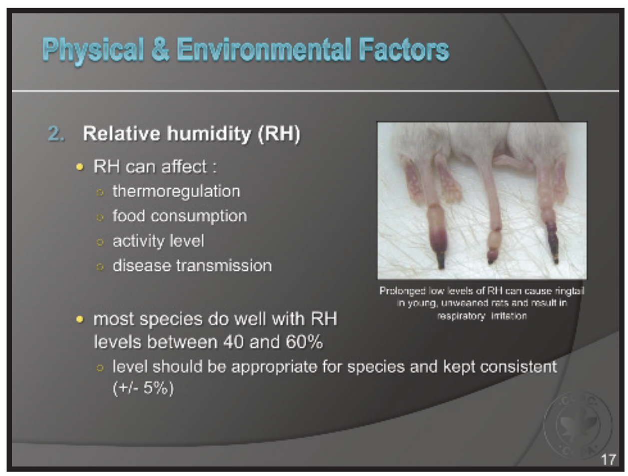


Small laboratory animals respond to temperature variations by changes in behaviour (e.g., shivering, huddling) and metabolic rate (including increased food consumption if they require more body heat production). These changes could affect several metabolic processes including drug metabolism. Daily animal room temperature fluctuations should therefore be limited (by good heating/ventilation design) to +/- 2°C. Daily temperature fluctuations should be monitored and recorded.

For some studies it may be necessary to measure cage parameters, and not just room environment. The temperature in an individual cage may be significantly warmer than the overall room temperature, especially if the cage houses a large number of animals and is placed on the top of a rack. The temperature within static microisolator cages will likely be higher than in open cages or individually ventilated cages.

Slide 17 Physical & Environmental Factors

2. Relative humidity

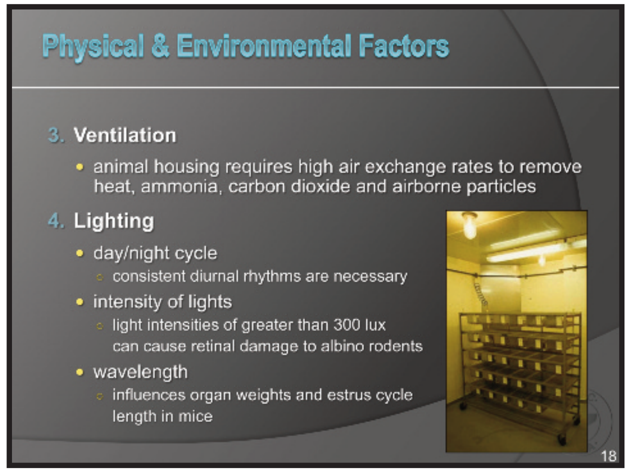


The relative humidity of the animal's immediate environment (microenvironment) is another factor that can impact research animals. It can affect thermoregulation, food consumption, activity level, and disease transmission.

Most species do well at a relative humidity of 40-60%. The humidity level should be chosen as appropriate for the species, and should be kept consistent (+/- 5%). Prolonged low levels of relative humidity can cause ringtail in young, unweaned rats, and result in respiratory irritation.

Slide 18 Physical & Environmental Factors

3. Ventilation



Animal housing requires high air exchange rates to remove animal generated heat, ammonia, carbon dioxide, and airborne particles (dust and allergens).

The environment within the cage may be quite different from that of the room, depending on the type of caging used. For example, static microisolator cages may contain high levels of ammonia and carbon dioxide despite good ventilation rates in the room itself. Air pressure gradients from animal rooms to corridors or between zones in an animal facility are also important for containment of micro-organisms.

Within a laboratory animal holding room there can be significant variations in ventilation, and levels of ammonia and CO_2, as well as air flow. Randomizing the location of animals in different treatment groups in a rack or in the room may help to avoid bias in the results due to these factors.

4. Lighting

The lighting cycle for the animals has several aspects; the day/night cycle, the intensity of the lights, and the wavelength.

Timer control of day/night cycle is necessary to maintain a consistent diurnal rhythm in the animal's metabolic state. Exposure to even brief periods of light during the dark phase of the cycle can disrupt the diurnal rhythm and affect reproduction and metabolism, which in turn can affect research results. For example, exposure to low light levels during the dark phase can alter the growth rate of tumors in rats.

The sudden onset of lights in the morning affects some hormone levels - effects that may last for several hours. The use of dusk/dawn lighting systems, which gradually change light intensity between dark and light phases is encouraged.

The intensity and wavelength are also important to animals. Albino rodents in particular experience retinal damage when room light intensities are above 300 Lux. Wavelength has been shown to influence organ weights and estrus cycle length in mice.

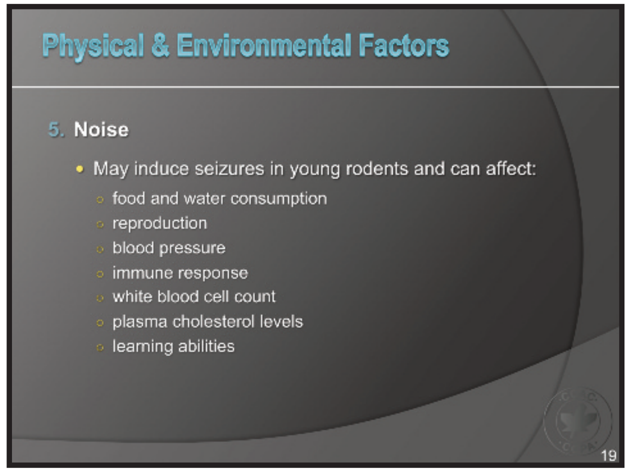
The amount of light perceived by animals in cages will depend on the cage type, the proximity of the cage to the light source, the presence of opaque environmental enrichment devices such as tunnels or houses, as well as the age of the light bulbs and the reflectivity of room surfaces. For studies where the light levels might influence the research results, randomizing the location of animals in different treatment groups on a holding rack may be useful in avoiding a light-induced bias in the results obtained.

Additional References and Resources:

Dauchy R.T., Sauer L.A., Blask D.E. and Vaughan G.M. (1997) Light Contamination During the Dark Phase in "Photoperiodically Controlled" Animal Rooms: Effect on Tumor Growth and Metabolism in Rats. *Laboratory Animal Science* 47(5):511-518.

Memarzadeh F., Harrison P.C., Riskowski G.L. and Henze T. (2004) Comparison of Environment and Mice in Static and Mechanically Ventilated Isolator Cages with Different Air Velocities and Ventilation Designs. *Contemporary topics in laboratory animal science* 43(1):14-20.

Slide 19 Physical & Environmental Factors

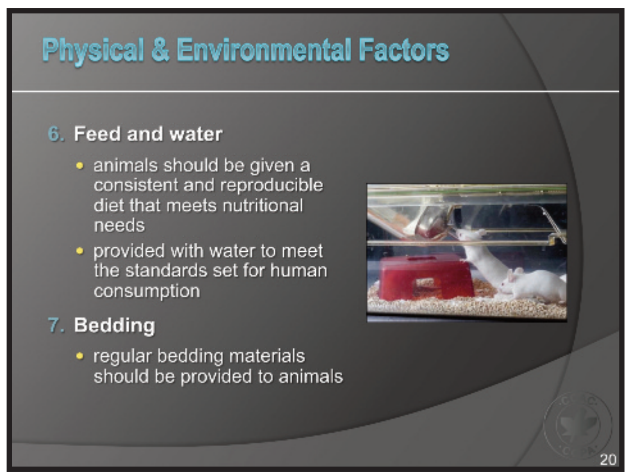


5. Noise

The impact of noise on the behaviour or responses of laboratory animals in biomedical research has received too little study. The fact that loud "buzzer" noises may induce seizures in young rodents (this has been used to create a model of audiogenic seizures) is well known.

Both intensity and sound frequency are important. Rodents and some other animals are particularly sensitive to ultrasonic frequencies, ones that we may not even be aware of since they are beyond the range of the human ear. Low frequency noises, for example from nearby construction, may also disturb the animals. Noise can impact many different types of research. For example, studies in lab animals have shown that noise affects food and water consumption, reproduction, blood pressure, immune response, white blood cell counts, plasma cholesterol levels, and learning ability.

Slide 20 Physical & Environmental Factors



6. Feed and water

A consistent and reproducible diet that meets the animal's nutritional needs is an important part of animal research. Purified diets with minimal nutrient variation are necessary for many types of research, since seasonal and batch-to-batch variation in components can affect the animal's physiology and metabolism. Many laboratory animal diets are also "certified" not to exceed maximal amounts of known contaminants or chemical residues that might affect research.

Laboratory animals should be provided with water that meets the standards set for human consumption. This is only a minimum requirement however, since there can be a significant amount of variation in quality of potable water from different sources. Water can contain impurities, including bacteria, heavy metals and trace chemicals such as pesticides that can affect research. Furthermore, the amounts of these contaminants can vary depending on the time of year or local weather conditions. For this reason, the use of reverse osmosis to purify water for laboratory animals is becoming the standard. In some facilities water is treated by acidification or chlorination to reduce bacterial growth. However, both of these processes can affect research. For example, acidification of water can lead to leaching of chemicals from the rubber stoppers used in water bottles, and by-products of chlorination may cause alterations in hematological parameters.

7. Bedding

Standard Operating Procedures (SOPs) in most animal facilities include provision of regular bedding materials for that facility. Any special bedding requirements have to be specified by the principal investigator.

The phenomenon of resins in softwood bedding (e.g., cedar shavings) activating some of the hepatic enzyme systems (P450 enzymes) is well known. This may complicate results if the experimental outcome is related to hepatic enzyme activity.

Additional References and Resources:

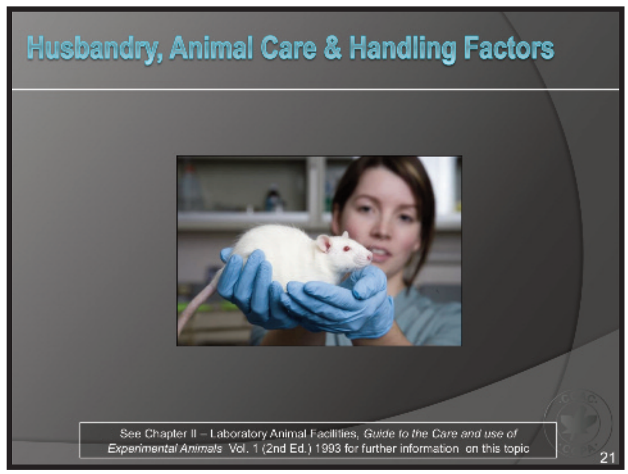
Barnard D.E., Lewis S.M., Teter B.B. and Thigpen J.E. (2009) Open- and Closed-Formula Laboratory Animal Diets and their Importance to Research. *Journal of the American Association for Laboratory Animal Science* 48(6):709-713.

Lauer A.M., May B.J., Hao Z.J. and Watson J. (2009) Sounds levels in modern rodent housing rooms are an uncontrolled environmental variable with fluctuations mainly due to human activities. *Laboratory Animals* 38(5):154-160.

Slide 21 Husbandry, Animal Care & Handling Factors

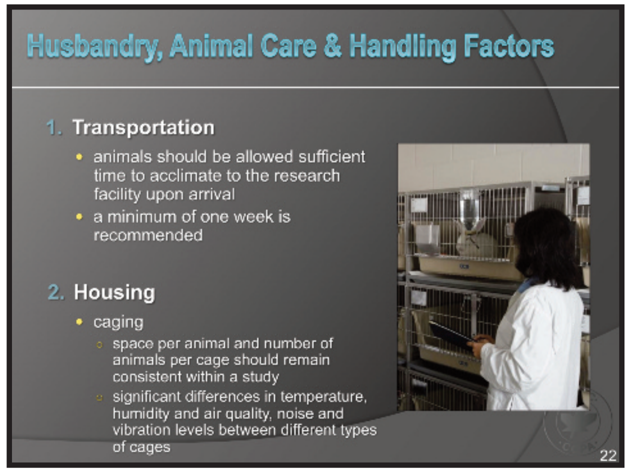
Stress from many different sources can affect the animal's physiology, biochemistry and behaviour. Sources of stress in the handling and care of the animals include transportation, housing, and the handling and manipulations done by animal care and research staff. For more detailed information on husbandry, care, and handling of laboratory animals:

Canadian Council on Animal Care (1993) Laboratory Animal Facilities. In: *CCAC Guide to the Care and Use of Experimental Animals*, vol. 1, 2nd ed. Ottawa ON: CCAC, Chapter II. pp. 44-51. Visit the CCAC website at www.ccac.ca to access and consult this guide.



Slide 22 Husbandry, Animal Care & Handling Factors

1. Transportation



Laboratory animals are rarely used in the same location where they are raised, so usually they are transported to the facility where the research will be done. A number of studies have shown that animals experience varying degrees of stress as a result of the transport, and that it takes some time upon arrival at the research facility to return to a normal physiological state. Eating, drinking and growth tend to return to normal levels in about a week after delivery to the new location. Some subtle physiological and immunological changes may last longer. Institutions should have SOPs for conditioning animals upon receipt that take into account the species and background of the animals.

2. Housing

Caging

The amount of space per animal, and the number of animals per cage may influence an animal's response in an experiment. Identical caging should be used for all animals in a study, to ensure that the space per animal and number of animals per cage remains consistent within a study. Cage design is also an important consideration, since there are significant differences in temperature, humidity and air quality, as well as noise and vibration levels between different cage types (open, static microisolator or individually ventilated cages).

Studies have shown that the number of rodents per cage affects the stress level (either isolation or crowding), and their growth. As a general rule small rodents should be housed in small groups to minimize stress, and for social enrichment. If the research requires individual housing, this should be scientifically justified to the institutional animal care committee.

Additional References and Resources:

Castelhano-Carlos M.J. and Baumans V. (2009) The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals* 43(4):311-27.

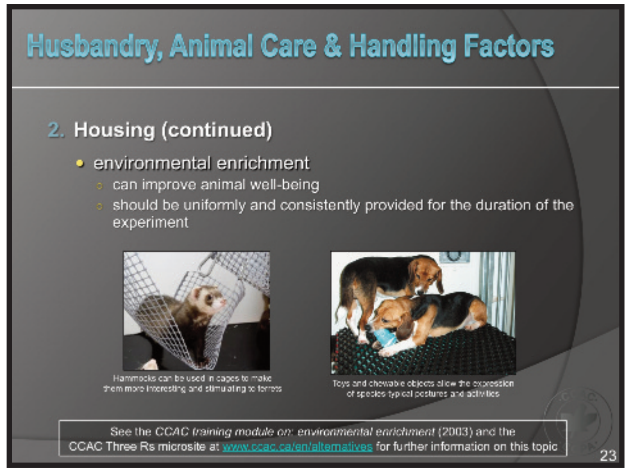
Lawlor M. (1990) The size of rodent cages. In: *Guidelines for the Well-Being of Rodents in Research*. (Guttman H.N., ed.).Bethesda MD: Scientists Center for Animal Welfare, pp.19-28.

Swallow J., Anderson D., Buckwell A., Harris T., Hawkins P., Kirkwood J., Lomas M., Meacham S., Peters A., Prescott M., Owen S., Quest R., Sutcliffe R. and Thompson K. (2005) Guidance on the transport of laboratory animals: report of the Transport Working Group established by the Laboratory Animal Science Association (LASA). *Laboratory Animals* 39(1):1-39.

Slide 23 Husbandry, Animal Care & Handling Factors

2. Housing (continued)

Environmental enrichment



The value of providing an enriched environment in improving the well-being of the animals must be emphasized. In the context of controlling research variables however, it must be noted that any changes (improvements) to the cages will have behavioural, physiological and anatomical effects, some of which result in permanent changes to the animal. Therefore any improvements or enrichments should be uniformly and consistently provided to all animals for the duration of an experiment. Changes in the cage's physical environment should only be made following consultation with the investigator.

For more detailed information on environmental enrichment:

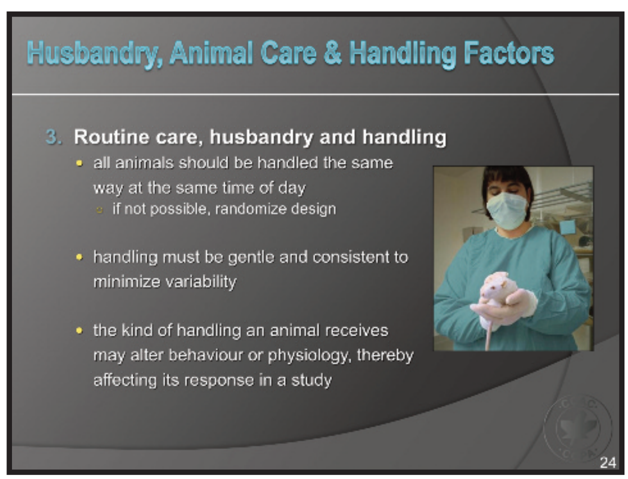
- *CCAC training module on: environmental enrichment* (2003). Visit the CCAC website at www.ccac.ca to access and consult this training module.
- Three Rs microsite: http://www.ccac.ca/en/alternatives/

Additional References and Resources:

Laber K., Veatch L.M., Lopez M.F., Mulligan J.K. and Lathers D.M. (2008) Effects of housing density on weight gain, immune function, behavior, and plasma corticosterone concentrations in BALB/c and C57BL/6 mice. *Journal of the American Association for Laboratory Animal Science* 47(2):16-23.

Slide 24 Husbandry, Animal Care & Handling Factors

3. Routine (daily/weekly) animal care, husbandry and handling



The daily routines of the animal care staff may have a profound impact on the way an animal responds in an experiment. As a general principle all animals should be handled the same way, and at the same time of day; if this is impossible, a randomized design should be used. Handling must be gentle, and consistent. Most animals quickly become familiar with their regular caretakers and their stress level rises when unfamiliar people handle them.

The kind of handling each animal receives may in fact alter its behaviour or physiology, and thereby affect its response in a study. One example of this comes from a handling study conducted in pigs. Hemsworth and co-workers published a paper in 1987 describing a study in which the responses of pigs to three kinds of handling; pleasant handling, unpleasant handling and minimal handling, were compared. The pigs exposed to pleasant handling approached people more quickly. The females receiving pleasant handling were different with respect to age at first estrus and sexual receptivity when bred, and had a significantly higher pregnancy rate than the other two groups. Although not a statistically significant results, the males receiving pleasant handling reportedly had larger testicles. Consistent handling, by animal care staff, by research technicians, and by the investigators, will ensure that this source of variability is minimized.

The weekly routine in any animal room must also be understood by the investigator. In many facilities, animal cages are cleaned on specific days. Taking samples just after the cages have been cleaned may result in altered results because of the disturbance of the animals at that time. In addition, it must be noted that weekend and holiday animal care routines are generally different than regular week day routines. This may alter the animals' responses on weekend days if sampling is scheduled then.

Additional References and Resources:

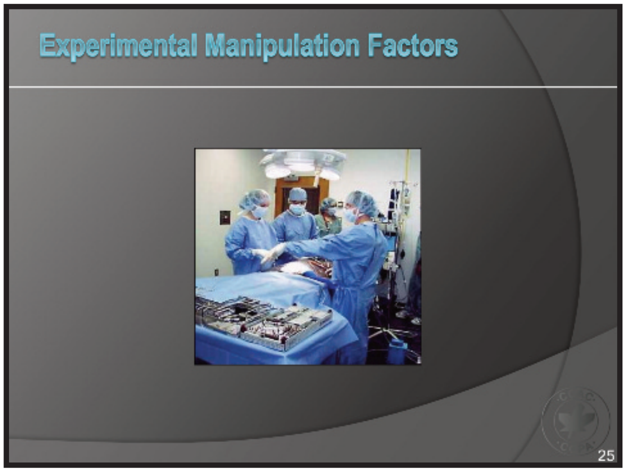
Balcombe J.P., Barnard N.D. and Sandusky C. (2004) Laboratory routines cause animal stress. *Contemporary topics in laboratory animal science* 43(6):42-51.

Duke J.L., Zammit T.G. and Lawson D.M. (2001) The effects of routine cage-changing on cardiovascular and behavioral parameters in male Sprague-Dawley rats. *Contemporary topics in laboratory animal science* 40(1):17-20.

Hemsworth P.H., Barnett J.L. and Hansen C. (1987) The influence of handling by humans in the behaviour, reproduction and corticosteroids of male and female pigs. *Applied Animal Behavioural Science*. 17(3): 245-252.

Slide 25 Experimental Manipulation Factors

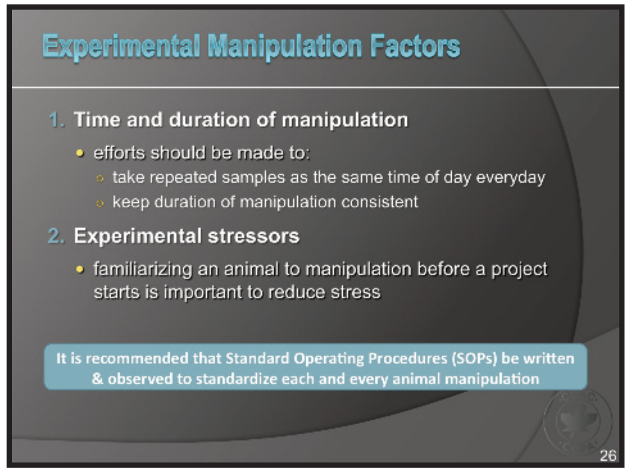
In addition to the many non-experimental variables that must be considered and controlled to ensure that the fewest animals need to be used and that the results are valid, there are the many research or protocol variables that the investigator and all research personnel must consider, and control.



For some studies such as the surgical induction of cerebral ischemia (stroke) in a rodent model, there may be many variables to control; duration and depth of anesthesia, body temperature during surgery and during recovery, duration of the application of the ischemia, timing of the analgesia, post-operative care and monitoring, etc. All of these may influence the outcome of the ischemia.

It is recommended that for each specific research procedure a SOP be written and observed by everyone involved, to standardize as much as possible each and every animal manipulation.

Slide 26 Experimental Manipulation Factors



1. Time and duration of manipulation

The normal biological diurnal rhythms in an animal's biochemistry and physiology alter its responses depending on the time of day that treatments are applied or samples are taken. Thus efforts should be made to take repeated samples at the same time of day, every day.

For each animal, the duration of the manipulation(s) should also be kept as consistent as possible. Biochemical and hematological changes start happening when an animal is taken from its cage. Studies have shown that some of these changes last for minutes to hours and will be reflected in the results obtained. For each animal in all treatment groups including control groups, time to sampling (from removing the animal from the cage) should be consistent.

Ordinary animal husbandry procedures such as moving rats to a clean cage can induce transient, but significant, cardiovascular and behavioural changes. Investigators and animal care staff should recognize that such routine procedures could confound experiments conducted shortly thereafter.

2. Experimental stressors

An animal learns from experience what will be happening to it when it is handled. Animals very quickly learn handling routines and procedures. Assuming the handling is competently done, not only will an animal's stress level be reduced, it will be much more likely to accept the manipulation being done.

During the conditioning period before an experiment begins, animals should be exposed to the routines that will be part of the study. This is particularly important if the study will involve special restraint, use of devices such as collars or jackets containing emerging catheters (e.g., rats with tethers for brain recordings; sheep in a metabolism crate). **Familiarizing the animal to manipulations or restraint before a project starts is important for both welfare and scientific reasons**.

Rewards for good behaviour are an excellent way to enhance a cooperative attitude in an animal. Rewards such as *Gummi Bears* or *Fruit Loops* are enjoyed by rats. Other examples are, giving a dog a dog biscuit, giving a cat a treat, giving a non-human primate a special treat. Of course these would need to be acceptable to the research project.

Additional References and Resources:

CCAC training module on: analgesia (2003). Visit the CCAC website at www.ccac.ca to access and consult this training module.

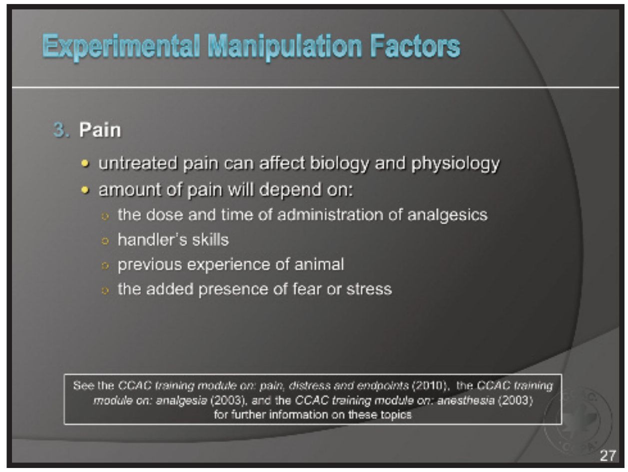
CCAC training module on: pain, distress and endpoints (2010). Visit the CCAC website at www.ccac.ca to access and consult this training module.

Conour, L.A., Murray A. and Brown M.J. (2007) Preparation of Animals for Use in the Laboratory, *Institute for Laboratory Animal Research Journal* 47(4): 283-293.

Slide 27 Experimental Manipulation Factors

3. Pain

Pain can also be an important variable in animal research, since **untreated pain affects many aspects of an animal's biology and physiology**. The amount of pain an individual animal experiences as a result of the experimental manipulations will depend on many different factors such as the choice, dose and time of administration of analgesic agents used; the skill of the surgeon or handler; the previous experiences of that animal; and the added presence of fear or stress. For more detailed information on pain:

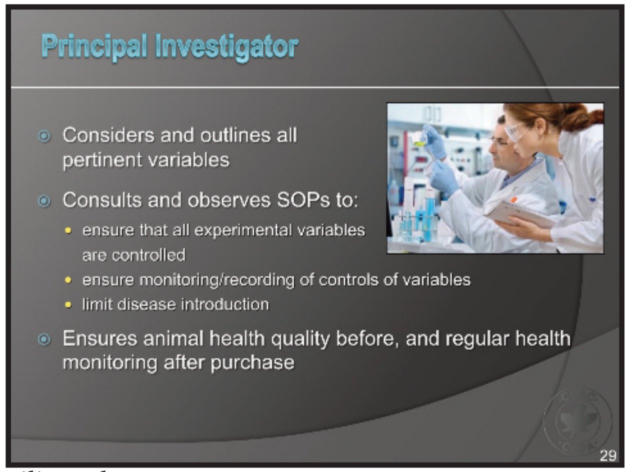


- *CCAC training module on: analgesia* (2003)
- *CCAC training module on: anesthesia* (2003)
- *CCAC training module on: pain, distress and endpoints* (2010)

Slide 28 Responsibilities of Research Team Members

The various members of the biomedical research team have different and important responsibilities in controlling both the non-experimental and experimental variables.

Slide 29 Principal Investigator



Responsibilities of the principal investigator include:

- considering all pertinent non-experimental variables
- outlining the need for controlling pertinent variables to all team members
- ensuring that all experimental variables are controlled or conducted using SOPs
- ensuring monitoring and recording of controls on variables (through SOPs)
- ensuring animal health quality before purchase
- assuring health monitoring is done according to facility rules
- observing all facility SOPs established to limit disease introduction

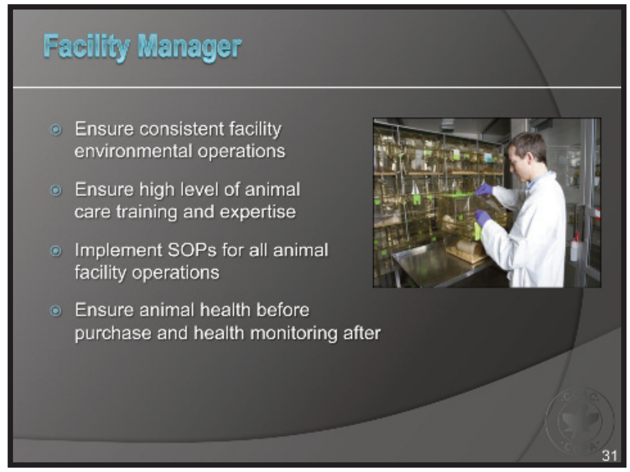
Slide 30 Graduate Students, Post-Doctoral Students and Research Technical Staff

Graduate students, post-doctoral students and research technical staff should:

- monitor and record controls on all variables
- conduct all experimental procedures according to SOPs
- employ skilled animal handling and manipulation techniques
- observe all facility SOPs established to limit disease introduction

Slide 31 Facility Manager

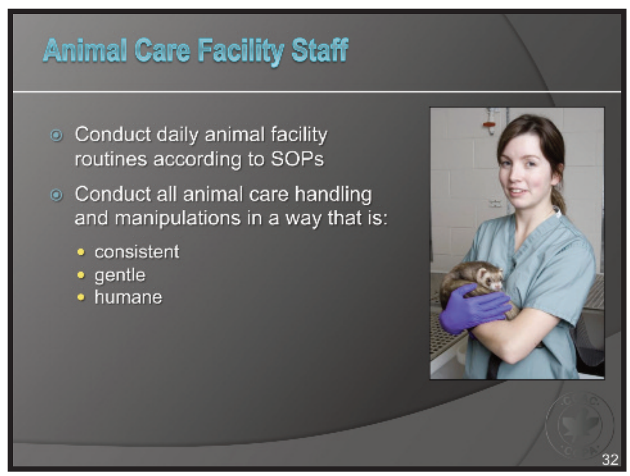
Responsibilities of the facility manager include:



- understanding demands for controls on non-experimental variables for all research underway
- ensuring consistent facility environmental operations
- ensuring high level of animal care training and expertise
- ensuring animal health quality before purchase
- ensuring health monitoring is done according to facility rules
- implementing SOPs for all animal facility operations

Slide 32 Animal Care Facility Staff

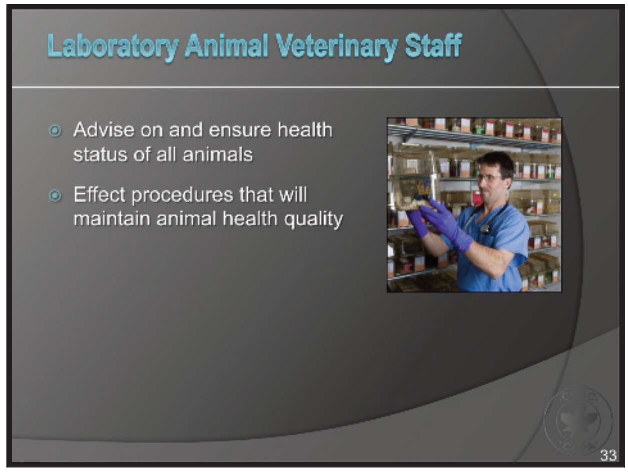
Animal care facility staff should conduct all daily animal facility routines in a consistent manner, according to SOPs and conduct all animal care handling and manipulations in a consistent, gentle, and humane way.



Slide 33 Laboratory Animal Veterinary Staff

Responsibilities of the laboratory animal veterinary staff include:

- advising on and ensuring health status of all animals
- effecting procedures that will maintain animal health quality

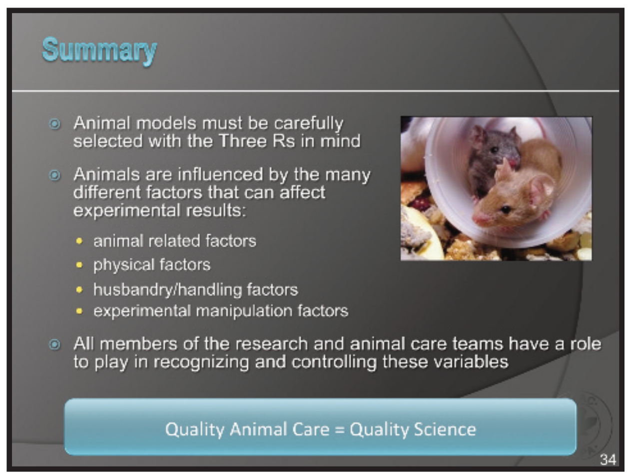


Slide 34 Summary

The importance of accomplishing both scientific and ethical goals by using the fewest animals to generate valid and reproducible scientific data was emphasized in this module. Animal models must be carefully selected with the Three Rs in mind.

Animals are affected by different factors (and in turn this affects experimental results). These include:

- animal related factors
- physical and environmental factors
- husbandry, animal care and /handling factors
- research manipulation factors



All members of the research and animal care teams have a role to play in recognizing and controlling these variables. Investigators and other animal users should strive to achieve their scientific goals in line with the best possible animal welfare standards.