NNMC Institutional Animal care and use policy

NNMC Institutional Animal Care and Use Committee

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**Table of Contents**

[Section 1: Overview of the IACUC Process 3](#_Toc510519407)

[**What is an IACUC?** 3](#_Toc510519408)

[**How does the IACUC process work?** 3](#_Toc510519409)

[**What research requires IACUC approval?** 4](#_Toc510519410)

[**Who may apply for IACUC approval?** 5](#_Toc510519411)

[**What animal research is not possible at NNMC?** 5](#_Toc510519412)

[Section 2: Justification of Animal Use 5](#_Toc510519413)

[**Definition of an animal** 5](#_Toc510519414)

[**What needs to be considered in the justification of animal use?** 6](#_Toc510519415)

[Section 3: Scientific Justification 8](#_Toc510519416)

[**Scientific Justification** 8](#_Toc510519417)

[Section 4: Pain and Distress 8](#_Toc510519418)

[**What is pain and distress?** 8](#_Toc510519419)

[**How are pain and distress measured?** 9](#_Toc510519420)

[**Minimizing Pain and Distress** 10](#_Toc510519421)

[Section 5: Animal Acquisition 11](#_Toc510519422)

[**Appropriate sources for animal acquisition** 11](#_Toc510519423)

[**Who responsible for acquiring animals?** 12](#_Toc510519424)

[**Reporting animal numbers** 13](#_Toc510519425)

[Section 6: Animal Care 13](#_Toc510519426)

[**Transportation** 13](#_Toc510519427)

[**Housing** 14](#_Toc510519428)

[**Care and Handling** 15](#_Toc510519429)

[**Environmental Enrichment** 15](#_Toc510519430)

[**Special Training** 16](#_Toc510519431)

[**Veterinary Care** 17](#_Toc510519432)

[**Care of Novel Species** 18](#_Toc510519433)

[Section 7: Recommendations for Invertebrates 19](#_Toc510519434)

[**Invertebrate Oversight** 19](#_Toc510519435)

[Section 8: Field Studies 20](#_Toc510519436)

[**Do field studies require IACUC approval?** 20](#_Toc510519437)

[Section 9: Genetically Modified Animals 21](#_Toc510519438)

[**Special Considerations for GMA’s** 21](#_Toc510519439)

[Section 10: Endpoint 21](#_Toc510519440)

[**Euthanasia** 21](#_Toc510519441)

[**Disposal** 22](#_Toc510519442)

[**Giving Animals to Other Researchers** 23](#_Toc510519443)

[**Adoption** 23](#_Toc510519444)

[Section 11: Personal Safety 23](#_Toc510519445)

[**Occupational Health and Safety** 23](#_Toc510519446)

[**Personnel Training** 23](#_Toc510519447)

[Section 12: Emergency Preparedness 24](#_Toc510519448)

[**Disaster Planning** 24](#_Toc510519449)

[Section 13: Non-Compliance 24](#_Toc510519450)

[**Consequences of Non –Compliance** 24](#_Toc510519451)

[Appendix I 25](#_Toc510519452)

[**USDA/AWA (Animals Welfare Act) PAIN/DISTRESS CATEGORIES** 25](#_Toc510519453)

[Glossary 27](#_Toc510519454)

[Index 29](#_Toc510519455)

**Section 1: Overview of the IACUC Process**

## **What is an IACUC?**

IACUC stands for Institutional Animal Care and Use Committee. The need for oversight of animal use in laboratory research has been clear to individuals and advocacy groups for over 120 years. However, prior to the 1960’s animal research policies in the U.S. were strictly the business of the researcher. In 1966, the Animal Welfare Act (AWA) became the first official law regarding the use and care of laboratory animals.[[1]](#footnote-1) The National Institute of Health (NIH) as well as the Public Health Service (PHS) began to create policies around the use of research animals in the 1970’s. It was from these policies that guidelines for local committees were first made.[[2]](#footnote-2)

Any institution in the United States using animals for research must have a local IACUC. It is a committee composed of scientists, college faculty, a community member and is overseen by a veterinarian. The IACUC creates institutional policies for animals used in research. These policies are based on federal and state guidelines. The IACUC is responsible for reviewing and approving / denying proposals for studies involving live animals based on these policies. It also monitors compliance and takes necessary action in the event of non-compliance.

NNMC’s IACUC was founded in 2018 as a response to an institutional need, to participate in animal research in laboratories and in the field. NNMC’s IACUC is meant to support primary investigators (PI), students and the institution in maintaining the highest ethical standards as we participate in a diverse array of research.

## **How does the IACUC process work?**

To apply for IACUC approval the PI must complete an animal use form. This form can be found at <https://nnmc.edu/home/facultystaff-gateway/IACUC>. Once the form is completed, it must be submitted by email to [IACUC@nnmc.edu](mailto:IACUC@nnmc.edu). PI’s can expect to hear the IACUC’s decision within 1-2 months.

If your application is rejected the PI is encouraged to revise and resubmit. Any questions about the process can be addressed by emailing [IACUC@nnmc.edu](mailto:IACUC@nnmc.edu) or by contacting the IACUC chairperson.

Once a protocol is approved, if any animal facilities are required, they may be set up and will be subject to a visual inspection biannually. The PI is required to supply documentation of animal numbers at that time (see section 5). Every 3 years the PI must resubmit their protocol for approval. This timeframe is subject to change if required by new state or federal regulations.

## **What research requires IACUC approval?**

At NNMC, all work with vertebrate animals, cephalopods and decapod crustaceans requires IACUC approval. This includes laboratory work, the raising of agricultural animals for observation and/or field studies. You may note that the Animal Welfare Act (AWA) does not cover certain vertebrates; however, it is U.S. Public Health Service (PHS) policy, and the policy of our institution that any research in which live vertebrate animals are used must be approved. As noted above, our institutional policy also includes cephalopods and decapod crustaceans. Work with eggs, embryos or fetuses does not require IACUC approval. However if those eggs, embryos or fetuses are being collected from live parent animals than the work does require IACUC approval.

Research using invertebrates, excluding cephalopods and decapod crustaceans,

such as crickets or cockroaches does not require IACUC approval. This topic is very controversial because there is very little research into pain in invertebrates. While no conclusive evidence exists that invertebrates (with the exception of cephalopods) feel pain, there is also no conclusive evidence that they do not.[[3]](#footnote-3) For this reason, Section 7 of this document addresses the care and use of such organisms. All researchers at our institution are urged to hold themselves to the highest of ethical standards regardless of whether their work requires IACUC approval.

## **Who may apply for IACUC approval?**

The Primary Investigator (PI) must be a faculty member. Students may be co-investigators. The PI takes full responsibility for the research throughout the application, approval and execution on the research. This includes compliance with state and federal regulations or laws, and relevant granting agencies. They are also responsible for complying with IACUC guidelines and decisions. Additionally, the PI is responsible for the training and supervision of researchers, students, and/or staff working under their approved protocol.

## **What animal research is not possible at NNMC?**

Certain activities are not possible in animal research at NNMC. These are mostly due to resource limitations.

Activities which will usually not be approved at NNMC include:

* Surgery
* Extreme pain or distress (Level D or E pain categories as defined by the USDA, see Sec.5)
* Work involving Hazardous biological agents (anything above BSL 2, see Sec. 13)

For information on the requirements for facilities conducting these kind of activities please see Chapter 5 of The Guide for the Care and Use of Laboratory Animals (*The Guide*).[[4]](#footnote-4) If a PI wishes to conduct research that includes the afore mentioned activities than the PI must meet with the IACUC chairperson to discuss steps for submitting a special protocol.

# **Section 2: Justification of Animal Use**

## **Definition of an animal**

An animal, for the purpose of this IACUC is any vertebrate animal, cephalopod or decapod crustacean to be used in research, some field studies, and agriculture. All studies involving animals by this definition must gain IACUC approval. Dead animals do not require IACUC approval unless they are being killed specifically for the research being conducted. Eggs, embryos and fetuses are not animals by this definition (see Section 1).

## **What needs to be considered in the justification of animal use?**

To justify animal research, the PI must present evidence that they have refined, reduced and replaced animals. This process is known as the three R’s.

Refinement refers to creating a protocol that minimizes pain and distress wherever possible. Examples of refinement could include enriching the environment, providing social contact, using less invasive procedures, or more intense monitoring of animals so that intervention is quicker in the event of pain or distress.[[5]](#footnote-5)

Reduction refers to using the least number of animals possible to obtain valid data. Using too few animals means that those animals are harmed without providing useful data. Too many animals also mean that animals could be harmed unnecessarily.[[6]](#footnote-6) Because the appropriate number of animals is very important it is, separate from the justification of animal use on the protocol form. Wherever possible the number of proposed animals should be statistically justified[[7]](#footnote-7). Generally, a power analysis or appropriate citations should be submitted with your proposal. Reduction also includes proof that the animal research is not an unnecessary duplication of previous work regardless of whether that work has been published.[[8]](#footnote-8)

Replacement refers to completely replacing animal models with non-animal options whenever possible, for example mathematical models, computer simulations, or *in vitro* cells.[[9]](#footnote-9) When a non-animal option is not possible, replacement refers to choosing a species that is lower on the phylogenic tree. The term “lower on the phylogenic tree” comes from PHS policy. Unfortunately, PHS does not provide a precise biological definition. Section 12:13 of The IACUC Handbook provides a discussion of replacement alternatives.[[10]](#footnote-10) To lower the rate of unnecessary procedures, the IACUC recommends that investigators use animals that are the most appropriate to answer their research questions. For example, animals that are traditional model systems in a specific field of research, animals that provide useful data in disease research, species specificity, etc.

The Animal Welfare Information Center (AWIC) is a very good resource when searching for alternatives to animal use.[[11]](#footnote-11) Their website includes national and international databases for searching animal alternatives. AWIC can be found at <https://www.nal.usda.gov/awic> . In research including pain level D or E or a field study which removes or alters animals in the wild, then PI’s must provide proof that they have considered all aspects of refinement, reduction, and replacement. This proof must include:

1. The name of the databases or other sources searched.
2. The date the search was performed
3. The range covered by the search
4. The strategy of the search (for instance what terms were used)[[12]](#footnote-12)
5. If painful or distressful procedures are proposed then each of those procedures must be individually searched for alternatives.[[13]](#footnote-13)

If you have a valid reason for not exploring one of these categories, please include a statement to that effect with your proposal.

When justifying the use of animals, the PI is expected to speak to the use of the specific species they are proposing to use. It is not necessary to use the particular strain or stock unless those details pertain to the choice of animal.[[14]](#footnote-14)When the committee is reviewing a proposal, the following questions will be used to judge the use of a particular species:

* Did the PI present evidence that alternatives were explored and ruled out?
* Is the species an established model for the type of research being performed?
* Is the species an appropriate size for the research being conducted?
* Can the species be easily, safely and humanely kept in the proposed facilities?
* Do the PI, assistant researchers and supporting staff have sufficient experience working with this species?[[15]](#footnote-15)

For more information about writing, appraising, or the discussion of justification of animal use please refer to Chapter 12 and 13 of The IACUC Handbook[[16]](#footnote-16) or visit: <https://www.niaid.nih.gov/grants-contracts/research-vertebrate-animals> .

# **Section 3: Scientific Justification**

## **Scientific Justification**

The PI should provide scientific justification for proposed research. It should contain a brief background and the hypothesis of the study, along with how the animals used in the research answer the question(s) of the research. The IACUC should not concern itself with the scientific merit of the study. Rather, the IACUC should examine the hypothesis, sample size, group numbers, and the adequacy of controls, *et cetera* to prevent unnecessary animal use. Per Section 2, animal sample size should be large enough to adequately test the hypothesis and small enough to minimize unnecessary use. If the sample size is too small, the results will be meaningless and animal use is therefore unjustified.

Additionally, there are certain situations that require special considerations. Those include any research involving a Pain and Distress Level D or E (See Appendix I). Any PI who plans on conducting research involving significant pain and distress must meet with the IACUC chairperson and discuss a special application. Please see Chapter 12 of The IACUC Handbook for information about scientific justification in these special circumstances.[[17]](#footnote-17)

# **Section 4: Pain and Distress**

## **What is pain and distress?**

Pain is defined in *The Guide* as, “a complex experience that typically results from stimuli that damage or have the potential to damage tissue; such stimuli prompt withdrawal and evasive action.”[[18]](#footnote-18) As such, it is clear that pain is experienced by many species, not only vertebrates. AWA defines a painful procedure as, “ (one) that would reasonably be expected to cause more than slight or momentary pain or distress in a human to which that procedure is applied.” The example given is a procedure that causes greater pain than an injection or other minor routine procedures.[[19]](#footnote-19)

Stress is an organism’s natural response to a disruption in psychological or physiological homeostasis. This disruption is not necessarily unpleasant as in the example of a dog playing a rigorous game of fetch. The purpose of a stress response is to give an organism the ability to adapt to the situation and return to homeostasis.[[20]](#footnote-20) Distress is defined in *The Guide* as, “an aversive state in which an animal fails to cope or adjust to various stressors with which it is presented.”[[21]](#footnote-21) Stress becomes distress when an organism cannot cope with, “severe, sustained or multiple combined stressors.”[[22]](#footnote-22) Causes of distress and the signs of distress vary greatly between species.[[23]](#footnote-23)

## **How are pain and distress measured?**

The best tool for judging pain and distress in a species is knowledge and experience with that species. That is why one factor the committee considers when analyzing a proposal is whether the PI and other staff have experience and/ or training with the species they propose to work with. Monitoring for pain and distress by qualified individuals is required for animal research.

When predicting pain or distress in a proposal the PI is asked to use the categories laid out by the USDA / AWA. Appendix I shows those categories in full. Categories B and C encompass routine care, holding, breeding and minor procedures such as injections. Categories D and E encompass painful procedures both with and without the use of pain relieving drugs. Categories D and E require special consideration. In these cases, the PI must meet with the IACUC chairperson and discuss submitting a special proposal.

Proposals that do not plan on Category D or E pain or distress still need to have a plan for unexpected outcomes. This is a clear plan for quick action should an animal develop unexpected pain or distress. This plan should include steps to alleviate the discomfort or euthanize the animal. Please see Section 13 of this document for more information on euthanasia.

## **Minimizing Pain and Distress**

Minimizing pain and distress falls under the principle of refinement. This is an important issue when dealing with the ethical use of animals, public concern, and the advancement of scientific knowledge. It is well documented that prolonged stress, either mental or physical, causes a number of adverse, sometimes chronic, effects.[[24]](#footnote-24) These effects can interfere with the collection of accurate data. The IACUC handbook has this to say about the prioritization of reducing pain and distress:

*It is important to acknowledge that an investigator would not be expected to shy away from additional time, effort or cost to attend to details that would make the experiment itself successful and that reluctance to improve animal welfare because of the work involved may inadvertently harm the external validity of the model in addition to posing an ethical dilemma.[[25]](#footnote-25)*

As the PI is creating their protocol, they must consider minimizing pain and distress. This can be as simple as following guidelines for best management practices of their proposed species or it may require looking for alternatives in the AWIC databases. A veterinarian may be necessary either to consult or use regularly for observation, see Section 6.

Once the protocol is approved, monitoring for pain and distress by qualified individuals in necessary. Training or experience with the species being used is very important so that species-specific signs of pain and distress can be recognized and appropriate action can be taken.

If the PI or the committee does not know if a particular, procedure should be considered painful or distressful they are encouraged to review Chapter 16 of the IACUC Handbook.

# **Section 5: Animal Acquisition**

## **Appropriate sources for animal acquisition**

Animals obtained for research at NNMC can be purchased from a licensed dealer / vendor or a farm. They may be acquired from another institution or from an inner institution-breeding colony. They may also be obtained at the end of studies from other researchers at NNMC. In certain circumstances, they may be obtained from the field. Different considerations apply to each of these options.[[26]](#footnote-26) The plan for animal acquisition must be listed in the PI’s proposal and be approved by IACUC.

If animals are being purchased from a dealer or vendor, that dealer / vendor must be licensed in compliance with all federal and state regulations. It is also highly recommended that the Association for Accreditation of Laboratory Care International (AAALAC) accredit them. The dealer / vendor must have a documented program of veterinary care. They must have a history of compliance with state and federal regulations. They must also have a history of providing healthy animals and accurate information (i.e. genotype, breeding etc.) The animals must come with verifications of health status.[[27]](#footnote-27) Animals acquired from farms should follow the same guidelines. Pet shops are not generally acceptable as sources for animals unless justification can be given. For example, proof that the animals are not available from any other credible source.

If the animals are coming from another institution, they must come from a facility with veterinarian oversight. They must come with complete records (for example breeding and genotype) and verification of health status. Animals may not be exposed to multiple painful procedures without special justification and IACUC approval. In considering getting animals from another institution, PI’s should note that IACUC would not approve the use of the same animals in painful or distressing procedures without justification even if some of those procedures were done at another institution.

Animals may be obtained from breeding colonies at NNMC. Breeding colonies are subject to IACUC oversight and must seek approval. They are subject to the same guidelines for care and use as other animal research. When applying to form a breeding colony the PI must prove knowledge and skills in the areas of breeding, pre-natal and post-natal care and colony management for the species to be bred.[[28]](#footnote-28) Researchers obtaining animals from inner institution breeding colonies are subject to the same process of IACUC approval as those acquiring them from other sources.

If animals are to be used in multiple studies at NNMC, each study must seek approval from IACUC. Animals must have a verification of health status prior to the transfer. In addition, as in the case of animals coming from other research facilities, animals may not be used for multiple painful procedures regardless of whether those procedures happened in different studies.

To obtain animals for research from the wild requires special consideration. Applicable international, federal, and regional laws can apply and must be fully researched and considered by the PI. Additionally, both the safety of the field personnel and disruption to the ecosystem must be considered. It is NNMC’s stance to reduce disturbance to ecosystems involved in field research (see section 8).

Various institutions regulate transfer of animals internationally, nationally, and regionally. The PI must follow all applicable laws both in the countries of export and in the United States. This includes, but is not limited to, crossing international borders, issues associated with the transmission of zoonotic and communicable diseases, the movement of endangered animals, and humane transport (see section 6).

## **Who responsible for acquiring animals?**

The PI is responsible for acquiring animals once the source of their animals has been approved by IACUC. Under no circumstances should a PI or lab at NNMC acquire animals prior to IACUC approval.

## **Reporting animal numbers**

After approval, there will be a visual inspection of research involving animals every 6 months. At the time of the inspection, the PI is responsible for providing a document, which states the number of animals they currently have. This includes all animals for which they are responsible. For example, those actively in research, in breeding colonies, or being housed for future use.

The number of animals the PI provides must be as exact as possible. Accuracy will vary depending on species. Mammals and birds must be counted exactly to the individual. Many fish and amphibians present distinct challenges when counting, particularly counting young. If an exact number is not possible, the PI is to provide their best estimate along with a brief written statement about why the animals are not countable.

# **Section 6: Animal Care**

## **Transportation**

Transportation of animals includes their travel to and from NNMC facilities as well as transfer between labs or buildings on campus. Transportation requires knowledge of state and federal regulations, consideration of the health of animals and humans involved in the process, as well as species-specific protocols for the successful minimization of travel stress. *The Guide* states that, “Careful planning for all types of transportation should occur to ensure animal safety and well-being. The process of transportation should provide an appropriate level of animal biosecurity, while minimizing zoonotic risks, protecting against environmental extremes, avoiding overcrowding, providing for the animals’ physical, physiologic, or behavioral needs and comfort, and protecting the animals and personnel from physical trauma.”[[29]](#footnote-29)

Transportation plans, which are specific to the species in the proposal, must be included in a protocol application along with plans for animal care. The PI must show that they are aware of and in compliance with all state and federal regulations regarding their transportation.[[30]](#footnote-30) The PI must also show that they (and their support staff) have knowledge of species-specific best practices for transporting the animals in their research.[[31]](#footnote-31) All efforts must be made to minimize transportation time. All vehicles involved in transportation are technically animal facilities and must adhere to the same guidelines for sanitation, ventilation, crowding etc. Transportation of animals in different phases of life, for instance pregnancy, may require special consideration.

For state and federal guidelines as well as best practices, please refer to Guidelines for the Humane Transportation of Research Animals. A link to this document can be found on the NNMC IACUC webpage.

## **Housing**

Providing appropriate housing is extremely important in the health and well-being of research animals. As was discussed in Section 4, compromising health and well-being may call to question the validity of scientific results in addition to bringing up ethical concerns.

PI’s must show the committee that they have knowledge and background with the species they propose to use and have a well-formulated housing plan. This plan must include the microenvironment, for instance the cage, tank, or paddock an individual is housed in. It must also include the macro-environment, for instance the room or barn that the cage or paddock is in. This plan should be specific to the species and to the type of research being performed. For instance, livestock may be kept in cages, paddocks or on pasture depending on what type of work is being done. NNMC uses the standards that are outlined in Chapter 3 of *The Guide* for designing terrestrial and aquatic housing plans(a link to this document may be found on the NNMC IACUC webpage).[[32]](#footnote-32) Appendix A of *The Guide* gives references for making these general guidelines specific to a given species, strain or circumstance.[[33]](#footnote-33) Specific references for designing and building animal facilities can be found in *The Guide* on page 196.[[34]](#footnote-34)

When considering housing the PI should consider whether animals will be housed individually or in groups. To avoid distress social animals must not be held individually unless it is necessary for the collection of valid data.[[35]](#footnote-35) Animals that are providing for social needs should be included in overall numbers when justifying the number of animals being requested.

## **Care and Handling**

Care and handling, or management includes feeding animals, changing bedding, disinfecting micro and macro environments, providing enrichment and in some cases giving supplements or routine medications. PI’s, student researchers or supportive staff, may perform this care. The PI is responsible for all persons performing care having appropriate knowledge of the species. The PI must ensure that all persons interacting with the animals are trained in best practices for whatever tasks they are performing. This protects the health and wellbeing of the animals as the researchers / staff. For specific guidelines, please use Chapter 3 of *The Guide*.[[36]](#footnote-36)

## **Environmental Enrichment**

Environmental enrichment refers to providing stimulation for animals to engage in species appropriate activities that promote psychological wellbeing. This is an important element in decreasing stress in a variety of animals. The stimuli can be structural like high shelves for cats, material that mice can burrow into or visual barriers that keep animals from being aggravated by neighbors. The stimuli may be resources like chew sticks for rodents, opportunities for foraging, walks for dogs, toys with manipulability for animals such as pigs, dogs, primates, or corvids. In addition to providing cognitive and motor stimulation, environmental enrichment offers animals some control over their immediate environment that can help them cope better with other stressors. [[37]](#footnote-37)

It is important that enrichment be specific to the species. Some animals will respond unfavorably to certain kinds of stimuli. Some animals may also experience stress if changes are sudden or happen too often. For this reason, environmental enrichment must be an approved part of the animal use proposal. The PI must show sufficient knowledge of the behavioral biology of the species they intend to work with so that animals can be monitored, any adverse effects can be identified, and appropriate action can be taken.[[38]](#footnote-38) A veterinarian may decide to remove an individual animal from an enrichment program for the health or well-being of that individual.[[39]](#footnote-39)

There are concerns among some researchers that environmental enrichment can decrease reproducibility and therefore validity of scientific results. However, research suggests that the danger of elevated stress skewing data is far greater than that posed by environmental enrichment.[[40]](#footnote-40) If the environmental enrichment is well-planned and based in a knowledge of the specific species as well as the needs of the study then it can be very beneficial.

## **Special Training**

Before beginning animal work, all personnel who will be in contact with the animals must complete the mandatory IACUC training that is available through NNMC’s IACUC webpage.

In addition, the AWA requires that all people engaged in animal work have proper training in the care and handling of the species they are working with. This training must include:

* The basic needs of each species being handled
* Proper handling and care for those species
* Proper care for expected special circumstances such as post procedural or pregnancy
* Safe and humane methods of cleaning microenvironments
* Safe and humane methods of administering routine medication such as vaccinations
* Information on the behavioral biology of the species and protocol for observed distress
* Proper methods for environmental enrichment
* Protocol for unexpected outcomes, such as pain or distress
* Emergency / Disaster Plan of the facility the research is being done in
* How to identify and report mismanagement of animals[[41]](#footnote-41)

All required training must be completed before an individual begins work with animals. PI’s must provide the plan for training other researchers or staff in their application.

## **Veterinary Care**

All research involving the use of animals must have an adequate veterinary care program. At the core of this program is an attending veterinarian (AV) who oversees all aspects of animal use. The AV has three primary goals. The first and most important is ensuring the well-being of animals in use. The second is to ensure compliance with institutional regulations. The third is to the PI to facilitate research.[[42]](#footnote-42)

The AV must have experience with research animals generally and with the species being used in particular. Usually the AV will attend on a consultant basis, and will often be the same veterinarian who is serving on the IACUC board. However, if a PI wants to use a species that the IACUC board veterinarian does not have experience with then use of a consultant veterinarian capable of making visits to the lab and who has that experience may be necessary.[[43]](#footnote-43),[[44]](#footnote-44)

The AV will use the American College of Laboratory Animal Medicines guidelines for adequate veterinary care (which can be found on the NNMC IACUC webpage), *The Guide’s* section on veterinary care[[45]](#footnote-45) , and Chapter 27 of the IACUC Handbook to govern the adequate veterinary care program. The *Guide* states that this program will encompass:

* Animal Procurement and Transportation
* Preventative Medicine
* Clinical Care and Management
* Surgery
* Pain and Distress
* Anesthesia and analgesia
* Euthanasia[[46]](#footnote-46)

The AV is to make regularly scheduled visits to all facilities with animals overseen by the IACUC. These visits will vary in frequency depending on the number of animals being used and the research being conducted. The frequency will be set by the AV and approved by the IACUC.[[47]](#footnote-47)

The AV will also make recommendations for proper animal care and appropriate environmental enrichment based on *The Guide* and personal experience. It is recommended that the PI schedule a pre-study veterinary consultation with the AV to discuss all aspects of their proposal prior to submission.[[48]](#footnote-48)

## **Care of Novel Species**

The housing protocol that is outlined in *The Guide* can be used as a baseline for most species. In Appendix A of *The Guide,* references can be found for researching the needs of specific species. PI’s must show in their application that they have done adequate research and created a reasonable care plan which includes transportation, housing, handling etc. In the case of species that have special needs the PI must show they can provide for them. They also must comply with any state or federal law or regulation regarding possession of such a species.[[49]](#footnote-49) Questions can be directed to [iacuc@nnmc.edu](mailto:iacuc@nnmc.edu).

# **Section 7: Recommendations for Invertebrates**

## **Invertebrate Oversight**

Within animal research, there are growing debates about invertebrate oversight. There is no federal regulation protecting invertebrates used in research. Institutions vary in whether or not they have policies regarding certain so called, “higher vertebrates” such as cephalopods. At this time, research involving invertebrates, with the exception of cephalopods and decapod crustaceans, does not require IACUC approval. However, there are still important considerations for anyone working with invertebrates.[[50]](#footnote-50)

There has been very little research done in the area of pain and invertebrates. It is clear that the classic assumption that no pain can be felt by animals whose nervous systems differ greatly from humans, was faulty. The lack of clear information should not be used as evidence that they do not feel pain or distress.[[51]](#footnote-51) As with any other animal in use at NNMC, researchers at our institution are urged to take into consideration the reduction of pain and distress when using invertebrates. This can be difficult due to lack of information about pain in invertebrates. For help please read Volume 52, Number 2 of the Institute for Laboratory Animal Research Journal which is devoted to the issue of invertebrates in research.[[52]](#footnote-52)

Researchers at NNMC should consider all aspects of their studies in terms of pain and distress to animals, if there is no proof that they cannot experience pain or distress, Issues such as proper anesthesia for vivisections, overcrowding and other housing concerns, humane endpoint disposal and post procedural pain reduction should all be considered.

# **Section 8: Field Studies**

## **Do field studies require IACUC approval?**

A field study is defined by AWAR as “a study conducted on free-living wild animals in their natural habitat. However, this term excludes any study that involves invasive procedure, harms or materially alters the behavior of an animal under study.” A study that meets this definition, in other words a study that is observing wild animals but in no way interacting with them, does not require IACUC approval.[[53]](#footnote-53)

*The Guide* says, “The Guide does not purport to be a compendium of all information regarding field biology and methods used in wildlife investigations, but the basic principles of humane care and use apply to animals living under natural conditions.” Therefore, studies, which manipulate wild animals in any way, are not distinguished from laboratory studies. These studies require IACUC approval and must meet the same requirements as a laboratory study. In addition, PI’s who wish to perform this work must show knowledge of state, federal and international regulations or permissions. They must also assure the IACUC that the field study will not compromise the health of persons or animals in the study. The PI must also consider any risk involving zoonotic diseases,[[54]](#footnote-54) or the spread of communicable diseases, such as Chytrid Fungus in amphibians.

Disturbance to ecosystems involved in field studies must be kept to a minimum. The PI must declare any anticipated disturbance and show that they have considered alternative, less disturbing strategies. Field studies should be performed hygienically to prevent the spread of known and unknown organisms into non-native ranges. For example, gear, including boats and trailers, used in aquatic habitats is known to spread invasive species and pathogens. As such, gear should be cleaned with appropriate disinfectants before being used in new habitats.

The PI and IACUC may wish to consult a wildlife biologist in addition to the AV.

# **Section 9: Genetically Modified Animals**

## **Special Considerations for GMA’s**

Use of Genetically Modified Animals (GMA) vertebrates are guided by the same standards as any other animal used in research. Their use requires IACUC approval. Use of GMA invertebrates, while not covered by IACUC, should still be guided by principles of humane care.

Additionally, there are a few special considerations for GMA’s. If they are, being bred during an animal study special attention to unexpected outcomes should be observed. Close monitoring of the first generation of a GMA from birth to early adulthood is necessary to ensure that unexpected pain or distress are not caused by novel phenotypes. If unexpected pain or distress is observed it must be addressed and then reported to the IACUC. If unexpected outcomes cause death or require euthanasia to end unexpected pain then a reassessment of animal use numbers may be necessary. This situation requires IACUC renewal. [[55]](#footnote-55)

The IACUC must be assured that there will be no environmental or occupational consequences due to working with GMA’s. *The Guide* provides suggested reading about the responsible use of GMA’s on pages 175-176.[[56]](#footnote-56)

# **Section 10: Endpoint**

## **Euthanasia**

Euthanasia is a method of killing, “in a humane manner that causes rapid unconsciousness or death without pain or distress.”[[57]](#footnote-57) The IACUC may approve euthanasia of animals involved in research during or at the end of a study for tissue or blood samples or for ethical reasons to end severe pain or distress that is untreatable. If the PI is unavailable or if there is a disagreement between the PI and AV then the AV has the authority to euthanize an animal to end pain or distress.[[58]](#footnote-58)

Techniques for euthanasia must be decided based on species, the individual animal’s disposition, the methods of restraint available and the physical space being used.[[59]](#footnote-59) All proposals must include a detailed plan for scheduled euthanasia as well for euthanasia in unexpected situations. Plans should be developed using the AVMA Guidelines for Euthanasia available at the AVMA’s website: <https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx> or on NNMC’s IACUC webpage. The Canadian Council on Animal Care also has a wealth of information on the subject of euthanasia that can be found on their website: <https://www.ccac.ca/en/training/modules/animals-housed-in-vivaria-stream/euthanasia-of-experimental-animals.html> .

According to the IACUC Handbook, besides humane concerns three other categories ought to be considered concerning euthanasia. These are regulatory, human and scientific. Regulatory refers to all euthanasia following policies in *The Guide[[60]](#footnote-60)* as well as state and federal law. Human, refers to all personnel performing euthanasia having proper training. It also refers to minimizing distress for humans witnessing procedures. Lastly, scientific refers to choosing techniques and agents that will interfere the least with the scientific objectives of the study.[[61]](#footnote-61)

## **Disposal**

Disposal methods will depend on the organism as well as the nature of the research. Prior to acquiring animals, a disposal plan must be in place and approved by IACUC. Large animals or animals that are contaminated by research materials will require special consideration. Please refer to local ordinances in the disposal of animal carcasses and/or infectious waste (see, for example, The City of Española Solid Waste

Ordinances: <https://ecode360.com/14541621>). The Veterinary Compliance Assistance organization has disposal resources listed by state that may be helpful, <http://www.vetca.org/lacd/nm-lacd.cfm>.

All disposal plans must comply with city, county, state and federal regulations.

## **Giving Animals to Other Researchers**

Animals that have been used in research may be given to other researchers at NNMC. Those researchers must obtain IACUC approval for the new study and all involved must follow IACUC policy for the acquisition of animals (see section 5) and transporting animals (see section 6).

## **Adoption**

At the end of a study, healthy animals may be adopted out to researchers, students or community members. Adoption policies are the responsibility of the lab. These policies must comply with all city, state and federal regulations. They must include assurance that the animal will be placed with a person competent to care for them and no money is to be exchanged.

# **Section 11: Personal Safety**

## **Occupational Health and Safety**

PI’s must be aware of and follow all applicable state and federal regulations about employee safety and well-being, including OSHA regulations. Safety guidelines must be posted in labs and other working spaces. Check <https://www.osha.gov/Publications/laboratory/OSHA3404laboratory-safety-guidance.pdf> for pertinent regulations.

## **Personnel Training**

1. All persons participating in studies involving animals must complete the IACUC training available through NNMC’s IACUC website.
2. PI’s are responsible for providing and requiring appropriate training to all researchers, students, and staff involved working in their laboratory or field site. This is a necessity in terms of both animal well-being and personnel safety. OSHA provides many trainings that may be appropriate, see <https://www.osha.gov/dte/ecd/course_otiec_search_public.html>. IACUC will require proof that personnel are adequately trained in both personnel and animal safety; for instance, euthanasia, administering medication, care and handling, and transportation all require training to prevent injury to both study organisms and personnel.

# **Section 12: Emergency Preparedness**

## **Disaster Planning**

In the event of a natural disaster, animals may be subject to pain and distress due to the failure of systems such as ventilation, cooling, or heating. The may also suffer if there is a sudden deficit of personnel due to a disaster. Therefore, a disaster plan should be made that takes into account disasters that are likely in our area, for instance, severe winter storms. Taking into account the ability to rescue animals and the potential limitations of our institution, the plan may include rescue, euthanasia or use of back-up systems. Certain animals may be priorities for rescue based on irreplaceability. The PI should identify persons involved in the study who are aware of and trained in what to do for the animals in case of an emergency. These plans need to be approved by IACUC.[[62]](#footnote-62)

# **Section 13: Non-Compliance**

## **Consequences of Non –Compliance**

Research that does not comply with IACUC will be ordered to cease immediately. In severe cases, the animals may be removed or destroyed. Anyone who is unsure about their own research or something they have witnessed may contact [iacuc@nnmc.edu](mailto:iacuc@nnmc.edu).

# **Appendix I**

The following is from the Research and Graduate Studies Department of the Utah State University. It was retrieved from:http://rgs.usu.edu/iacuc/wp-content/uploads/sites/16/2015/07/PainCategory.pdf on February 3, 2018.

## **USDA/AWA (Animals Welfare Act) PAIN/DISTRESS CATEGORIES**

NOTE; Definition of Painful Procedures (AWA)

"As applied to any animal, pain means any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures."

**Category B** - Management Procedures - Animals being held, bred, or conditioned for use in Teaching, Testing, Experiments, Research, or Surgery but not yet used for such purposes.

Examples;

* Animal breeding, pregnancy, parturition, and lactation
* Physical restraint and preventative medical procedures such as vaccination
* Husbandry procedures such as non-stressful transporting animal from one housing location to another

**Category C** - No or minimal painful Procedures - Animals used where no or minimal pain/distress is produced and no pain relieving drugs are used.

Examples;

* Physical or chemical restraint and husbandry procedures, such as applying identification tags, ear notching, tattoos, etc.
* Transporting of animals from one housing location to another over several hours
* Insertion of per-cutaneous catheters
* Positive reinforcement behavioral modification
* Venous Blood Sampling
* Management procedures in agriculture species as listed in the Ag Guide
* Euthanasia alone using AVMA approved methods

**Category D** - Painful procedures with pain relieving drugs - Animals used where pain and distress to the animal is present and in which appropriate local or general anesthesia, analgesic, or tranquilizer drugs are used. See Note

Below.

Examples:

* Approved Euthanasia methods following terminal procedures with anesthesia
* Surgeries with local and or general anesthesia
* Painful or stressful post-operative circumstances with analgesics
* Stressful transport of animals with tranquilizers
* Ocular and skin irritancy testing with local anesthesia

**Category E** - Painful procedures without pain relieving drugs - Animals used where pain and distress to the animal is present and for which the use of appropriate anesthetic, analgesic, or tranquilizer drugs would adversely affect the procedure results, or interpretation of the results. (A justification of the procedures producing pain or

distress and the reasons pain-relieving drugs were not used must be attached.) See Note Below.

Examples:

* Negative reinforcement behavioral experiments
* Use of adjuvants which cause death of tissue resulting in tissue sloughing
* Induction of radiation sickness
* Restraint for long periods of time (days to weeks)
* Death as endpoint study
* Induction of self-mutilation

NOTE: Category D & E experiments present an explicit responsibility on the investigator to search for alternatives such as replacement of a live animal model with non-living systems; refinement of methods which are less painful or distressful and reduction in number of animals used to ensure that animal pain/distress is minimized. For help, see the following website:

AWIC Alternatives and Database Searches: <http://www.nal.usda.gov/awic/alternatives/alternat.htm>

# **Glossary**

**AAALAC,** Association for Assessment and Accreditation of Laboratory Animal Care International. An organization providing voluntary accreditation to institutions, researchers and dealers/vendors. http://www.aaalac.org/

**ACLAM,** The American College of Laboratory Medicine, an organization of veterinarians who set the standards of veterinary care used by the PHS and NIH, <https://grants.nih.gov/grants/olaw/ACLAM_Adequate_Vet_Care.pdf>

**AV,** Attending Veterinarian, this veterinarian, usually the same one that serves on the IACUC board is responsible for the development and oversight of an adequate veterinary care program for all animals used in research at NNMC

**Animal,** for the purpose of this document, any vertebrate animal, cephalopod or decapod crustacean to be used in research including field studies and agriculture. All studies involving animals must gain IACUC approval.

**AWA,** The Animal Welfare Act. The first and only U.S. law regarding the care and use of laboratory animals.

**AWIC,** The Animal Welfare Information Center, mandated by AWA , AWIC provides information, databases and support for researchers to improve animal care. https://www.nal.usda.gov/awic

**Cephalopod,** a mollusk of the class *Cephalopoda*, characterized by their arms, for example octopi and squids

**Distress,** A negative state in which an organisms is unable to cope or adapt and therefore unable to return to homeostasis. Distress may be physical or emotional.

**End Point,** The final stage of a research project.

**Euthanasia,** A method of killing which is quick and humane, causing little or no pain or distress.

**IACUC,** Institutional Animal Care and Use Committee. A committee composed of scientists, community members and veterinarians which oversees all components of its institutions animal research.

**Macroenvironment,** (From *The Guide* pg.42), The immediate physical environment surrounding the animal (i.e., the environment in the primary enclosure such as the cage, pen, or stall).

**Microenvironment,** (From *The Guide* pg.42), The physical environment of the secondary enclosure (e.g., a room, a barn, or an outdoor habitat).

**NIH,** National Institute of Health. The part of the U.S. Dept. of Health and Human Services which, through its Office of Laboratory Animal Welfare, creates policies and monitors for compliance in all PHS conducted or supported animal research. <http://grants2.nih.gov/grants/olaw/>

**Pain,** physical suffering or discomfort often caused by injury or illness

**PHS**, The United States Public Health Service, part of the Department of Health and Human Services. <http://www.usphs.gov/>

**PI,** Primary investigator. This is the researcher who is responsible legally and ethically for a particular animal project. At NNMC all PI’s must be faculty members.

# **Index**

Adoption, 22

amphibians, 11, 19

animal acquisition, 9

animal alternatives, 5

animal carcasses, 22

Animal Welfare Act (AWA), 1, 2

Animal Welfare Information Center, 5, 28

Association for Accreditation of Laboratory Care International, 9

attending veterinarian (AV), 16

AWA, 1, 2, 7, 8, 15, 25, 28

AWIC, 5, 9, 27, 28

Breeding colonies, 10

Care and handling, 14

cephalopods, 2, 18

Disaster Planning, 23

Disposal, 21, 22

distress, 3, 4, 6, 7, 8, 9, 13, 15, 16, 18, 20, 21, 23, 25, 26, 27, 28

duplication of previous work, 4

Eggs, 4

embryos, 2, 4

Emergency Preparedness, 23

Endpoint, 20

Environmental enrichment, 14

Euthanasia, 17, 20, 21, 26, 28

fetuses, 2, 4

Field Studies, 19

field study, 19

fish, 11

Genetically Modified Animals (GMA), 20

Hazardous biological agents, 3

housing, 13, 17, 19, 25

*in vitro*, 5

infectious waste, 22

invasive species, 19

invertebrates, 2, 18, 20

management, 9, 10, 14

medications, 14

Minimizing Pain and Distress, 8

multiple painful procedures, 10, 11

National Institute of Health (NIH), 1

NIH, 1, 28, 29

Non –Compliance, 24

Novel Species, 17

number of animals, 4, 11, 13, 17, 27

Occupational Health and Safety, 22

OSHA, 22, 23

pain, 2, 3, 4, 6, 7, 8, 9, 16, 18, 20, 23, 25, 26, 27, 28

pathogens, 19

PHS, 1, 2, 5, 28, 29

PI, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 19, 20, 21, 22, 23, 29

Preventative Medicine, 17

primary investigators, 1

Public Health Service (PHS), 1, 2

reduction, 5, 18, 19, 27

refinement, 4, 5, 8, 27

replacement, 5, 27

scientific justification, 6

Surgery, 3, 17, 25

Transportation, 12, 17

veterinary care, 9, 16, 28

zoonotic diseases, 19

1. Murthy,S., Silverman, J., & Suckow, M.A.(2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.1. [↑](#footnote-ref-1)
2. Ibid.4. [↑](#footnote-ref-2)
3. Harvey-Clark, C. (2011). IACUC Challenges in Invertebrate Research. *ILAR Journal,* 52 (2), 217. [↑](#footnote-ref-3)
4. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.133. [↑](#footnote-ref-4)
5. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.317. [↑](#footnote-ref-5)
6. Ibid.237. [↑](#footnote-ref-6)
7. Ibid.227. [↑](#footnote-ref-7)
8. Ibid.222. [↑](#footnote-ref-8)
9. Frequently Asked Questions- PHS Policy on Humane Care and Use of Laboratory Animals – Office of Laboratory Animal Welfare. (n.d.). Retrieved February 09, 2018, from https://grants.nih.gov/grants/olaw/faqs.htm#1655 [↑](#footnote-ref-9)
10. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.223. [↑](#footnote-ref-10)
11. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.228. [↑](#footnote-ref-11)
12. Ibid.226. [↑](#footnote-ref-12)
13. Ibid.339. [↑](#footnote-ref-13)
14. Ibid.219. [↑](#footnote-ref-14)
15. Ibid.219. [↑](#footnote-ref-15)
16. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.211. [↑](#footnote-ref-16)
17. Ibid.211. [↑](#footnote-ref-17)
18. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.120. [↑](#footnote-ref-18)
19. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.321. [↑](#footnote-ref-19)
20. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.320. [↑](#footnote-ref-20)
21. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.121. [↑](#footnote-ref-21)
22. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.320. [↑](#footnote-ref-22)
23. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.121 [↑](#footnote-ref-23)
24. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.318. [↑](#footnote-ref-24)
25. Ibid.319. [↑](#footnote-ref-25)
26. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.253. [↑](#footnote-ref-26)
27. Ibid. 253-258. [↑](#footnote-ref-27)
28. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.265. [↑](#footnote-ref-28)
29. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.107. [↑](#footnote-ref-29)
30. *Guidelines for the humane transportation of research animals.* (2006) Washington D.C.: National Academy Press. 11. [↑](#footnote-ref-30)
31. Ibid.33. [↑](#footnote-ref-31)
32. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.41. [↑](#footnote-ref-32)
33. Ibid. 176. [↑](#footnote-ref-33)
34. Ibid.196. [↑](#footnote-ref-34)
35. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.64. [↑](#footnote-ref-35)
36. Ibid.65. [↑](#footnote-ref-36)
37. Ibid. 53. [↑](#footnote-ref-37)
38. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.53. [↑](#footnote-ref-38)
39. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.659. [↑](#footnote-ref-39)
40. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.54. [↑](#footnote-ref-40)
41. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.483. [↑](#footnote-ref-41)
42. Ibid.617. [↑](#footnote-ref-42)
43. Ibid. 618 [↑](#footnote-ref-43)
44. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.131. [↑](#footnote-ref-44)
45. Ibid.131. [↑](#footnote-ref-45)
46. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.131. [↑](#footnote-ref-46)
47. Murthy, S., Silverman, J., & Suckow, M.A. (2014).*The IAUCU Handbook* (3rd ed.). Boca Raton: CRC Press.620. [↑](#footnote-ref-47)
48. Ibid.620. [↑](#footnote-ref-48)
49. Ibid.41. [↑](#footnote-ref-49)
50. Harvey-Clark, C. (2011). IACUC Challenges in Invertebrate Research. *ILAR Journal,* 52 (2),213-220.doi:10.1093/ilar.52.2.213 [↑](#footnote-ref-50)
51. Ibid.217. [↑](#footnote-ref-51)
52. Ibid.213. [↑](#footnote-ref-52)
53. Murthy, S., Silverman, J., &amp; Suckow, M.A. (2014).The IAUCU Handbook (3rd ed.). Boca Raton: CRC Press. Pg. 231. [↑](#footnote-ref-53)
54. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.32. [↑](#footnote-ref-54)
55. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.28. [↑](#footnote-ref-55)
56. Ibid. 175. [↑](#footnote-ref-56)
57. Ibid.123. [↑](#footnote-ref-57)
58. Murthy, S., Silverman, J., &amp; Suckow, M.A. (2014).The IAUCU Handbook (3rd ed.). Boca Raton: CRC. Press.377. [↑](#footnote-ref-58)
59. Ibid. 378 [↑](#footnote-ref-59)
60. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.123. [↑](#footnote-ref-60)
61. Murthy, S., Silverman, J., &amp; Suckow, M.A. (2014).The IAUCU Handbook (3rd ed.). Boca Raton: CRC. Press. 378. [↑](#footnote-ref-61)
62. *Guide for the care and use of laboratory animals.* (2011). Washington, D.C.: National Academies Press.35. [↑](#footnote-ref-62)