PERFORMANCE STANDARD: Tracking of the number of animals used is crucial for ensuring that no more animals are used on a project than were approved by the IACUC. Accurately evaluating animal numbers is central to the mission of the IACUC since animal reduction is one of the committee’s responsibilities. Principal investigators with concerns about animal numbers should contact the IACUC office iacuc@colorado.edu

1. Tracking animal use:
   a. Justify the number of animals in the protocol (See “Guidance for Justifying Animal Numbers” on page 3 for more details).
   b. Once a protocol is approved, the PI or designee will use a spreadsheet or database to track animal use and counts (see step 3).
   c. Before purchasing, breeding, capturing, or transferring animals, the PI or designee will check the # needed against their records of the approved # of animals remaining.
   d. PI or designee will submit an addendum for IACUC review and approval before exceeding the previously approved number.
   e. For animal orders, obtaining animals from an outside collaborator, and transferring animals from one protocol to another, the PI or designee must:
      • Email the order or transfer request to the IACUC office (iacucoffice@colorado.edu) with the number of animals, species, and protocol number prior to placing the order or transferring animals. Animal orders cannot be placed until the IACUC has provided written approval to place the order or transfer the animals. It may take up to 48 hours during normal working days to hear from the IACUC, so make allowance for any purchasing deadlines. The investigator may choose to email the IACUC an electronic record of their animal tracking; approval by the IACUC is still required.
      • Email the number of animals ordered to the facility manager to ensure there is enough space. If it is an unusually large order, notice to facility animals before the order is placed is advised.
      • Notify the IACUC in writing (iacucoffice@colorado.edu) of any animals received in excess of the number ordered and the protocol that the animals will be added to. Once animals are received ensure that any extra animals received are also counted against a protocol (e.g. 10 animals were ordered, but 12 live animals arrive. All extra animals received need to be subtracted from either the protocol under which the animal order was placed or transferred to another protocol having sufficient numbers of animals remaining to cover them, so that all received animals are fully accounted for).
   f. For breeding animals the PI or designee must email the number of animals that are weaned or used each month to the IACUC so that the IACUC office can ensure there are enough animals approved on the protocol.
   g. For field researchers the PI or designee must:
      • Indicate in the annual review form the number of animals captured each year and the number anticipated for capture for the next field season and email this form to the IACUC so that the IACUC office can ensure there are enough animals approved on the protocol.
      • Keep track of the remaining number of animals during the field season and submit an addendum to the IACUC for review and approval before going over the approved number of animals.
• At the end of the field season email an update to the IACUC on the actual number of animals obtained per protocol.

h. Once Animals are received for housing, the facility managers, PI or PI designee will write the protocol number or code for the protocol number on the cage cards to facilitate tracking.
• If transferring animals to another protocol, add the new protocol number to the cage card.
• PI or designee must notify the facility manager in writing before protocol transfers, and include the original protocol number and the receiving protocol number.

i. Submit the annual review form each year before the anniversary of approval. Protocols that will be renewed must be submitted 2-3 months before expiration to ensure the protocol does not lapse (see “4. Annual review form” below on page 3 for details).

2. Accounting for multiple uses of animals:
   a. All procedures including multiple procedures on a single animal must be reviewed and approved by the IACUC.
   • Multiple survival surgeries must be scientifically justified and approved by the IACUC before implementation. Animals with previous survival surgery on one protocol cannot be used for survival surgery on a second protocol unless approved by the IACUC.
   • Animals must be counted once against each protocol if multiple procedures are conducted under different protocols.
   b. If a terminal procedure is conducted under one protocol, and tissue is taken for use on another protocol, the animal is only counted against the first protocol.

3. Counting Procedure:
   a. What must be counted? All animals generated or obtained for research, testing, or teaching must be counted. Animals generated or obtained for research, testing, or teaching are considered ‘used’. Animals are reported as either:
      • Adults, or
      • Embryos/Neonates (See “Definitions of Animal Age” on page 3 for details)
   b. When should you count animals as individuals or as estimated numbers? While this definition may be modified depending upon species, generally:
      • Mature larger-sized species (e.g. frogs, large fish, mice, rabbits, etc.): adults and embryos/neonates are counted as individuals.
      • Smaller-sized community species (e.g., medaka, zebrafish, etc.): adults may be estimated based upon tank size and density.
   c. When should counting occur? Animals are generally counted and applied to the approved protocol at the point of first use for animals bred in the facility (e.g. euthanized, used in a procedure, shipped to other researchers) or weaned, or for ordered animals at the point of receipt or transfer from another protocol. When originally entered, USDA-covered animals should be listed under their approved or intended pain category. USDA-covered species exclude fish, birds, rats and mice of the genus Rattus and Mus bred for research, reptiles and amphibians.
      • How do I count animals purchased from a vendor? Count as ‘used’ all animals that arrive alive from a commercial vendor or other source.
      • How do I count individuals in an in-house breeding colony? All breeding colony animals produced must be counted, even if only a sub-set of those animals were used for actual experimentation. For example, 20 mice are produced from a selected mating, but genotyping revealed only 5 were the right genotype for the project. All 20 mice are counted as ‘used.’ Animals generated within a breeding colony may be reported as ‘Adults’ or ‘Embryos/Neonates,’ depending upon the age of the animal at the time it is was
genotyped (first-use), euthanized (first-use) or engaged in an experimental study (first-use). Any animals purchased as breeders from an outside vendor or received from colleagues are counted against the breeding protocol.

d. **When should you report animals used as Adults?** All animals that are born and reach weaning age should be counted as adults. These animals may or may not have been used for research, testing, or teaching.

e. **When should you report animals used as Embryos/Neonates?**
   - All animals born and euthanized prior to weaning are considered embryos or neonates.
   - These animals may or may not have been used for research, testing, or teaching. All animals produced and/or used for research must be counted, even if only a sub-set of those animals were used for experimentation. For example, extraction of the uterus revealed 8 embryonic pups. Only 3 were needed for the research. All 8 embryonic pups are counted as used.
   - **Early Embryonic Animal (See page 4 for definitions):** While not ‘reportable’ in the same sense as adults or embryos/neonates, the number of early embryonic animals needed are approximated on the protocol application. The reason is so that estimations may be made regarding the number of pregnant females and breeding males required to produce the required number of early embryonic animals.

f. **How do I count animals in a Field Study?** Unless otherwise stated within the protocol, animals should be counted at point of capture, whether they are the specific animal needed for that field assessment or not.

4. **Annual Review Form :**
   a. The annual review form is due at least a month before the anniversary of the protocol approval. The Annual Review Form shall include:
      - The number of Adult animals used
      - The number of Embryo/Neonates used.
      - The number of animals anticipated for use in the next year.

Note: Early embryonic stages are not reported on the Annual Progress Reports.

5. **Verification:**
   a. Before the investigator orders or transfers animals, the IACUC will independently verify that the approved number of animals will not be exceeded.
   b. The IACUC may request review of investigator records.

6. **Holding protocol:** The Institutional Veterinarian has an approved holding protocol for rare situations such as expired protocols or for other situations that warrant transferring to this protocol for daily care only. No experimental procedures can be conducted on the holding protocol. An addendum would still need to be reviewed by the IACUC for this protocol to account for the specific situation.
Guidance for Justifying Animal Numbers:

a. The Animal Welfare Regulations and PHS Policy require justification of the number of animals to be used as well as justification of the rationale and appropriateness of the species.

b. The proposed number of animals to be used must be clearly stated in the justification along with the method of determining those numbers, such as:
   - Required for statistically significant results based on power analysis (tests used or statisticians consulted should be included);
   - Based on scientific literature or past experience (references should be cited); based on results of a pilot study; required by FDA or other federal agency (federal code, regulation or standard, etc., must be cited);
   - Required by international testing requirements (code, regulation, standards, etc., must be cited);
   - Based on the experiments (e.g. n/experiment, variables, etc).
   - Based on the number of students/animal and procedures needed to learn.

c. Breeding Protocols To assist the IACUC in reviewing breeding colony protocols, the following information should be included:
   - Estimated number of breeders needed to produce desired numbers;
   - Estimated number of replacement breeders held (i.e., for rodents, this is usually 2 to 3 x number of breeders used at one time);
   - Estimated number of animals (pre-weaned, weaned, or adult) to be subject to manipulations.

Definition of Animal Age: while no definition will be 100% accurate for all species or all circumstances, the working definitions for the University of Colorado Boulder Animal Care Program includes the following:

a. Early Embryonic Animal: Defined as:
   - Prior to 50% of its normal gestation (mammals), or
   - No swimming motion and no evidence of absorbing the yolk sac (aquatics), or
   - Greater than 7 days prior to anticipated birth from an egg (avian, reptile species).

b. Embryos/Neonates: Defined as:
   - Beyond 50% of its normal gestation (mammals), or
   - Has swimming motion and is absorbing the yolk sac (aquatics), or
   - Is within 7 days of anticipated birth from an egg (avian, reptile species).

c. Adult: Defined as:
   - Those animals which are ‘mature,’
   - Have aged beyond the weaning date (for the species), or
   - Are of an age able to reproduce.
   - Free swimming and no longer dependent upon the yolk sac (aquatics).
REFERENCES:
1. United States Department of Agriculture, 9 CFR Parts 1, 2, and 3.
2. PHS Policy on the Care and Use of Laboratory Animals, OPRR, 1996.
3. 8th Edition: The Guide for the Care & Use of Laboratory Animals
4. OLAW Web Site: http://grants.nih.gov/grants/olaw/
   - “Is it necessary for our IACUC to track the numbers of animals used in the various protocols it has approved to ensure that the numbers do not exceed those that were approved in the proposal review process? If so, could you provide guidance on the best means for accomplishing this?” *Contemporary Topics* 36(2):47-50, 1997
   - “Is the IACUC responsible for tracking animal usage?” Frequently Asked Questions PHS Policy on Humane Care and Use of Laboratory Animals