

Arizona State University
Institutional Animal Care and Use Committee
STANDARD INSTITUTIONAL GUIDELINE

COUNTING ANIMALS FOR CALCULATING ANIMAL USE NUMBERS IN A PROTOCOL

Purpose:

Regulations require that all live vertebrates used in teaching and research be covered by an Institutional Animal Care and Use Committee (IACUC) protocol, and AAALAC International requires accredited institutions, which includes Arizona State University (ASU), to report the number of vertebrates used in the program each year. In most cases, determining the maximum number of animals to be used under a protocol is straight forward. However, this can be less clear when considering breeding colonies or the use of prenatal and perinatal animals. AAALAC is not definitive regarding when during development an animal should be counted – e.g., embryo, fetus, newborn/hatchling, weanling/fledgling). This guideline defines the point of development at which perinatal animals are counted at ASU and provides considerations for research on embryos and fetuses.

Time point for counting individuals of viviparous (e.g., live-bearing) species:

- 1) Animals are not counted until they have been detected after birth.
- 2) To avoid jeopardizing maternal care and possible abandonment or cannibalization by the dam as a result of disturbing her and her recently produced litter, newborn rodent pups do not need to be counted until the next cage change (up to 7 days after birth depending on the timing of the birth relative to the cage change schedule).
- 3) All live pups detected at the time of first count are counted toward the number of animals approved in the protocol even if the animals are not used for an experiment. This enables assessment of the appropriateness of the production volume given the experimental needs.
- 4) The production of offspring not used in experiments should be accounted for when calculating the number of animals needed for a protocol. Production beyond the number to be used in the experiment can be justified for numerous reasons, including undesired genotype, undesired sex, mismatch between timing of birth and timing of the experiment, long-term colony subsistence even when there is an experimental hiatus, and the need for a buffer to ensure adequate numbers of experimental animals are produced.

Time point for counting individuals of oviparous (e.g., egg-laying) species:

- 1) For most egg-laying species, neonates should be counted on the day of hatching.
- 2) For studies that have less than daily observations (e.g., field research), neonates should be counted at first detection.
- 3) For species with very large clutch sizes (>100 eggs) and extremely small hatchlings, which includes most fish fry and amphibian larvae, an estimate of the number of hatchlings is acceptable provided the larvae have not yet been used experimentally. Once used experimentally, an exact number of those used should be reported in addition to the estimated number that hatched.
- 4) For non-experimental fish kept in large raceways or outdoor ponds, where counting the total number of fish, let alone detecting new fry, is impossible, then an estimate of the carrying capacity for that species in the given enclosure type will suffice.

Considerations for the use of embryos or fetuses in experiments:

- 1) Prenatal individuals do not count against the IACUC protocol's approved number of animals, even if used in experiments.
- 2) Even though prenatal individuals are not counted against the number of animals used under a protocol, the protocol must contain information regarding the use of these individuals:
 - a. The protocol must include a justifications for the number of dams that will produce the prenatal individuals, and this number should be based on the number of prenatal individuals needed for the study and the anticipated average litter size of the dams.
 - b. As the sensitivity to pain is uncertain in prenatal individuals and likely depends on multiple factors including stage of development and species, the protocol must include a consideration of pain management for prenatal procedures.
- 3) When the target subjects of an experiments are embryos or fetuses and therefore these individuals are not counted in the number of animals approved for the protocol, there must be processes in place to ensure that any unused pregnant dams do not give birth and any unused eggs do not hatch. The preventative measures need to be described in the protocol. Unintended births or hatchings must be promptly reported to the IACUC and processes revised to avoid recurrence.