# **When animal offspring count towards numbers in protocols**

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| **Title** | When animal offspring count towards “numbers” in protocols | | |
| **Scope** | IACUC and investigators | | |
| **Responsibility** | IACUC, Attending Veterinarian, Animal Managers | | |

## Position statement

The Chapman IACUC has adopted these biological parameters to establish when an animal, by species, meets the criteria for counting against approved numbers in protocols. All animals and their usage count in an ethical sense. However, it's not always practical to count every animal in every circumstance. Animal care providers are still obligated to use good judgement and obtain reasonable numbers.

## Background

One of the responsibilities of the IACUC is to evaluate the number of animals to be used in research, testing, or teaching activities. Investigators are asked (actually obligated) to estimate (prospectively) the amount and adhere to the principles of the 3Rs to limit the numbers to those needed to obtain valid results. PHS policy requires that protocols specify a rationale for the approximate number of animals to be used. This may involve statistical tests, other published data, a pilot study, etc. Occasionally, animals (offspring) are born or hatched, so they are alive, but they do not satisfy the needs of the animal activity. Institutions, per OLAW (see references 1, 3, 4, and 5), have some flexibility in how they track animal usage and this document is meant to provide guidance for Chapman.

From OLAW (3): “The PHS Policy is applicable to live vertebrate animals used in research, research training, and biological testing, and clearly applies to pre-weanling animals.”

The breeding of animals requires a protocol. Offspring born or hatched in a breeding protocol are counted initially as an estimate. Later they may be transferred to an experimental (research) or a teaching (training) protocol, at which time they are counted officially for that purpose.

The purposes and objectives behind animal use protocols are varied; thus, counting animals may be combined, such as for both breeding and experimental studies, or they may be separated into individual protocols. Regardless, investigators are obligated to describe in the protocol the expectations of survival and usage rates during the study. Such information is recognized to come from personal experience and references (published and vendor/supplier). Investigators are also required to reflect on the number of animals used in their annual/continuing reports reviewed by the IACUC.

It is recognized that rodent embryos die *in utero* and are resorbed. These therefore do not count.

Unfertilized avian and fish eggs do not count.

Fertilized unused avian eggs which are not incubated do not count.

As part of its ethical review processes, the IACUC will look at breeding schemes, animal health and mortality reports, and other factors contributing to animal usage numbers. The IACUC may stipulate the process of how to count offspring upon approval of a specific protocol. Examples include when lethal phenotypes are being studied or *in utero* manipulations occur. In consideration of animal welfare and IACUC oversight, the counting of animals used cannot be “hidden” in another protocol. The intent is to understand and consider the numbers of animals being used in the Chapman animal care and use program.

When offspring do count:

* General:
  + Regardless of species, they count when used as embryos before birth or hatching.
  + An estimated count must be made – at species-defined intervals - while the young animals are dependent upon external resources, unable to live on their own. The estimated count, during maturation in breeding circumstances, informs about animal care, health, environmental conditions, and potential genetic vigor before study objectives occur.
  + The count is made official and with accuracy when the offspring live on their own. This count may be the same, greater, or less than the estimated count depending upon circumstances.
  + Animals, regardless of age, count upon receipt by the animal facility after purchase or importation.
  + For observational/wildlife studies, no distinction about offspring is made. All animals count.
* Zebrafish: Only live embryos are applicable to the PHS Policy – and count only after they hatch.
  + Larvae typically hatch from their chorion or eggshell between 3 and 4 days post-fertilization (dpf). Larvae are not counted if they have embryonic development defects and die at, say, 6 dpf.
  + Embryo defects are generally visually obvious, for example, non-spherical embryo formation, lack of hatching 3 dpf, and abnormalities and malformations after hatching like curved spine.
* Mouse:
  + Mouse pups are to be counted by or at the first manipulation (i.e., cage change, genotyping). (Wolff, ref. 4). This estimated count is recorded and compared to numbers in the approved breeding, research, or training protocol as appropriate.
  + Estimated counts can be taken within days of birth to inform the research staff (e.g., in early developmental studies). Care needs to be taken not to disturb the litter because cannibalism can occur. The official count is made later, based upon the discretion of both husbandry and research staff.
  + Pups are usually weaned at 21 days of age. Pups remain as part of the estimated count if they are too weak to be weaned, die, or are cannibalized.
  + For late maturing pups (up to 28 days), counts can be taken at many growth milestones. The official count occurs when weaned.

* Rat:
  + Circumstances for counting rats are similar to mice and therefore apply to them, too.
* Japanese quail:
  + An estimated count is made upon hatching.
  + The official count is made when no external resources (when they no longer require artificial heat for example) are needed for survival.
  + They count officially when used experimentally prior to week 3 or when used for embryology studies (*in ovo*)*.*
* Zebra finch:
  + An estimated count is made upon hatching.
  + The official count is made at the fledgling state (around 3 weeks after hatching)..
  + They count officially when used experimentally prior to week 3 or when used for embryology studies (*in ovo*)*.*

## References

1. University of Iowa. Zebrafish. [https://animal.reserach.uiowa.edu](https://animal.reserach.uiowa.edu/)
2. OLAW. Zebrafish 101 for IACUCs. Webinar broadcasted March 12, 2015
3. “Should you count your mice before they’re weaned?” (2006) Lab Animal 35(1), <https://grants.nih.gov/grants/olaw/references/laba06v35n1.htm>
4. Wolff, Dr. Axel, OLAW, email conversation text (January 24, 2020): “*The PHS Policy and the grant’s Vertebrate Animal Section have always stated that the* ***approximate*** *number of animals used must be listed.  OLAW has further stated that all mouse pups are to be accounted for at the first manipulation (i.e., cage change, genotyping).  Upon birth, the pups are live vertebrate animals used or intended for use in research, testing, training.  These numbers will not be exact, but should be as close as possible.  As for fish, the numbers can be estimated by how many fish per volume of water.  All animals are to be subtracted from the protocol upon use, including ones that were born/hatched but not used (unwanted genotype)*.  [https://olaw.nih.gov/guidance/faqs](https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Folaw.nih.gov%2Fguidance%2Ffaqs&data=02%7C01%7Cbrkennedy%40chapman.edu%7Ca4d1a7d6b8804a852b7308d7a0c4cb22%7C809929af2d2545bf9837089eb9cfbd01%7C1%7C0%7C637154639399283465&sdata=EOZ6Y8MOzq%2BDXC489odmKn%2FTIpGlu8g5OmTUjmwENBg%3D&reserved=0)   See F-2”
5. OLAW FAQ F-2 (accessed January 27, 2020) “Is the IACUC responsible for tracking animal usage?” Although the PHS Policy does not explicitly require a mechanism to track animal usage by investigators, it does require that proposals specify a rationale for the approximate number of animals to be used and be limited to the appropriate number necessary to obtain valid results. This implicitly requires that institutions establish mechanisms to document and monitor numbers of animals acquired and used, including any animals that are euthanatized because they are not needed. Monitoring should not exclude the disposition of animals inadvertently or necessarily produced in excess of the number needed or which do not meet criteria (e.g., genetic) established for the specific study proposal. Institutions have adopted a variety of administrative, electronic, and manual mechanisms to meet institutional needs and PHS Policy requirements.
6. Brown University (April 5, 2019) IACUC Guidelines for Counting Animals Used in Research & Justification for Animal Numbers, <https://www.brown.edu/research/sites/research/files/policies/Guidelines%20for%20Counting.pdf>