

# ACUC Guideline

## The Use and Euthanasia Procedures of Chicken/Avian Embryos

(version: January 19, 2012; ACUC guideline on the use and euthanasia procedures of chicken-avian embryos.doc)  
(approved by the ACUC February 9, 2012)

### Scope:

Avian embryos are not considered live animals under PHS policy. However, there is a consensus in the scientific community that at a certain point in development, avian embryos can experience pain. Because that exact point is not known for chicken embryos, chicken use and euthanasia guidelines differ across institutions. Cal Poly Pomona has chosen to adopt a guideline with the belief that pain occurs on or after gestation day 13, in anticipation of reviewing protocols including them. Therefore, with the adoption of this policy, studies involving the use of chicken embryos on or after gestation day 13 will require submission of a full ACUC *Animal Use Protocol Application* for review and approval.

While other institutions develop their guidelines/policies on the embryo's potential to hatch, Cal Poly Pomona's Guideline is based upon the embryo's potential to feel pain. According to the Canadian Council on Animal Care, "There is emerging evidence that indicates precocial oviparous species are conscious at hatching and during the last few days prior to hatching." This guideline takes this period before hatching into account and pertains to all use of chicken embryos, including research, teaching, or testing.

The use of chicken embryos at gestation day 12 and younger does not require an ACUC *Animal Use Protocol Application*. However, PIs should be prepared for any potentials as outlined in this guideline.

Principal Investigators must include in the protocol their procedures and means for euthanasia, as well as a contingency plan for euthanasia in the event that the embryos hatch or are not euthanized at the intended time. This plan must include a list of trained persons (such as, teaching assistants and research assistants) responsible for euthanasia in the event that the chicken embryos age past their intended date of use. The personnel responsible for euthanasia and the methods of euthanasia in which they have been trained must both be listed in the protocol. The use of carbon dioxide, carbon monoxide and inhalant anesthetics carry risks to personnel and training should be agent specific. Barbiturates are controlled substances and can only be administered by a veterinarian, licensed registered veterinary technician or researcher with a DEA license. The plan can be either included in the protocol in the section about euthanasia (preferred) or be referred to in a separate document such as a standard operating procedure, SOP (but it must be submitted with the protocol).

PIs must recognize that when a chicken embryo hatches, the resulting chick is consequently covered under PHS policy and must be reported and then euthanized according to the *AVMA Guidelines on Euthanasia*. Failure to comply with federal regulations can endanger the funding and conduct of animal research at Cal Poly Pomona.

If embryos of another species (bird, fish, amphibia, etc.) are used, it is the responsibility of the PI to consider that species' developmental rate and develop an appropriate means of euthanasia in compliance with the *AVMA Guidelines on Euthanasia*.

### Euthanasia Procedures and Rationale:

1. Hypothermia is not an acceptable procedure for euthanasia according to the *AVMA Guidelines on Euthanasia*. However, chick embryos younger than embryonic day 13 (E13) are assumed unable to experience pain. Therefore, E13 and younger embryos may be euthanized by hypothermia (typically conducted by placing the eggs in a -20°C freezer or < 4°C for 4 hours) or via other means. Death should be confirmed by decapitation, membrane disruption, or maceration.
2. It should be assumed that chick embryos between E13 – E17 can experience pain and must be euthanized by an acceptable method outlined in the *AVMA Guidelines on Euthanasia*. (Cervical dislocation, decapitation, barbiturate injection, carbon dioxide inhalation, carbon monoxide inhalation, inhalant anesthetic and maceration using specialized equipment).
3. Chick embryos at or older than embryonic day E18 may be euthanized by methods such as carbon dioxide (CO<sub>2</sub>), anesthetic agents, or decapitation. Pulmonary respiration begins by the seventh day of embryo development; therefore, the embryonic environment may have a CO<sub>2</sub> concentration as high as 14%. Considering the natural state of the embryo, CO<sub>2</sub> concentrations for euthanasia should

be high. The *AVMA Guidelines for Euthanasia* reports a CO<sub>2</sub> concentration of 60% to 70% with a 5-minute exposure time as optimal while other sources recommend a 90% CO<sub>2</sub> for at least 20 minutes. *Dry ice is not acceptable as a source for CO<sub>2</sub> euthanasia.*

4. When utilizing chick embryos at day E18 or later, the *ACUC Animal Use Protocol Application* must include methods for humane euthanasia of animals in the event of unexpected hatching. Avian embryos that hatch are live vertebrate animals and are regulated by PHS Policy. The method of euthanasia should be selected and named from the approved methods.
5. According to NIH policies, after euthanasia occurs all content must be disposed of as Medical Pathological Waste.
6. Any deviations from the outlined guidelines and procedures must be reviewed and approved by the ACUC.

#### References:

- Avian Embryo Use. *IACUC Policies*. Case Western Reserve University, July 2008.  
<http://casemed.case.edu/ora/iacuc/policies/Policy%20for%20Use%20of%20Avian%20Embryos.pdf>
- AVMA Guidelines on Euthanasia. AVMA, 2007. [http://www.avma.org/issues/animal\\_welfare/euthanasia.pdf](http://www.avma.org/issues/animal_welfare/euthanasia.pdf)
- CCAC guidelines on: euthanasia of animals used in science. *Canadian Council on Animal Care in Science*. 2010. <http://www.ccac.ca/Documents/Standards/Guidelines/Euthanasia.pdf>
- Guidelines for the Euthanasia of Rodent Feti and Neonates." *ARAC Guidelines*. NIH OACU.  
[http://oacu.od.nih.gov/ARAC/documents/Rodent\\_Euthanasia\\_Pup.pdf](http://oacu.od.nih.gov/ARAC/documents/Rodent_Euthanasia_Pup.pdf)
- Guidelines for Use of Zebrafish in the NIH Intramural Research Program. *ARAC Guidelines*. NIH OACU.  
<http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf>
- Perkins, Jennifer. "Use of Zebrafish Embryos." Message to [IACUC-ADMIN@LIST.MSU.EDU](mailto:IACUC-ADMIN@LIST.MSU.EDU). December 19, 2011. E-mail
- Policy for Use of Avian Embryos. *Animal Care and Use Committee Guidelines*. Florida State University, 2005.  
[http://www.research.fsu.edu/acuc/Policies\\_Guidelines/Avian%20Embryo%20Policy.pdf](http://www.research.fsu.edu/acuc/Policies_Guidelines/Avian%20Embryo%20Policy.pdf)
- Poultry: Reproduction and Incubation. *Mississippi Agriculture and Forestry Exp. Station*. Mississippi State University Extension Service. 2010. [http://msucares.com/poultry/reproductions/poultry\\_chicks\\_embryo.html](http://msucares.com/poultry/reproductions/poultry_chicks_embryo.html)
- The Public Health Service Responds to Commonly Asked Questions. *ILAR News* 33.4 (1991): 68-70. *Office of Laboratory Animal Welfare*. <http://grants.nih.gov/grants/olaw/references/ilar91.htm>
- The Use of Chicken/Avian Embryos. *IACUC Policies and Procedures*. University of Louisville, 2010.  
[https://louisville.edu/research/iacuc/policies/Use%20of%20ChickenAvian%20Embryos.pdf/at\\_download/file](https://louisville.edu/research/iacuc/policies/Use%20of%20ChickenAvian%20Embryos.pdf/at_download/file)
- Wheler, Colette. "Euthanasia of 4 day old chick embryo." Message on the Comparative Medicine List to Kunwar Srivastava. April 6, 2011. E-mail