**Policy for Use of Avian Embryos\***

**Purpose:** To provide guidance on the use of avian embryos in research, teaching, training, and testing. To describe euthanasia procedures for chick embryos in various stages of development as well as to ensure euthanasia procedures are in place for inadvertently hatched chicks.

**Scope:** This policy applies to all avian embryos used in research, teaching, training, or testing at the University of Arkansas - Division of Agriculture.

**Policy:** While avian embryos are not considered live animals by U.S. regulatory agencies, there is a consensus in the scientific community that avian embryos that have attained ~50% incubation have developed a neural tube sufficient for pain perception and demonstrate sustained EEG activity at ≥80%1. This is, in part, due to the fact that certain avian species (chickens, quail, etc.) are precocial and are more advanced morphologically during development than altricial species. Also note, vascular gas exchange begins by the seventh day of embryo development; therefore, the embryonic environment may have a CO2 concentration as high as 14%. Considering the natural state of the embryo, CO2 concentrations for euthanasia should be high. The AVMA Guidelines for Euthanasia reports a CO2 concentration of 60%-70% with a 5-minute exposure time as optimal while other sources recommend a 90% CO2 for at least 20 minutes. Embryos and chicks up to 72 hr. old are resistant to CO2.

Consequently, the University of Arkansas System – Division of Agriculture has adopted the following guidelines:

*Chicken embryos, which hatch in approximately 21 days, are considered the model species for this policy. If other avian species are used, then the guidelines should be adjusted based on relative time to hatching (<85% of expected incubation + hatching period).* *These guidelines are specific for poultry and/or other species produced for food/fiber.*

**Guidelines:**

* Investigators using avian embryos ≤ E17 (or ≤ 80% of expected incubation period), must inform the Ag-IACUC by means of the “*Notice of Intent to Use Avian Embryos*” form (<https://aaesbusinessoffice.uada.edu/agiacuc/Forms>). This serves as a record of avian embryo use for the Ag-IACUC. If embryos will be euthanized prior to 4 days before hatching (i.e. day </=17), the research is not subject to Ag-IACUC review, unless specifically requested by the investigator. **No formal Animal Use Protocol (AUP) need to be filed.**
  + All avian embryos that have been set “incubated” for any duration up through day 17 (E17) must be euthanized by an acceptable method as outlined in the AVMA Guidelines on Euthanasia 2020, Ag-Guide 2020, or Ag-IACUC Policy

* + - Prolonged exposure to CO2 (90% for 20 minutes1), followed by chilling or freezing
    - Freezing (-20oC/-4oF for minimum of 4 hours1,2)
    - Chilling/Cooling (≤ 4oC/40oF for 24hours1,2)
  + Death must be confirmed by breakout of a reasonable subset of embryos (at least 20 or up to 5% of total) prior to incineration (Never leave embryos in a cold incinerator). Additional humane methods of euthanasia may be considered. ***All courses and demonstrations utilizing embryos of any age require an approved animal use protocol (AUP).***
* Studies using embryos within three days of hatching (i.e. day ≥ 18 or ≥ 85% of expected total incubation period for species other than chickens), or using hatchlings, **must be reviewed by the normal Ag-IACUC procedure for animal use.** Investigators must include in the protocol their procedure 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.
  + All avian embryos E18 and greater must be euthanized by an acceptable method as outlined in the AVMA Guidelines on Euthanasia 2020, Ag-Guide 2020, or Ag-IACUC Policy
    - CO21,2
      * Exposure to 90% CO2 for at least 20 minutes, followed by either chilling or freezing. Death must be confirmed1 in a reasonable subset of embryos (at least 20 or up to 5%) prior to incineration (Never leave embryos into a cold incinerator).
    - Chilling (≤ 4oC/40oF for 24hours) 1,2
  + Pipped eggs and/or Hatchlings (Death must be confirmed)
    - CO2 (90% for 20 minutes)1,2
    - Cervical Dislocation1
    - Decapitation1,2
* Avian embryos that hatch (intentionally or unintentionally) are live vertebrate animals; therefore, any project in which avian embryos may hatch must have an approved AUP on file. Investigators are asked to describe their methods for humane euthanasia of hatchlings.

**Note:** Any deviations from these outlined guidelines and procedures must be reviewed and approved by the Ag-IACUC.

1AVMA Guidelines on Euthanasia 2020

(<https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>)

2Ag-Guide, 4th ed. 2020 (<https://www.adsa.org/Portals/0/SiteContent/docs/AgGuide4th/Ag_Guide_4th_ed.pdf>)

*\*All use of warm-blooded animals in research, teaching, training, and testing with the Division of Agriculture is regulated by the University of Arkansas System – Division of Agriculture Institution Animal Care and Use Committee (Ag-IACUC).*