Institutional Animal Care and Use Committee

San Francisco State University

**Policy for Use of Live Embryonated Eggs of Egg-Laying Vertebrate Species**

**(“Intent to Use Embryos” Form)**

All use of vertebrate animals in research, teaching and testing is regulated by the Institutional Animal Care and Use Committee (IACUC). Federal policy requires institutions to have policies and procedures in place that address the care or euthanasia of live embryonated eggs of avian or other egg-laying vertebrate species which hatch unexpectedly.

Principal Investigators (PIs) who already have an active protocol should submit a modification to the existing protocol to include the use of live embryonated eggs. If the PI does not have an active protocol, they must complete the “Intent to Use Embryos” form, to ensure that appropriate plans are in place to address the care and euthanasia of animals that may hatch unexpectedly. (In this case, CITI training is not necessary.) The form will be reviewed by HAP staff and the Attending Veterinarian (AV). The PI must obtain clearance from HAP staff before engaging in the proposed activities.

The use of offspring of avian and other egg-laying vertebrate species *after* hatching, including zebrafish larvae 4 days post-fertilization (dpf) and chicken embryos after 16 days of incubation, must receive prior IACUC review and approval via submission of an animal use protocol.

**For avian embryos specifically:**

Avian embryos are not considered live animals by U.S. regulatory agencies. Nonetheless, there is consensus in the scientific community that avian embryos greater than two-thirds of the way to hatching can experience pain. If avian embryos hatch, intentionally or unintentionally, they are live vertebrate animals and are regulated by the IACUC. Consequently, the SFSU IACUC has adopted the following guidelines. Chick embryos are considered the model species. If other avian species are used, then the guidelines should be adjusted based on relative time to hatching.

1. Research involving avian embryos that will be sacrificed prior to 3 days before hatching does not require IACUC review, as these are not considered to be live vertebrate animals, though the “Intent to Use Embryos” form must be registered. On the other hand, the IACUC does require submission of a complete animal protocol for projects utilizing pre-hatched avian embryos at or after 80% of the mean incubation period.
2. Chick embryos younger than embryonic day 15 (E15) are assumed to be unable to experience pain. It is recommended that E14 or younger embryos be euthanized by hypothermia, typically by placing the eggs in a -20°C freezer.
3. Chick embryos from E15 to E18 can experience pain and should be euthanized by decapitation or other rapid and humane method.
4. Embryos E19 and older must be euthanized by humane methods such as CO2, anesthetic agents, or decapitation. It should be noted that embryos are resistant to CO2. If this method is chosen, the embryos must be exposed to 90% CO2 for at least 20 min. Dry ice is unacceptable as a source of CO2 for euthanasia.
5. The IACUC recognizes that inadvertent hatching may occur. Investigators are asked to describe their methods for humane euthanasia of hatchlings and to provide documentation of training in the use of this euthanasia.

These guidelines are based on recommendations of ILAR, the NIH intramural recommendations for rodent neonates, and the AVMA Panel on Euthanasia.

**Intent to Use Embryos (form to be completed in IRBManager)**

**IACUC Registration Number**

**Protocol Title**

**PI Name**

**SFSU Faculty Member (yes/no)**

**Names of students, staff, and/or other faculty who will be involved in the study**

**State the purpose of the activity and describe procedures.**

**State the name of the species being used.**

**State the building and room number where the embryo use will occur.**

**State the length of average incubation time until hatching.**

**State the age of embryos at planned use.**

**State the age at which embryos (used and unused) will be discarded.**

**Describe the method of euthanasia for embryos and provide documentation of training in this method of euthanasia.**

*(See AVMA Guidelines for the Euthanasia of Animals (2020)* or consult Attending Veterinarian)

**If an embryo inadvertently hatches, the PI is required to notify the IACUC within 3 days of hatching. In addition:**

1. **who will handle the animal?**
2. **what method of humane euthanasia will be used? Provide documentation of training in this method of euthanasia.**

*(See AVMA Guidelines for the Euthanasia of Animals (2020)* or consult Attending Veterinarian)

**Applicant’s Assurance. Please confirm that you agree with the following:**

**(Assurance is the same as for all animal protocols.)**