I. Purpose: This policy applies to all avian embryos used in teaching and research at Brown University. The intent of this policy is 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.

II. Background: Avian embryos are not considered live animals by U.S. regulatory agencies and many universities do not regulate their use in research\(^1\). Nonetheless, 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. Also, if avian embryos hatch, intentionally or unintentionally, they are live vertebrate animals and thus, are regulated by the IACUC.

Consequently, the Brown University IACUC has adopted the following guidelines. These guidelines were developed based on recommendations of the Institute for Laboratory Animal Research (ILAR)\(^2\) and the AVMA Guidelines for the Euthanasia of Animals: 2013 edition\(^3\). Chicken embryos, which hatch in approximately 21 days, are considered the model species. If other avian species are used, then the guidelines should be adjusted based on relative time to hatching.

III. Guidelines

Investigators using avian embryos must inform the IACUC by means of the “Notice of Intent to Use Avian Embryos” form below. This form serves as a record of avian embryo use for the IACUC. If embryos will be sacrificed prior to 3 days before hatching (i.e. day ≤18), the research is not subject to IACUC review, unless specifically requested by the investigator. Studies using embryos within three days of hatching (i.e. day ≥ 19), or using hatchlings, must be reviewed by the normal IACUC procedure for vertebrate animals.

Chick embryos younger than embryonic day 10 (E10) are assumed to be unable to experience pain. It is recommended that E10 or younger embryos be euthanized by...
hypothermia, typically by placing the eggs in a −20°C freezer for a minimum of 4 hours.

Chick embryos from E11 to E18 may perceive pain and therefore should be euthanized by rapid decapitation. Additional humane methods of euthanasia may be considered3.

Embryos E19 and older must be euthanized by CO₂, decapitation or prolonged exposure to anesthetic agents through the air cell. Avian embryos are resistant to CO₂. Therefore, embryonated eggs must be exposed to 90% CO₂ for a minimum of 20 minutes. Dry ice is unacceptable as a source of CO₂ for euthanasia.

The IACUC recognizes that inadvertent hatching may occur. Investigators are asked to describe their methods for humane euthanasia of hatchlings.

Notice of Intent to Use Avian Embryos

Please submit completed form to ACF Office (BMC 222) or send to animal_care@brown.edu.

Project Title:
Principal Investigator or Course Instructor:
Department:
Email:
Phone:

Avian Embryo Use Summary

1. Avian Species to be Used.
   (Specify all species, typical incubation for each, and incubation at planned use)

<table>
<thead>
<tr>
<th>Species</th>
<th>Length of Normal Incubation</th>
<th>Embryo Age(s) at Planned Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>21 days</td>
<td></td>
</tr>
<tr>
<td>Other - Specify</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   (Note: Add or delete rows as necessary)

2. Building and room number where avian embryo use will occur

3. Method of euthanasia of embryos < 50% incubation (<10 days for chickens)
   - Not applicable. Embryos will be used after 50% incubation

4. Method of euthanasia of embryos > 50% incubation (>11 days for chickens)
   (Specify for all species, in the event planned use is delayed for some reason)

5. Procedure for euthanasia of inadvertently hatched chicks
   (See AVMA Guidelines for the Euthanasia of Animals: 2013 and/or consult veterinarians)

Investigator Assurance

I have read the IACUC "Policy for Use of Avian Embryos" and agree to abide by it.

Signature ___________________________  Date ___________________________