1.0 Policy Purpose

The purpose of this policy is to describe euthanasia procedures for chick embryos in various stages of development and to ensure euthanasia procedures are in place for inadvertently hatched chicks.

2.0 To Whom the Policy Applies

This policy applies to all individuals using avian embryos in teaching and research at Brown University under an IACUC-approved animal use protocol.

3.0 Policy Statement

Avian embryos are not considered live animals by United States regulatory agencies and many universities do not regulate their use in research. Nonetheless, there is a consensus in the scientific community that avian embryos that have attained > 80% 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) and the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 edition. 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.

3.1 Guidelines

Investigators using avian embryos must inform the IACUC by means of the “Notice of Intent to Use Avian Embryos” form (see section 7.3). This form serves as a record of avian embryo use for the IACUC. If embryos will be sacrificed prior to three days before hatching (i.e., day ≤ 18 for chicken embryos), 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 for chicken embryos), or using hatchlings, must be reviewed by the IACUC in accordance with its procedures for all vertebrate animals.

Chicken eggs < 80% incubation (i.e., younger than embryonic day 16 (E16)) must be destroyed by prolonged exposure (> 20 minutes) to CO2, cooling (< 4°C for 4 hours), or by freezing (-20°C).
Chicken embryos **E16 and older** should be treated like avian neonates and must be euthanized by prolonged exposure (>20 minutes) to CO2, anesthetic overdose, or decapitation. 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.

Additional humane methods of euthanasia may be considered after consultation with a Brown veterinarian.

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 and cannot be used as a source of CO2 for euthanasia.

The IACUC recognizes that inadvertent hatching may occur. As such, Principal Investigators are asked to describe their methods for humane euthanasia of hatchlings in IACUC animal use protocols.

**4.0 Definitions:** N/A

**5.0 Responsibilities**

All individuals to whom this policy applies are responsible for becoming familiar with and following this policy. University supervisors are responsible for promoting the understanding of this policy and for taking appropriate steps to help ensure compliance with it.

**6.0 Consequences for Violating this Policy**

Violation of this policy may be considered a serious event of noncompliance that is reportable to the IACUC, funding and accrediting agencies, as well as other regulatory agencies. Violation of this policy is a serious matter that may adversely affect both the ability to perform animal work and acquire funding sources.

Failure to comply with this and related policies is subject to disciplinary action, up to and including suspension without pay, or termination of employment or association with the University, in accordance with applicable (e.g., staff, faculty, student) disciplinary procedures.

**7.0 Related Information**

Brown University is a community in which employees are encouraged to share workplace concerns with University leadership. Additionally, Brown’s Anonymous Reporting Hotline allows anonymous and confidential reporting on matters of concern online or by phone (877-318-9184).

The following information complements and supplements this document. The information is intended to help explain this policy and is not an all-inclusive list of policies, procedures, laws and requirements.
7.1 Related Policies: N/A
7.2 Related Procedures: N/A
7.3 Related Forms:
   • Avian Embryo Use Form
7.4 Frequently Asked Questions (FAQs): N/A
7.5 Other Related Information: References

8.0 Policy Owner and Contact

8.1 Policy Owners: Vice President for Research
8.2 Policy Approved by: Vice President for Research
8.3 Subject Matter Contact: Brown University Animal Research Compliance
   • Telephone: 401-863-3050
   • Email: IACUC@Brown.edu

9.0 Policy History

9.1 Policy Effective Date: February 5, 2016
9.2 Policy Last Reviewed: February 1, 2021
9.3 Policy Update/Review Summary: This policy is not new; it was converted to the University's new policy template and re-reviewed by the IACUC at its convened meetings on July 10, 2020 and December 4, 2020. Prior version of this policy was:
   • IACUC Policy for Use of Avian Embryos, date of IACUC review and approval: January 4, 2019.