

GUIDE ON THE USE OF AVIAN, INCLUDING POULTRY, EMBRYOS

1. Policy statement

The NACLAR Guidelines cover all live vertebrates; including fish, amphibians, reptiles, birds and non-human mammals, as well as cephalopods. As a general guide, when the embryos and foetuses of these animals are at the developmental stage where they begin to feel pain, they become covered by the NACLAR Guidelines. NACLAR does not prescribe this exact day or stage of development. The Principal Investigator (PI) and the Institutional Animal Care and Use Committee (IACUC) are responsible for developing policies in line with current best practices and scientific literature.

Eggs must be destroyed before hatching, unless hatching is a requirement of the experiment. The IACUC must approve the arrangements made for hatchlings.

These guidelines were developed based on the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 edition.

2. Definitions

Avian, including poultry, embryos refer to embryos from all bird species.

3. Scope

This applies to all NUS personnel who use avian and poultry embryos for scientific purposes.

4. Policy

The use of vertebrate animals for research, teaching and testing (scientific purposes) is regulated by the Animal and Birds Act – Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004.

However, if eggs used for scientific purposes hatch intentionally or unintentionally, they become live vertebrate animals and their use are subject to oversight by the IACUC. There is also increasing scientific evidence that chick embryos greater than two thirds of the way to hatching can experience pain. The following guidelines address chicken embryos and should be adjusted for other avian species according to hatching time.

- a. Researchers using chick embryos must inform the IACUC by means of the “Notice of Intent – Use of Avian Embryos for Scientific Purposes” form (Appendix B). If the embryos will be euthanized before embryonic day 16 (ED16), the research will not subject to IACUC review, unless specifically requested by the researcher. If they are euthanized at ED16 or older, the research is subject to IACUC review.
- b. The IACUC recognizes that inadvertent hatching may occur. In such cases only AVMA approved euthanasia methods may be used and researchers are asked to describe their method for humane euthanasia of hatchlings.

AVIAN:

Avian embryo that have attained > 80% incubation should be euthanized by similar methods used in avian neonates such as anesthetic overdose, decapitation, or prolonged (> 20 minutes) exposure to CO₂.

Eggs at < 80% incubation may be destroyed by prolonged exposure (> 20 minutes) to CO₂, cooling (<

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4°C for 4 hours), or freezing. Freezing method in a -20°C freezer.

POULTRY:

Embryonated poultry eggs after 80% incubation (e.g. chicken eggs $\geq$ E16) may be destroyed by prolonged exposure (20 minutes) to CO₂.

Embryonated poultry eggs before 80% incubation (e.g. chicken eggs $<$ E16) may be destroyed by cooling (4 hours at 40°F) or freezing.

5. Procedures and Documentation

1. Appendix A: AVMA Guidelines for euthanasia of poultry and avian embryos.
2. Appendix B: Notice of Intent – Use of Avian, including poultry, Embryos for Scientific Purposes.

6. References

1. [Animals and Birds Act \(Chapter 7\) - Animals and Birds \(Care and Use of Animals for Scientific Purposes\) Rules 2004](#)
2. [Guidelines on the Care and Use of Animals for Scientific Purposes. National Advisory Committee for Laboratory Animal Research. 2022](#)
3. IACUC website <http://nus.edu.sg/research/iacuc>
4. AVMA Guidelines for the Euthanasia of Animals: 2020 edition
<https://www.avma.org/sites/default/files/2020-02/Guidelines-on-Euthanasia-2020.pdf>

Appendix A

From AVMA Guidelines for the Euthanasia of Animals: 2020 edition.

For POULTRY

From page 78, S3.4.4 Embryos and Neonates

The following methods are acceptable with conditions for euthanasia of embryos or neonates. Embryonated eggs may be destroyed by prolonged exposure (20 minutes) to CO₂ or before 80% of incubation, cooling (4 hours at 40°F), or freezing. In some cases inhaled anesthetics can be administered through the air cell at the large end of the egg. Egg addling can also be used. Embryos that have been exposed can be decapitated.

Note: for information on maceration, please refer to

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

For AVIAN

The following comments and recommendations pertain to pet, aviary, falconry, racing, research, and zoo birds.

From page 82, S5.3 Eggs, Embryos, and Neonates

Bird embryos that have attained > 80% incubation demonstrate EEG activity that is sustained, with increases in amplitude suggesting the potential for pain perception in conscious embryos; therefore they should be euthanized by similar methods used in avian neonates such as anesthetic overdose, decapitation, or prolonged (> 20 minutes) exposure to CO₂. Eggs at < 80% incubation may be destroyed by prolonged exposure (> 20 minutes) to CO₂, cooling (< 4°C for 4 hours), or freezing. Because research is still evolving and there are species-specific differences in development, euthanasia of embryos should be performed based on the best available data and with attention to assuring, as best as possible, that conscious suffering does not occur.

Appendix B

Notice of Intent Use of Embryos for Scientific Purposes

Instructions

1. This form is for the use of embryos obtained from oviparous animals. Examples of applicable embryos include:
 - (a) zebrafish embryos up to 5 days post fertilization and medaka embryos up to 3 days post hatching (please read the IACUC [Guidelines on the Use of Zebrafish and Medaka Embryos/Larvae](#), and
 - (b) avian embryos up to embryonic day 16 (please read the IACUC [Guidelines on the use of Avian Embryos](#)).

If the research extends beyond these times, or involves the use of embryos obtained from viviparous animals, please submit the “Use of animals for research” form.

2. Please provide all the information required and contact IACUC Office tel: 6516 2861 or email: dprbox33@nus.edu.sg if you need assistance in completing this form.
3. Please email the completed form to dprbox33@nus.edu.sg. The **Principal Investigator must make the email submission**. A signed hard copy is not necessary.
4. Unless embryos are sourced from an IACUC protocol, a notice of intent may be valid for up to 4 years. Please email dprbox33@nus.edu.sg if you wish to renew.

Notice of Intent Use of Avian, including Poultry, Embryos for Scientific Purposes
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For IACUC Office Use Only		
Notice of Intent No.	Date Received	Date Acknowledgement sent to PI

1. Proposed *length of study:

Starting Date:		*Ending Date:	
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**Note that where embryos are obtained from animals in an IACUC protocol, the 'Ending Date' cannot be beyond the protocol expiration date.*

2. Principal Investigator:

Name:	
Academic Title:	Phone No:
Department:	Email:

3. List the animal species/strains of embryo:

4. Source of embryos:

(a) ☐ Commercial source. Provide name of supplier:

(b) ☐ Other sources. Please describe:

5. Office of Risk Management and Compliance (ORMC)

Under ORMC's safety and health procedures, all projects must undergo a risk assessment to determine the appropriate medical surveillance, vaccinations and personnel protective equipment (PPE) against potential health hazard to be provided to members involved in the project.

(a) Have you provided the details of the use of the embryos to ORMC for Risk assessment?

☐ Yes. ORMC Risk Assessment approved.

☐ No. Please submit and be reminded that ORMC risk assessment approval is required before your work can commence.

☐ N.A. Please explain:

(b) Does the use of embryos also involve the use of human cells/tissues?

- ☐ No.
☐ Yes. Please answer the following questions:

(i) Have you provided the details of using human cells/tissues in your risk assessment?

- ☐ Yes.
☐ No. Please submit.

(ii) Have you informed IRB for the use of human cells/tissue?

- ☐ Yes. IRB approval obtained.
☐ No. Please submit. Please be reminded that IRB approval is required before your work can commence.

(c) Bio-safety level (BSL) of the project:

- ☐ BSL1 ☐ BSL2 ☐ BSL3

6. What is the goal/specific aim of this project?

7. Provide a description on how the embryos will be used.

8. Where will the embryos be used?

Location:

Building Number	Building Level and Room Number
<input type="text"/>	<input type="text"/>

9. What is the age of embryo when they are euthanized and what is the method of euthanasia?

10. Provide describe the procedures for *humane euthanasia if hatching occurs inadvertently:

*Note that methods described must comply in AVMA Guidelines for the Euthanasia of Animals (current edition)
<https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf>

11. Declaration: I have read and agree to abide with the

☐ **IACUC Guidelines on the use of Avian Embryos**

<https://inetapps.nus.edu.sg/iacuc/documents/policies/Guidelines%20on%20use%20of%20avian%20embryos.pdf>

☐ **IACUC Guidelines on the Use of Zebrafish and Medaka Embryos / Larvae**

<https://inetapps.nus.edu.sg/iacuc/documents/policies/Guidelines%20on%20use%20of%20zebrafish%20and%20medaka%20embryo-larvae.pdf>

Signature : _____

Name of PI : _____

Date : _____

Document History

1. Created
2. **P301.01.** Revised: Unknown
3. **P301.02.** Revised: Updated policy with AVMA Euthanasia Guidelines 2020, added Appendices A and B and embryo age changed from 18days to 16days for IACUC review.
4. **P301.03.** Reviewed to:
 1. ensure policies are in compliance with the NAELAR Guidelines 2022 and the updated Animals and Birds (Care and Use of Animals for Scientific Purposes) Rules 2004; and
 2. up-to-date with current practicesChanges made:
 1. under section 1 to add "The NAELAR Guidelines cover all live vertebrates unless hatching is a requirement of the experiment. The IACUC must approve the arrangements made for hatchlings."; and
 2. undersection 4 to remove "Avian embryos are not considered 'live vertebrate animals' under the Act."; and
 3. under section 6, updated to "Guidelines on the Care and Use of Animals for Scientific Purposes. National Advisory Committee for Laboratory Animal Research. 2022"

Author

Dr Foo Tet Wei, John
Unknown
IACUC
Dr Chew Li Li
IACUC

IACUC approval date

Unknown
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3 January 2023