**APPLICATION FOR THE USE OF EXPERIMENTAL FISH UNDER THE CARE OR SUPERVISION OF THE**

**BIOLOGICAL RESOURCE CENTRE (BRC), ARES**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| For IACUC Secretariat Use Only | | | | | |
| IACUC # |  | Submission date | Click to enter a date. | Approval date | Click to enter a date. |
| IACUC Chair |  | | Approval duration | Start Date to End Date | |

## Please complete ALL SECTIONS clearly in soft-copy, stating N/A if appropriate, and use the boxed spaces and check boxes provided. Please consult with [IACUC Secretariat](mailto:iacuc@brc.a-star.edu.sg) if you need help in completing any part of this form. Email this application with all supporting documents as soft copy to the IACUC Secretariat. Signature of Principal Investigator would only be required in the final revised copy. Incomplete forms will be returned to you AND WILL cauSE delays in processing OF APPLICATION.

**Please note –**

The Animal & Veterinary Service (AVS) Rules and the National Advisory Committee for Laboratory Animal Research (NACLAR) require the following information to be completed and submitted for review by the Institutional Animal Care and Use Committee (IACUC).

The IACUC office will appoint a reviewer to review your application, to ensure that the care and use of laboratory animals at the BRC are scientifically, technically and humanely appropriate. During the review process, the reviewer may contact you from time to time for clarifications. It is important that you can respond to the clarification in timely manner, in order to get your application presented to the committee and stands a good chance of approval without delay.

The application should be based on each individual project rather than laboratory-based. IACUC strongly discourage multiple disparate projects in a single application.

The IACUC will endeavour to consider and approve applications within 4-6 weeks. However, due to the high volume of applications received, this may not always be possible for the more complex protocols. Therefore, clarity of submission and inclusion of all supporting documents will greatly assist the IACUC in its consideration of your application.

**FORMAL OFFICIAL Approval from the IACUC OFFICE is required beforE commencing animal experiments.**

IACUC Email: [iacuc@brc.a-star.edu.sg](mailto:iacuc@brc.a-star.edu.sg)

**SECTION 1: ADMINISTRATION**

|  |  |
| --- | --- |
| **Title of Project:** |  |

**I.**

**II. Type of Project:**

**2A.**  New Application  Renewal Application

Please indicate the old IACUC number

**2B.**  Research Project  Service/Training Project

**III. Duration of Project:** Choose the Number of Years

**IV. Funding:** Please indicate the source of the funding for this project:

BMRC  NMRC  NRF  MOH

SingHealth  MOE  NUS  NUH

Others – please specify

**V. Principal Investigator (PI):**

**VA. PI Details**

|  |  |
| --- | --- |
| Name |  |
| Research Institution / Company |  |
| Department |  |
| Address |  |
| Work telephone number |  |
| Hand phone number |  |
| E-mail address |  |
| RCULAC Cert. No (Theory) &  Date/ Year obtained |  |

**VB. PI will be handling and HAVE CONTACT WITH ANIMALS:**

|  |  |
| --- | --- |
| ☐ **YES** | ☐ **NO** |

If “**YES”**, please complete the following table:

|  |  |  |
| --- | --- | --- |
| RCUF/ Training Details (Hands-on) | Vaccination History1 | Experience working with lab animals |
| Cert. No.: Text Field  Date/Year obtained: Text Field | Tetanus  ☐ Yes ☐ No  Hepatitis B  ☐ Yes ☐ No  Tuberculosis  ☐ Yes ☐ No | Where  When  What species  What procedures |

**VI. Information of ALL staff who will have contact with animals:**

***\*Please copy and paste the table row if additional personnel are required.***

|  |  |  |  |
| --- | --- | --- | --- |
| Personnel Details | RCULAC/ RCUF | Vaccination History1 | Experience working with lab animals2 |
| Name: Text Field  RI/Company: Text Field  Contact no.: Text Field  E-mail: Text Field  Role/Responsibility:Text Field | Cert. No. (Theory):  Text Field  Date/Year obtained:  Text Field  Cert. No. (Hands-on):  Text Field  Date/Year obtained:  Text Field | Tetanus  ☐ Yes ☐ No  Hepatitis B  ☐ Yes ☐ No  Tuberculosis  ☐ Yes ☐ No | Where  When  What species  What procedures |
| Name: Text Field  RI/Company: Text Field  Contact no.: Text Field  E-mail: Text Field  Role/Responsibility:Text Field | Cert. No. (Theory):  Text Field  Date/Year obtained:  Text Field  Cert. No. (Hands-on):  Text Field  Date/Year obtained:  Text Field | Tetanus  ☐ Yes ☐ No  Hepatitis B  ☐ Yes ☐ No  Tuberculosis  ☐ Yes ☐ No | Where  When  What species  What procedures |

1 If **“NO”** is checked, please indicate in the box below when (approx. date) staff intends to be vaccinated, or contact the [BRC Safety Office](mailto:safety@brc.a-star.edu.sg) if staff decides to opt out of the vaccination program.

|  |
| --- |
|  |

2 If staff included does not have any experience, please indicate the person who would be providing the training in the box below. Please note that the trainer should have experience working with lab animals and must be included in this protocol.

|  |
| --- |
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**SECTION 2: EXPERIMENTAL PROCEDURES**

**VII. Rationale**

Summarize in lay terms the rationale, purpose and scientific goals of the proposed research. Briefly describe how this proposal will have relevance to human or animal health, advancement of knowledge or benefit to society.

*For renewal of applications, please include a brief description of the findings over the past 3 years and provide justification for the renewal****.***

**Please limit response to 200 words:**

|  |
| --- |
| What is the need for this project?  What is the plan for this project?  How will success be measured in this project? |

**VIII. Experimental Procedures**

Please provide a list of all experimental procedures to be performed on the animals. This list should be written clearly, be easily understood by all IACUC members (including the layperson) and contain **concise sequential information** of all experimental procedures to be performed on animals. Please select the pain or distress classification (C, D or E) for each procedure.

***\*Please copy and paste the table row if additional row is required.***

|  |  |  |
| --- | --- | --- |
|  | List of Procedures | Pain Classifications |
| 1. | *e.g. Mating or Breeding of transgenic fish (C)* | Please select |
| 2. | *e.g. Fin clips for genotyping (D)* | Please select |
| 3. | *e.g. Chemical mutagenesis of adult fish (E)* | Please select |

**NOTE: After the application has been approved, any change and additional procedures MUST be submitted to the IACUC as an amendment and obtain written approval PRIOR to implementation.**

**IX.** **Non-surgical Procedures**

Provide a brief, clear description of each non-surgical procedure listed in section VIII. (Surgical procedures should be described in Section X below). Please avoid using jargon, abbreviations etc. and where possible use language that an intelligent and educated layperson would be expected to understand. You are strongly encouraged to use a diagram or chart if it will help to explain complex experimental designs.

|  |
| --- |
| *Please include details of the following, where applicable:*   * *Injections or inoculations (substance, dose, site, volume)* * *Identification* * *Breeding* * *Any other procedures* |

**NOTE: After an application has been approved, any change and additional procedures MUST be submitted to the IACUC as an amendment and obtain written approval PRIOR to implementation.**

**X. Procedures**

|  |  |
| --- | --- |
| Blood Collection |  |
| Surgical |  |
| Behavioral |  |
| Field Study |  |
| Others Please specify |  |

**Description of the Procedures**:

|  |
| --- |
|  |

TYPE of STUDY

Terminal (Acute): Animal never awakens from initial procedure.

|  |  |
| --- | --- |
| **YES** | **NO** |

Survival (Chronic): Animal awakens and survives for \_\_\_\_ hours/days after initial procedure.

SPECIAL CONSIDERATIONS (Check if applicable)

Multiple Surgeries:

|  |  |
| --- | --- |
| **YES** | **NO** |

If **“YES”**, explain:

|  |
| --- |
|  |

Breeding Colony:

|  |  |
| --- | --- |
| **YES** | **NO** |

If **“YES”**, describe the standard operating procedure for care and breeding as well as the number of adult animals that you plan to generate from any in-house breeding:

|  |
| --- |
|  |

**XI. Proposed Number of Animals for the Study and Pain/Distress Classification Level**

Indicate in the table below, the species, source, and number of animals you will be using during the duration of this project in relevant to the pain categories C, D or \*E:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Animal Species (Common Name) | Source | Pain Classification | Number of Animals used each year | | | 3 year total number of animals |
| Year 1 | Year 2 | Year 3 |
|  |  | Please select |  |  |  |  |
|  |  | Please select |  |  |  |  |

It is reasonable to assume that procedures which cause pain in humans will cause pain/ distress in animals too. Please consider all procedures and complete the Table below.

PAIN CATEGORY (Indicate species and number of animals in each pain category):

**Classification C:**

Animals upon which teaching, research, experiments, or tests will be conducted, involving no pain, distress or use of pain- relieving drugs.

Examples:

* Procedures that are considered to produce minimal, transient, or no pain or distress when performed by competent individuals e.g. using non- motile fish embryos\*\*.
* Euthanasia performed in accordance with the recommendations of the most recent AVMA Panel on Euthanasia, utilizing procedures that produce rapid unconsciousness and subsequent humane death *e.g.* buffered Tricaine overdose in fishes.
* Manual restraint or handling that is no longer than would be required for a simple manipulation or handling.

**Classification D:**

Animals upon which experimental, teaching, research or surgical tests will be performed, involving accompanying pain or distress to the animal – for which appropriate anaesthetic, analgesic or tranquilizing drugs will be given.

Examples:

* Using surgical procedures conducted by trained personnel in accordance with standard veterinary practices, such as biopsies, fin clips.

**Classification E: \***

Animals upon which teaching, experiments, research, surgery or tests will be conducted, involving accompanying pain or distress to the animals and for which the use of appropriate anaesthetics, analgesics or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery or tests.

* E.g. chemical mutagenesis of adult fishes.

\*\*In general, once fish embryos become motile, they will be considered to feel pain and will require anaesthesia during painful procedures.

**\*Please provide scientific justification if animals are to be subjected to Pain/Distress Classification E.**

|  |
| --- |
|  |

**XII. Justification for the Choice of Species:**

|  |
| --- |
|  |

**XIII. Justification for Animal Numbers:**

|  |
| --- |
|  |

**XIV. Use of Multiple species**

Will you be conducting the same experience in more than one species?

|  |  |
| --- | --- |
| **YES** | **NO** |

If **“YES”**, please justify:

|  |
| --- |
|  |

**XV. Housing**

Where do you intend to house the animals?

|  |  |
| --- | --- |
| IMCB Fish Facility |  |
| Other Please specify location, including room number |  |

**A. Single Housing**

Do you require the animals to be singly housed?

Additional enrichment will be provided to singly housed fish unless otherwise justified

|  |  |
| --- | --- |
| **YES** | **NO** |

If **“YES”,** please justify and specify the additional enrichment that will be provided.

|  |
| --- |
|  |

If enrichment would not be given, please provide scientific justification to support.

|  |
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|  |

**XVI. Death as an Endpoint**

Will death be used as an experimental endpoint?

|  |  |
| --- | --- |
| **YES** | **NO** |

If **YES**, please provide scientific justification.Also describe the criteria that will be used to decide when euthanasia will be performed (e.g. when the animal becomes moribund; tumour size exceeds a certain diameter, percentage body weight loss etc.):

|  |  |
| --- | --- |
| Scientific Justification |  |
| Criteria for Euthanasia |  |

**XVII. Euthanasia**

Zebrafish should be euthanized by methods consistent with the AVMA Guidelines for the Euthanasia of Animals: 2022 Edition. The method chosen depends on the researcher, facility, or the intended use of the fish after euthanasia.

**Standard Methods of Euthanasia for adult fish include:**

1. For Zebrafish: Overdose of buffered Tricaine methanesulfonate (MS222, 200-300 mg/l) by prolonged immersion. Fish should be left in the solution for at least 30 minutes following cessation of opercular movement.
2. For Zebrafish where chemicals may alter experimental data, euthanasia by rapid chilling (2° to 4°C) until loss of orientation and operculum movements and subsequent holding times in ice-chilled water, specific to fish size and age is acceptable.

*Zebrafish adults (approx 3.8 cm long) can be rapidly killed (10 to 20 seconds) by immersion in 2° to 4°C (36° to 39°F) water. Adult zebrafish should be exposed for a minimum of 10 minutes and fry 4 to 7 dpf for at least 20 minutes following loss of operculum movement to ensure death*

**Standard Methods of Euthanasia for fish embryo include:**

* 1. *Use of rapid chilling and use of buffered MS 222 alone have been shown to be unreliable euthanasia methods for zebrafish embryos < 3 dpf. To ensure embryonic lethality these methods should be followed with an adjunctive method such as use of dilute sodium or calcium hypochlorite solution at 500 mg/L.*
  2. Immersion in diluted sodium or calcium hypochlorite solution is acceptable for embryos up to 7 days of age
  3. *MS 222 alone is not effective for euthanasia of zebrafish eggs, embryos, or larvae (< 14 days old), and other methods should be used for these life stages*

**Which standard method or other will be utilized?**

|  |
| --- |
|  |

\*Formation of ice crystals on the skin and in tissues of an animal may cause pain or distress. Quick freezing of deeply anesthetized animals is acceptable.

**XVIII. Tissue Sampling**

Will tissues/organs be collected prior to euthanasia?

|  |  |
| --- | --- |
| **YES** | **NO** |

If YES, list all the tissues that you plan to sample:

|  |  |  |
| --- | --- | --- |
| 1. | 2. | 3. |
| 4. | 5. | 6. |

Provide details regarding the anaesthesia you will follow: agent concentration used, and whether the fish will be euthanized *immediately* following tissue sampling.

|  |
| --- |
|  |

**XIX. Will tissue/organs be collected from euthanized animals?**

|  |  |
| --- | --- |
| **YES** | **NO** |

If YES, list all the tissues that you plan to harvest:

|  |  |  |
| --- | --- | --- |
| 1. | 2. | 3. |
| 4. | 5. | 6. |

**XX. Fate of any animals remaining after completion of the study**

Will the animals be euthanized at the end of the study?

|  |  |
| --- | --- |
| **YES** | **NO** |

If **YES**, the method for euthanasia should be the same as in Section XVII.

If **NO**, explain why the animals will not be euthanized and describe what will happen to them:

|  |
| --- |
|  |

**SECTION 3: JUSTIFICATIONS**

**XXI.**  **Avoidance of Duplication of Previous Research**

Describe the database and literature searches that have been carried out to ascertain that the proposed work is not an unnecessary duplication of previous research; include a list of the key words / terms and databases that were used for the searches:

|  |  |
| --- | --- |
| Date of the most recent search to avoid duplication: |  |
| Years covered by search: |  |
| Key words used: |  |
| Search strategy used: |  |
| Result of search, e.g. number of hits: |  |
| Conclusion / justification to proceed: |  |

Based on the information obtained from database and literature searches, does the proposed work duplicate any previous research?

|  |  |
| --- | --- |
| **YES** | **NO** |

If “**YES”,** provide justification for proceeding with the proposed studies:

|  |
| --- |
|  |

**XXII. The 3 R’s**

Provide written assurances that the following 3R’s were given due consideration when planning the project:

**Replacement –** Alternative to using live animals, or the use of invertebrate species. Indicate clearly which non-animal alternatives were considered for the study:

|  |
| --- |
|  |

**Reduction –** Reduction in the number of animals used (e.g. using appropriate statistical methods in the design and analysis of the study), without compromising scientific validity of the project. State clearly the reasons for the numbers of animals you wish to use:

|  |
| --- |
|  |

**Refinement –** Do you intend to use alternative or improved techniques or procedures to minimize potential pain, distress and discomfort of the animals?

|  |
| --- |
|  |

**Use one of the following web-sites to assist / justify your searches:**

1. USDA Animal Welfare Information Centre, Alternatives and Searches: <https://www.nal.usda.gov/services/literature-searching-animal-use-alternatives>
2. Johns Hopkins University Centre for Alternatives to Animal Testing: <http://caat.jhsph.edu/>
3. Fund for the Replacement of Animals in Medical Research (FRAME)

<https://frame.org.uk/resources/searching-for-alternatives/>

1. National Centre for the Replacement Refinement & Reduction of Animals in Research <https://nc3rs.org.uk/who-we-are/3rs#anchor_1>

**Please retain your search data on file for the duration of the project, so that AVS may inspect if they wish to.**

**Database searched - provide details of any database searches relating to the 3R’s:**

|  |  |
| --- | --- |
| Date of the most recent search for 3R’s: |  |
| Years covered by search: |  |
| Key words used (should relate to the project and the 3R’s): |  |
| Search strategy used/ Database searched: |  |
| Conclusion / justification to proceed: |  |

**SECTION 4: SAFETY**

**XXIII. Potentially Hazardous Materials**

Ifthis proposal entails the use of any of the following materials in animals, indicate the nature of the material used and include the necessary authorization documents. Also, indicate the biological safety level at which experiments will be conducted.

|  |  |  |
| --- | --- | --- |
| **Hazard** | **Documentation from Safety Office** | **Nature of hazardous material** |
| Recombinant DNA/ transgenic animal | GMAC approval documentation |  |
| Radioactive material | Bio safety documentation  CRP Licence No. |  |
| Hazardous chemicals  e.g. toxins, carcinogens, teratogens etc. | Bio safety documentation.  Provide copies of relevant SDS sheets. |  |
| Human-derived materials | Bio safety and IRB documentation |  |
| Other biological agents | Bio safety documentation |  |

|  |  |  |  |
| --- | --- | --- | --- |
| Animal Bio Safety Level (ABSL) | ABSL1 | ABSL2 | Comment |

**XXIV. Disposal of Contaminated Materials**

Describe the practices and procedures required for the safe handling and disposal of contaminated animal tissues / carcasses, cages, waste and materials associated with this study. Also, describe methods for removal of radioactive waste and, if applicable, the monitoring of radioactivity**:**

|  |
| --- |
|  |

**If available, Standard Operating Procedures (SOP’s) or Method Statements should be attached, to describe safety procedures.**

**XXV. Staff Health**

It is the responsibility of the Principal Investigator (PI) to inform BRC staff of any risks to staff health.

Will the procedure result in the release of infectious or non-infectious organisms?

|  |  |
| --- | --- |
| **YES** | **NO** |

If **“YES”**, provide information how you intend to handle spills and unused stocks:

|  |
| --- |
|  |

**XXVI**. **Animal Welfare**

It is the responsibility of the Principal Investigator (PI) to inform BRC staff of any risks to animal well-being.In the case of mutant, transgenic or gene KO/KI animals please indicate if the genetic modification will impact the well-being of the modified animal (if known):

|  |  |
| --- | --- |
| **YES** | **NO** |

If **“YES”**, please provide information on how you plan to address this handicap to the animals:

|  |
| --- |
|  |

If **NO,** but the modification subsequently affects the well-being of the animals, it is the responsibility of the investigator to immediately inform the IACUC of any such handicaps and describe how they will be addressed in an annex to this research proposal.

**XXVII. Assurance and Declaration**

PIs – Please read the following and inform all staff involved in this application.

1. It is the PI’s responsibility to make the approved application available and understood by all the staff listed in this application. PI to ensure that the staffs only perform approved animal experimental procedures in this application. Any misconduct will be considered as Non-Compliance which may result in suspension of the application.
2. PIs must provide the IACUC with an annual update report at the end of the year, tabulating the number of animals used under each Pain or Distress classification for the full year. PIs should also apply for a renewal of the project three months before the project’s end date.
3. For this proposed project, I have provided, as accurately as possible, the description of the animal care and use that will be followed. I have also briefed all the staff involved in this project of the contents of the protocol and safety measures to be followed while working on this project. I will update the Biological Resource Centre and IACUC of any changes or termination of the project.
4. I will obtain IACUC approval prior to any changes in the approved protocol.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Click to enter a date

**Signature of the Principal Investigator Date**

|  |
| --- |
| *You have reached the end of this form. Please ensure that you have responded to every question on this application, even if your response is “N/A”.* |