Approval Date _____

1st Renewal Due Date _____

2nd Renewal Due Date _____

Expiration Date _____

(For Office Use Only)

(For Office Use Only)

ANIMAL USE APPLICATION TO THE INSTITUTIONAL ANIMAL CARE & USE COMMITTEE (IACUC) UNIVERSITY OF OREGON

ZEBRAFISH FORM

(Must be typewritten - Diskette available upon request in IACUC, OVSAC and some departmental offices)

Date Submitted _____

I. **TITLE, DATES, AND PERSONNEL**

Title of Research Project _____

_____

Project Dates _____ to _____

Principal Investigator _____ Title/Rank _____

Dept/Institute _____ Ext. _____ Emergency Phone _____

Co-Investigator _____ Title/Rank _____

Dept/Institute _____ Ext. _____ Emergency Phone _____

Senior Technician _____ Title/Rank _____

Dept/Institute _____ Ext. _____ Emergency Phone _____

Other Personnel _____

II. **PURPOSE** (Check if applicable):

___ Research Project ___ Pilot Project ___ Teaching ___ Student Special Project

III. **FUNDING (POTENTIAL AND AWARDED)**

A. **PROTOCOL STATUS**: _____ New _____ Amendment _____ Annual Renewal

B. **FUNDING PROPOSAL TYPE:** ___ New ___ Continuation ___ Renewal ___ Revision

Was this project originally funded or reviewed without the use of animals, or are there new significant changes involving animals which were not previously outlined in the grant proposal? **YES**____ **NO**____. If yes, you will need to send a letter addressed to the program officer of the granting agency detailing the proposed significant changes involving the use of animals. The letter requires a counter-signature by the institution. The Office of Research Services & Administration (ORSA) will sign on behalf of Richard Linton, Vice Provost for Research. You may attach Section IX of the protocol application to satisfy funding agency requirements and IACUC policies and procedures. If you have any questions regarding the requirements for the letter, please contact ORSA, 346-5131, or OVSAC, 346-4957. Please attach a copy of the letter with the appropriate signatures to this application.

C. **EXTRAMURAL FUNDING**: (When more than one funding source is solicited, a single IACUC animal use request may be submitted provided the species, number, and procedures are the same for each grant proposal application.)

Agency _____ Grant # _____

Grant Title _____

Proposed Dates: _____ to _____

Agency _____ Grant # _____

Grant Title _____

Proposed Dates: _____ to _____

D. **INTRAMURAL/NON-COMPETITIVE FUNDING** (See page 2 of instructions)

Funding Source _____

Proposed Dates _____ to _____

E. **COOPERATIVE RESEARCH** (Is this a cooperative research project (are there principal investigators from more than one institution involved)? ____Yes ____No

If yes, please see the UO's Policy to Address Cooperative Research which states that all Principal Investigators engaged in cooperative research with another institution must have approval by the UO's IACUC for all those projects which utilize vertebrate animals. Additionally, if the animals are housed at a cooperating facility, then there must be approval by the cooperating institution's IACUC.

F. **PEER REVIEW OF UNSPONSORED RESEARCH** (1. For teaching applications. 2. Other)

1. Departmental Curriculum Committee Review:

_____ _____ _____

(Department) (Committee Chair) (Date Approved)

2. Other Peer Review:

I have reviewed the attached animal use application and find it to be scientifically valid and consistent with University of Oregon policy.

_____ _____

Signature (Authorized reviewer) Name & Title (typed)

_____ _____

Signature (Authorized reviewer) Name & Title (typed)

IV. ANIMAL REQUIREMENTS AND FACILITIES

Note: Approval does not necessarily guarantee that housing is available. It is the investigator's responsibility to make housing arrangements with the manager of the zebrafish facility.

Common Name Zebrafish Source University of Oregon Zebrafish Facility

Strain Various # of embryos per year _____ # of adults per year* --

(*Adults used for experiments, not for maintaining the breeding colony.)

Housing Location Huestis Zebrafish Facility Lab Room# _____

Will special housing be needed? No If yes, explain: _____

Will animals be held more than 12 hours outside of OVSAC? Yes If yes, explain: Facility housing locations are outside of OVSAC; adults and larvae are kept within these locations. Embryos are generally delivered to the user laboratories where they are kept at constant temperature (25E-33E) until the time of hatching 3-5 days when feeding is first required.

Will facilities outside of University of Oregon campus be used? No If yes, explain: _____

Are animals wild or laboratory-bred? laboratory bred

Is live feed required? yes If yes, explain: Prepared fish food for adults (supplemented 2-3 times/week with other *food sources* such as adult brine shrimp or Drosophila larvae). Small fish, larvae & juveniles, are fed the following as is appropriate to their size: paramecia, rotifers, vinigar eels, daphnia, brine shrimp nauplii & early instar *Drosophila* larvae. Some of these *food sources* may be nutritionally enriched by feeding them vitamins and fatty acids immediately prior to them being fed to the zebrafish.

V. **WHAT IS THE OBJECTIVE OF THIS STUDY? HOW IS THIS STUDY RELEVANT TO HUMAN OR ANIMAL HEALTH, THE ADVANCEMENT OF KNOWLEDGE, OR THE GOOD OF SOCIETY?** (In lay terms)

VI. **DOES THIS STUDY UNNECESSARILY DUPLICATE PREVIOUS EXPERIMENTS? IF SO, EXPLAIN.**

CHECKLIST. (Please give details in Section IX).

VII. **TYPE OF PROJECT** (If necessary, please consult OVSAC for further information concerning pain categories. This section is only a checklist.)

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

C _____
Procedures that are considered to produce minimal, transient, or no pain or distress when performed by competent individuals (e.g. all zebrafish embryos**)

D _____
Procedures or tests involving the administration of appropriate anesthetic, analgesic, or tranquilizer drugs to avoid pain or distress (e.g., fin clips, MS222-tricaine on adults from which sperm & eggs are squeezed, ENU)

E * _____
Procedures or tests that, for scientific validity, are performed involving pain or distress without administration of appropriate anesthetic, analgesic, or tranquilizer drugs (e.g.,chemical mutagenesis of adults: ENU).

***** Please note that when a protocol falls into the "E" category, the investigator must attach a written justification for the procedure and may be requested to attend an IACUC meeting to discuss the proposed research.

**** In practice, tricaine anesthesia is sometimes used to facilitate capture and handling of the fish at any stage after the embryos become motile even though the procedures produce no or minimal discomfort. Even invasive procedures done with embryos could not produce discomfort because the neural centers mediating pain sensation are still undeveloped.**

PROCEDURE

___ Blood Collection _X_ Surgical ___ Non-Surgical ___ Behavioral ___ Field Study _X_ Other (Describe): Care and maintenance of adults; breeding and obtaining gametes and embryos (including parthenogenetic embryos); raising larvae, cryogenic preservation of sperm; strain record-keeping; fin clips, mutagenesis, quarantine and other procedures relating to disease control; and euthanasia

TYPE OF STUDY

_X_ Terminal (Acute): Animal never awakens from initial procedure.
_X_ Survival (Chronic): Animal awakens and survives for
_____ hours/days after initial procedure.

SPECIAL CONSIDERATIONS: (Check if applicable)

___ Multiple surgeries (If yes, explain in Section IX)
___ Restraint device(s) (If yes, explain in Section IX)
___ Neuromuscular blocking agents (If yes, explain in Section IX)
___ Complete Freund's Adjuvant (If yes, include signed copy of the U of O Adjuvant Policy)
_X_ Breeding Colony (If yes, include the standard operating procedure for care and breeding)
___ Food or Water Deprivation (If yes, explain in Section IX)

VIII. **ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS**

Are any hazardous agents including infectious agents, biohazards, carcinogens (ENU for mutagenizing), toxic chemicals, or radioisotopes, gamma rays for mutagenizing used on live animals for this study? ___ Yes ___ No

If hazardous agents are being used, attach a use authorization from the appropriate committee or office.

Authorized by:
Biosafety Committee (Infectious agents and biohazards)? ___ Yes ___ No
Environmental Health & Safety Office (Carcinogens and toxic chemicals)? ___ Yes ___ No
(EHS has reviewed and acknowledges the use of ENUfor zebrafish mutagenesis.)
Radiation Committee (Radioisotopes)? ___ Yes ___ No

NOTE: Since the use of animals in experimentation involving hazardous agents requires special consideration, the procedures and the facilities to be used must be reviewed by both the Office of Environmental Health and Safety and the IACUC. Formal safety programs should be established to assess the hazards, to determine the safeguards needed for their control, and to ensure that the staff is competent, and that the facilities are adequate for the safe conduct of the research (PHS *Guide*).

IX. **PLEASE PROVIDE DETAILED INFORMATION FOR THIS SECTION ON A SEPARATE SHEET**
(see next page)

This application form has been reformatted in order to accommodate the Vertebrate Animal Section of the Research Plan of the Public Health Service Grant Application form, PHS 398. Items 1-5 in the bold print are quoted directly from the PHS Application Packet. The light print is to serve as a guide (check sheet) in preparing your response to meet funding agency and IACUC requirements. This format is applicable for all animal use protocols, even when the funding source is other than PHS.

If PHS is the funding source, please answer the following questions and attach a copy of the Vertebrate Animal Section of the Research Plan. For funding other than PHS, please answer the following questions and attach a copy of all relevant portions of the grant application pertaining to animal care and use.

1. **Provide a detailed description of the proposed use of animals in the work previously outlined in the experimental design and methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.**

   Experimental/Non Surgical Study: Identify procedure and duration of study.
   Behavioral Study: Describe any conditioning, deprivation, or stimulation that might be involved.
   For Surgical, Blood & Tissue Collection, Address:

   - Drugs and/or antigens used — Quantity
   - Route of administration — Frequency
   - Injection sites — Pain associated with procedures

   For Surgical Procedure, Address:

   - Pre-operative care < Methods to prevent dehydration/hypothermia
   - Surgical procedure < Anticipated duration of surgery
   - Multiple surgeries < Anticipated duration of surgeries/type
   - Use of paralyzing drugs < Anticipated duration of study/endpoint/pain
   - Post-operative care < Anticipated nursing care medication & duration

   Field Study: For capture or any invasive procedure

2. **Justify the use of animals, the choice of species, and the numbers used. If the animals are in short supply, costly, or to be used in large numbers, provide additional rationale for their selection and their numbers.**

3. **Provide information on the veterinary care of the animals involved.** (Note: It is not necessary to complete this section for the IACUC. It is only necessary to state that veterinary service is being provided by the Office of Veterinary Services & Animal Care as described in routine facility standard operating procedures or PHS-approved assurance statements.)

4. **Describe procedures of ensuring that discomfort, distress, pain and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices where appropriate to minimize discomfort, distress, pain and injury.**

   Address:

   - Analgesic/anesthetic/tranquilizing drugs
     - Dose
     - Route of administration
     - Frequency
     - Criteria to assess pain/discomfort
   - Describe use of comfortable restraining devices
     - Dimensions and/or type
     - Duration of confinement (continual observation required)
   - Describe any other animal manipulations that may produce pain, discomfort, or anxiety not mentioned previously
   - Describe any physical or psychological impairment of the animal resulting from experimental manipulation (e.g. blindness, loss of motor abilities)
   - Describe the methods used to assess adequate levels of anesthesia
   - Describe indices used to help assess possible signs of pain, distress or discomfort

5. **Describe any euthanasia method to be used and the reasons for its selection. State whether this method is consistent with the recommendations on the panel of euthanasia of the American Veterinary Medical Association available in the OVSAC library and the *Researcher's Handbook*. If not, present a justification for not following the recommendations.**

   A. For chemical or gas euthanasia, please include the agent, dose and route.
   B. For physical euthanasia, please indicate the specific method.

IX. Detailed Information

If PHS is the funding source, please answer the following questions and attach a copy of the Vertebrate Animal Section of the Research Plan. For funding other than PHS, please answer the following questions and attach a copy of all relevant portions of the grant application pertaining to animal care and use.

INTRODUCTION: Standard Description of the Proposed Use of Animals

Species: Zebrafish, *danio rerio*
Sex: Both sexes
Ages: All ages
Number: _____

The zebrafish group has prepared its own detailed user manual that describes these standard procedures. This was done because of the special requirements of the zebrafish, and how they were used, as contrasted with use of other vertebrates at Oregon (particularly birds and mammals), that are covered by OVSAC's Standard Operating Procedures Document. The zebrafish standard operating procedure manual is *The Zebrafish Book* (ed. 3, 1995). The manual has been approved by the IACUC and is currently included within the IACUC packet.

The Zebrafish Standard Operating Procedures covered by this application does cover all procedures, including invasive ones, carried out with embryos, either done within the facility (e.g. DNA injection into early embryonic cells) or in the user laboratories (e.g. cell labelling, microsurgery, laser microablation, cell transplantation, donors for cell & tissue culture). In addition, all usual facility operations are included: care and maintenance of adults, breeding and obtaining gametes and embryos (including parthenogenetic embryos), raising larvae, cryogenic preservation of sperm, fin clips, mutagenesis, strain record keeping, sending fish to and receiving fish from other laboratories, quarantine and other procedures relating to disease control, and euthanasia.

1a. **Briefly summarize the methods to be used in achieving the objectives of your proposal. Please emphasize any procedures not covered by the *The Zebrafish Book* (please give a brief description in the space provided below).**

2a. **Standard justification for the use of animals and choice of species:**

The zebrafish has become widely accepted throughout the world as a particularly useful preparation to analyze how vertebrate development is regulated at the cellular, genetic, and molecular levels. There are a number of reasons for this assessment: (1) the fish are easy to maintain in large numbers and readily reproduce under laboratory conditions; (2) adult fish can be subjected to mutagenesis and mutations can be screened in the first generation by analyzing haploid embryos; (3) the zebrafish embryo has few cells relative to other vertebrates, thus making it a "simple" model for more complex vertebrates such as ourselves; (4) the embryos are optically clear and develop very rapidly and externally (not inside the mother or an eggshell) so that the events involved in the differentiation of tissue, such as the nervous system, can be readily observed; (5) direct access to the developing embryos make it possible to introduce foreign genetic material and to perform cell labelling and other experimental manipulations; and (6) the zebrafish is a small animal so that large numbers, required for genetics, can be kept and studied.

2b. **Are there any other justifications for this project not outlined above? If yes, please list.**

2c. **Justify the number of animals proposed:**

3. **Veterinary Care:**

Veterinary care is provided by the Zebrafish Facility staff; the ZIRC veterinarian, Dr. Jen Matthews; and a consulting fish pathologist, Dr. Mike Kent, as described in routine standard operating procedures. They consult with OVSAC as required.

4a. **Standard Procedures for Alleviation of Pain, Discomfort, Distress, and Injury:**

Most of the procedures on embryos will be done at very early developmental stages before the nervous system has matured. Indeed, the neural crest cells that we study are the *source* of sensory neurons that ultimately develop in these organisms. We feel, therefore, that without the structures necessary to detect pain, embryos at this stage are unlikely to be susceptible to painful stimuli. On the other hand, the developing muscle cells in the embryos twitch spontaneously causing the embryos to move. To prevent such movements, which make observations of cells more difficult, embryos older than 17 hours will be anesthetized in Tricaine, also called MS 222, added to the water. Tricaine is the best anesthesia available for lower (aquatic "cold-blooded") vertebrates. The dosage is age dependent. Anesthesia is administered by immersing the animal in the anesthetic to facilitate handling of the fish, e.g. during procedures to obtain gametes from adults which involves handling of the fish but produces minimal discomfort even if the fish were alert. There is no permanent impairment.

4b. **Which standard procedures outlined above or any others not mentioned will be utilized to ensure minimization of pain, discomfort, distress and injury?**

5a. **Standard method of euthanasia:**

Standard methods of euthanasia include:

1) Immobilization by submersion in ice water immediately followed by cranial concussion and decapitation via an in-sink garbage disposal.

2) Overdose of tricaine methanesulfonate (MS-222, 200-300mg/l) by prolonged immersion. Fish should be left in the solution for at least 10 minutes following cessation of opercular movement.

3) Anesthesia with tricaine methanesulfonate (MS-222, 168mg/l) followed by rapid freezing in liquid nitrogen.

5b. **Which standard method or other will be utilized?**

X. PERSONNEL[1] Please fill out **section A** with the P.I.'s information; complete **section B** by listing each research assistant, student, postdoc, and **primary** lab employee who will be involved in this study.

A. P.I. QUALIFICATIONS AND TRAINING

NAME _____ Campus Phone _____

Position _____ Institute/Department _____ Work Location _____

Credentials/Experience _____

Signature _____ Emergency Phone _____

In accordance with federal regulations, please provide the following information. **Be advised that the P.I. must assure that all persons participating have demonstrated competence for those techniques that they will be performing as part of this study.**

B. LAB PERSONNEL QUALIFICATIONS AND TRAINING
I have read the protocol and understand my responsibilities outlined therein. I have also read the University of Oregon's Animal Care & Use Training Handbook.

Name	Credentials/Experience	Personnel signature	Trained By	Training Required (Y/N)	OHP Review Date

1. NOTE: Federal regulations require that all personnel involved with animal care and use (including antigen preparation for antibody production) be qualified to perform their duties. Those personnel with any live animal contact must also be a part of the University of Oregon's Occupational Health Program. **In order for this animal use application to be approved, the IACUC must have on file training information and qualifications for each individual and documentation of participation in the Occupational Health Program for those individuals with any live animal contact.**

XI. ASSURANCE STATEMENTS

A. **ALTERNATIVES.** The following alternatives must be addressed prior to the use of animals in accordance with Federal policy:

1. Replacement:
   I have considered the use of alternatives to the present species, i.e. the use of other species and/or the use of non-animal models and have found them to be unacceptable. ____ Yes ____ No

2. Reduction:
   I have designed my experimental protocol with careful attention to using the appropriate number of animals and have considered appropriate statistical methods used to reduce the number of animals in this study.
   ____Yes ____ No

3. Refinement:
   I have planned this project to assure that animals are subjected to the minimum amount of pain and distress by the adequate administration of anesthetics, tranquilizers; humane euthanasia; that they receive careful scrutiny of behavioral indices of pain or distress; and that noninvasive imaging technologies are used when appropriate.
   ____Yes ____ No

4. Alternative Methods: (The following is from USDA Policy #12, June 21, 2000)
   Alternatives or alternative methods are generally regarded as those that incorporate some aspect of replacement, reduction, or refinement of animal use in pursuit of the minimization of animal pain and distress consistent with the goals of the research. These include methods that use non-animal systems or less sentient animal species to partially or fully *replace* animals (for example, the use of an in vitro or insect model to replace a mammalian model), methods that *reduce* the number of animals to the minimum required to obtain scientifically valid data, and methods that *refine* animal use by lessening or eliminating pain or distress and, thereby, enhancing animal well-being. Potential alternatives that do not allow the attainment of the goals of the research are not, by definition, alternatives.

   The USDA believes that the performance of a database search remains the most effective and efficient method for demonstrating compliance with the requirement to consider alternatives to painful/distressful procedures. However, in some circumstances (as in highly specialized fields of study), conferences, colloquia, subject expert consultants, or other sources may provide relevant and up-to-date information regarding alternatives in lieu of, or in addition to, a database search. When other sources are the primary means of considering alternatives, the Institutional Animal Care and Use Committee (IACUC) and the inspecting Veterinary Medical Officer should closely scrutinize the results. Sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge of the availability of alternatives in the specific field of study. For example, an immunologist cited as a subject expert may or may not possess expertise concerning alternatives to *in vivo* antibody production.

   When a database search is the primary means of meeting this requirement, the narrative must, at a minimum, include:
   1. The names of the databases search;
   2. The date the search was performed;
   3. The period covered by the search; and
   4. The key words and/or the search strategy used.

   The Animal Welfare Information Center (AWIC) is an information service of the National Agricultural Library specifically established to provide information about alternatives. AWIC offers expertise in formulation of the search strategy and selection of key words and databases, access to unique databases, on- and off-site training of institute personnel in conducting effective alternative searches, and is able to perform no-cost or low-cost electronic database searches. AWIC can be contacted at (301) 504-6212, via e-mail at *awic@nal.usda.gov,* or via its web site at *http://www.nal.usda.gov.awic.* Other excellent resources for assistance with alternative searches are available and may be equally acceptable.

   Regardless of the alternative source(s) used, the written narrative should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives or alternative methods. If a database search or other source identifies a *bona fide* alternative method (one that could be used to accomplish the goals of the animal use proposal), the written narrative should justify why this alternative was not used.

In accordance with the information provided on the preceding page from USDA Policy #12, please provide in the space below a written narrative description of the methods and sources used to determine that alternatives were not available or appropriate for this study.

B. **ASSURANCE FOR THE HUMANE CARE AND USE OF ANIMALS USED FOR TEACHING AND RESEARCH**

1. I agree to abide by the University of Oregon policies for the care and use of animals; the provisions of the NIH *Guide to the Care and Use of Laboratory Animals*; and all federal, state, and local laws and regulations governing the use of animals in research. I understand that emergency veterinary care will be administered to animals showing evidence of pain or illness, in addition to routine veterinary care as prescribed for individual species in the Standard Operating Procedures.

2. I declare that all experiments involving live animals will be performed under my supervision or that of another qualified biomedical scientist listed on this protocol.

3. I certify that all personnel having direct animal contact, including myself, have been trained in humane and scientifically acceptable procedures in animal handling, administration of anesthetics, analgesics, and euthanasia to be used in this project. **I assure that personnel will be allowed adequate time to attend training sessions**.

4. I understand that personnel with live animal contact are required to participate in the Occupational Health and Safety Program.

5. I further declare that the information provided in the accompanying protocol is accurate to the best of my knowledge. Any proposed revisions to the animal care and use data will be promptly forwarded in writing to the IACUC for approval, **including changes in personnel and location**.

6. I am aware that any deviation from an approved protocol or violations of pertinent policies, guidelines or laws could result in immediate suspension of this project.

I have read and understand the assurance statements.

P.I. Signature	Name and Title (typed)
Co-P.I. Signature, if applicable	Name and Title (typed)
Senior Technician, if applicable	Name and Title (typed)

NOTE: Person applying for an animal use approval must be eligible for Principal Investigator status.