Approval Date_____

1st Renewal Due Date_____

2nd Renewal Due 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 and Sponsored Programs (ORSP) will sign on behalf of Steadman Upham, 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 ORSP, 346-5131. 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 _____

Agency _____ Grant # _____
Grant Title _____
Proposed Dates _____ to _____

D. **INTRAMURAL/NON-COMPETITIVE FUNDING** (See page 2 of instructions)
Funding Source _____
Proposed Dates _____ to _____

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.

_____	_____
Name & Title (typed)	Signature (Authorized reviewer)
_____	_____
Name & Title (typed)	Signature (Authorized reviewer)

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 OVSAC.

Common Name Zebrafish embryos, Rabbits, Mice Source University of Oregon Zebrafish Facility
Strain Various # of embryos per year 680,000 # of adult zf per year[1] 5 rabbits, 34 mice
[1] Adults used for experiments, not for maintaining the breeding colony.
Housing Location 16 & 215 Huestis, Quonset Huts 120 & 122 Lab Room # various
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 (25°-33°) 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: Fish "chow" is supplemented on a daily basis with live invertebrates including brine shrimp obtained commercially and one-celled animals, paramecia, that are raised in the facility. Live food is required for maintenance of adults in breeding condition and rearing of larvae.

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 All ZF Embryos**
Procedures that are considered to produce minimal, transient, or no pain or distress when performed by competent individuals.

D _____
Procedures or tests involving the administration of appropriate anesthetic, analgesic, or tranquilizer drugs to avoid pain or distress (e.g., fin clips).

E * zebrafish for ENU
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).

***** 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; sending fish to and receiving fish from other laboratories; fin clips, mutagenesis, quarantine and other procedures relating to disease control; and euthanasia.

TYPE OF STUDY

____ 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 summary)
____ Restraint device(s) (If yes, explain in summary)
____ Neuromuscular blocking agents (If yes, explain in summary)
____ 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 summary)

VIII. **ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS**
Are any hazardous agents including infectious agents, biohazards, carcinogens, toxic chemicals, or radioisotopes used on live animals for this study? __X__ 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)?	__X__ Yes	____ No
Radiation Committee (Radioisotopes)?	__X__ 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*)

ON A SEPARATE SHEET(S) PLEASE PROVIDE THE INFORMATION REQUESTED ON THIS PAGE.

NAME _____ Protocol # _____

PROJECT TITLE _____

IX. **PLEASE PROVIDE DETAILED INFORMATION FOR THIS SECTION**

This application form has been reformatted in order to accommodate Section 6 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 Page 23 of 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 Section 6 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
- Criteria to assess pain/discomfort
- Describe use of comfortable restraining devices
- Dimensions and/or type
- Duration of confinement (continual observation required)
- Describe the methods used to assess adequate levels of anesthesia
- 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 indices used to help assess possible signs of pain, distress or discomfort

IX. Detailed Information

1a. **Standard Description of the Proposed Use of Animals**

Species: Zebrafish, *Brachydanio rerio*
Sex: Both sexes
Ages: Embryos and adults

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 we use them 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* (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 labeling, microsurgery, laser microablation). 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 Zebrafish Book* (please give a brief description in the space provided below.)

2a. Standard Justification for the Use of Animals, Species:

The zebrafish has become widely accepted throughout the world as a particularly useful preparation to analyze how the development of the vertebrate nervous system 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 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 labeling and other experimental perturbations; and (6) the zebrafish is a small animal so that large numbers, required for genetics, can be kept and studied.

2b. **Which specific justification(s) stated above or other justifications not outlined above are there for this project?**

None

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

3. **Veterinary Care:**

Veterinary care is provided by the Zebrafish Aquarium Facility staff, 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 one 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?**

N/A

5a. **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.**

Freezing is the preferred method of euthanasia since the animals show no signs of pain or distress and since drug use is avoided. Zebrafish come from the tropics and appear to have no adaptive behaviors for response to cold temperatures. Fish, within a beaker of water, are placed into a freezer (e.g. the freezer compartment of a standard refrigerator). As the temperature slowly falls, the metabolism of the fish gradually slows. They become quiet and then completely immobile and drop to the bottom of the vessel and are frozen solid.

Alternately, very rapid freezing may be required: e.g. for euthanasia in order to isolate unstable molecules from the fish. The fish is quickly and completely immersed in liquid nitrogen and is frozen solid within seconds.

5b. **Will the standard method or another method be utilized?**

Protocol # _____

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. __X__ 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. __X__ 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.

__X__ Yes ____ No

4. Alternative Methods: (Written response required below.)

What are the alternative research methods that you have explored that do not involve animals? **Please provide, in the space below, a written narrative description** of the methods and sources (e.g. the Animal Welfare Information Center, Medline, etc.) used to determine that alternatives were not available or appropriate for this study. (**NOTE:** If for instruction, address the use of media aides/preserved specimens.)

Protocol # _____

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 substantial animal or animal tissue 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.

Protocol # _____

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. PERSONNEL QUALIFICATIONS AND TRAINING

NAME _____ Campus Phone _____

Position _____ Institute/Department Institute of Neuroscience

Work Location _____ Emergency Phone _____

In accordance with federal regulations, please provide your training, experience, and skills in the animal care, use, and techniques **to be performed in this study**. Be advised that the P.I. must assure that all persons participating have demonstrated competence required to perform the study.

B. LAB PERSONNEL QUALIFICATIONS AND TRAINING

Name	Credentials/Experience	Employee Signature	Trained By	Training Required? (Y/N)
				N
				N
				N
				N
				N
				N
				N

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 significant 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 a separate personnel page for each individual and documentation of participation in the Occupational Health Program for those individuals with significant animal contact.**