Case Western Reserve University
Institutional Animal Care and Use Committee
Protocol Form

Instructions

The form is modular in construction. A Core form (this document) must be completed fully. Attachments may need to be completed depending on your responses to the checklist in Section V of the Core.

Submit the signed Core, the completed Attachments, and any additional documentation to the CWRU IACUC Office, Room WG-77, School of Medicine, Telephone (216) 368-3815, Fax (216) 368-4805, http://iacuc.cwru.edu.

- ✓**Enter your responses in the white cells only.**
- ✓**Submit only completed Attachments.**
- ✓**On some systems, users may need to "control + click" links.**

Rationale

Revisions were made to facilitate form completion and protocol review. Information on IACUC policies are provided in the form at relevant locations. Questions designed to elicit the specific information that reviewers need to understand the science, procedures, outcomes, justifications, and expected clinical condition of the animals have been added.

Your Input

We recognize that a new form, no matter how well designed, is an inconvenience to investigators and reviewers, but expect that the net result will be positive. We are committed to improving the form, and ask that you help us in this task by providing constructive criticism.

The last section of the core document is provided for your comments and criticisms on the forms and the review process.

Table of Contents

CWRU IACUC WG-77 School of Medicine (216) 368-3815	Date:	Species: Protocol Number: Designated or Full Review:

Notice Of Intent To Use Vertebrate Animals

Protocol Version 2.3 Form Date 10/2004

I. Contact Information

Principal Investigator:	**Dr. Jennifer O. Liang**				
Project Title:	**Development and function of the vertebrate nervous system**				
Department:	**Biology**				
Building:	**Millis**	Room:	**126**	Location code:	
E-mail:	**jol@case.edu**				
Phone:	**216-368-5428**	Fax:	**216-368-4672**	Pager:	**216-570-5412**
Animal Emergency Contact*:	**Jennifer Liang**			Phone:	**216-368-5428**
E-mail:	**jol@case.edu**			Pager:	**216-570-5412**
Name of External Funding Agency or Source:	**Mallinckrodt Foundation and March of Dimes**		Or Internal Funding Source†:		
New Submission?			Or Renewal of Protocol Number:		**020075**

* This individual will be contacted when animal morbidity or welfare requires immediate action.

II. Animal Use Summary

List the total number of animals of each species required for the entire protocol: **9,000 adult zebrafish (Danio rerio)**

Type of use: X Research ___ Teaching ___ Other (specify): ___

Check all that apply to the use of animals in this submission

X	Breeding		Survival (Chronic) Study	X	Chemical Mutagenesis
X	Tissue Collection	X	Transgenic/KO Production		Infectious Agents
X	Genetic Mapping		Viral Therapeutics		Surgery
	Nutritional Studies		Protein/DNA Therapeutics		Multiple Surgeries
	Aging		Drug Therapeutics		Inducement of Disease State
	Exercise Physiology		Nude Mouse Implants		Inducement of Stress
	Behavioral Tests		Neuromuscular Blockade		Prolonged Restraint
	Antibody Production		Ascites Production		Tumor Implants
	Terminal (Acute) Study		Irradiation		Cell Implants

Primary Reviewer	Title	Signature	Date
Veterinary Reviewer	Title	Signature	Date
IACUC Executive	Title	Signature	Date

Other (specify):

III. Investigator Assurances

- I agree to abide by the policies of the CWRU Institutional Animal Care and Use Committee (IACUC) and all applicable federal regulations.
- I will adhere to the protocol as described and as modified.
- I will submit any modifications of the protocol to the IACUC for review.
- I will notify the IACUC of changes in the location of the animal research.
- I will assist the IACUC in verifying compliance with the regulations.
- I will notify the IACUC of any unexpected results that affect the welfare of the animals. I will report any unanticipated pain or distress, morbidity or mortality to the attending veterinarian and the IACUC.
- I understand and agree that emergency veterinary care including euthanasia will be administered to animals exhibiting unbearable pain, distress or illness. An effort to contact me or my representative (the animal emergency contact identified on page 1) will be made by the veterinary staff prior to any emergency treatment.
- I declare that all experiments involving live animals will be performed under my supervision or that of another qualified scientist. All other personnel involved in animal use in this project have been or will be trained in proper procedures in animal handling, administration of anesthetics and analgesics, aseptic technique, post-operative monitoring, and euthanasia.
- I declare that the information provided in this application is accurate. If this project is to be funded by extramural source(s), I certify that this application accurately reflects all procedures involving laboratory animal subjects described in the proposal.
- I declare that the studies described here do not unnecessarily duplicate previous work by myself or others.

Do you have a financial interest* in the funding source named that could be perceived as a conflict of interest?		Yes	X	No

**A financial interest is a "significant financial interest" which must be disclosed if income from one company is expected to exceed $10,000 in one year, or represents 5% or more ownership interest (total ownership interest of the faculty member, spouse and dependent children).*

If yes, was this interest reported on the most recent conflict of interest disclosure form?		Yes		No

Is there any possibility that the data collected in this study will be submitted to, or reviewed by, the Food and Drug Administration?		Yes	X	No

For further information about FDA policies go to http://ora.ra.cwru.edu/case_glp.asp or contact Anne Duli at anne.duli@case.edu.

Are the results of the studies in this protocol to be used in any of the following circumstances? Please check all that apply.

New drug application	
New animal drug application	
Research or marketing permit	
Notice of claimed exemption for a new animal drug	
Notice of claimed investigational exemption for a new drug	
A biological product license	
An investigative device exemption	
Permit approval of a medical device	
Product development protocol for a medical device	

Signature of Principal Investigator **Date**

IV. Departmental Assurance:

This research protocol has been evaluated based on the following criteria:

1. Scientific design of the study and adequacy of methods.
2. Scientific value of the study.

Name and Signature of Department Chairman **Date**

V. Justification:

1) What is the objective of these experiments? *Please respond in language that can be understood by a layperson. This means minimal use of technical terms and a brief explanation of any specialized terms which you must use.*

The purpose of this research is to understand the development and function of the vertebrate nervous system. Our research will focus on the pineal organ. In all vertebrates, the pineal organ is the major site of synthesis of melatonin, which acts to regulate circadian behaviors such as the sleep/wake cycle. In lower vertebrates, such as fish, it contains cells that sense light, and a clock that keeps track of the organism's position in the 24 hour daily cycle. Although the pineal organ develops and begins functioning very early during development, very little is known about how pineal development is controlled. In addition, the pathways that control circadian rhythm within the pineal are complex, and their regulation is not well understood. We plan to use genetic and cellular techniques in zebrafish to uncover the mechanisms that govern pineal development and circadian function.

2) How is society likely to benefit, or what new knowledge will be gained from these studies?

The pineal organ is a major source of the sleep hormone melatonin. Because of this, disruption in pineal circadian rhythms in a major cause of human sleep disorders. Understanding the pineal organ and melatonin synthesis will lead to better understanding and treatment for blind people and others with sleep disorders. Further, there are many similarities between the pineal organ and the eye. Therefore, studies on pineal development are likely to give insights into the development of the human eye.

3) Why must this species be used? *Justify the selection of this animal model. The IACUC is mandated to encourage the substitution of species more acceptable to society. Describe the characteristics of the species, stock, strain, or mutant which are important for your investigations.*

Zebrafish (Danio rerio) are ideal for these studies. Zebrafish embryos are clear and develop rapidly, making it possible to observe development in live animals. The adult fish are small and easy to maintain in a laboratory setting, and produce large numbers of progeny. There is a large community of researchers who work with zebrafish and many techniques available to study their nervous system.

4) Briefly summarize the design of the animal experiments. *Include all procedures using animals and manipulations of animals. Give your best estimate of how many animals will undergo each procedure or manipulation described. For complicated experimental designs, a flow chart, diagram, list or table is strongly recommended to help the IACUC understand what is proposed. Additional documents may be attached to the form.*

Natural matings

The majority of fish in the facility are used only for natural matings between single pairs. Natural matings are carried out to obtain embryos for experiments, to maintain fish stocks, and to determine the genotype of the fish (the phenotype of the progeny reveals whether the parent is carrying a mutation). Male/female pairs are moved from their home tank using a fish net into a 1L breeding tank. As some fish can be aggressive, artificial foliage is included as a refuge. Fish are maintained in the mating tank for no more than one day. If fish need to be maintained for a longer time outside the aquatic system, they are changed into a new tank every day. They are returned to their home tank unharmed. To minimize stress, and maximize egg production, fish will not be set up to mate more than once a week. **Animal number:** typically, 10-200 single pair matings will be set up each week. Each natural mating produces between 0-200 eggs.

Mutagenesis

Adult males will be placed in water containing the chemical mutagen N-ethyl-N-nitrosourea (ENU) for three one hour treatments (0.15mM) spread over a week, or for a higher dose (1-2 mM), one time treatment for one hour. Protocols to maximize mutation rate and minimize harm to fish have been developed by other researchers, and their protocols will be closely followed. After treatment, mutagenized fish will be returned to the quarantine rack, and monitored carefully for health. Most fish recover fully from this treatment. However, some can fall ill and die. The fish will be monitored at least twice daily for signs of illness, and treatment (part 10) or interventional euthanasia will be performed as needed. **Animal number:** It is expected that one mutagenesis screen will be carried out during this three year protocol, which will require the mutagenesis of approximately 20 adult males.

In vitro fertilization

It is sometimes necessary to fertilize eggs in vitro. In these cases, adult males and females will be anesthetized to immobilize them, and then gentle pressure will be applied to release sperm or eggs, which will then be used to generate fertilized eggs in a petri dish. Fish are returned to their home tank unharmed. Sperm or eggs will not be harvested from any fish more than once a month. **Animal number:** Between 10-200 adult fish will be used for in vitro fertilization each year.

Fin clip

The tail fins of anesthetized adult fish will be clipped using a scissors for the purpose of making DNA. There does not appear to be any long term effects from this procedure, and the fins regenerate over time. **Animal number:** Between 10-200 adult fish will be fin clipped each year.

Transgenesis

To generate transgenic zebrafish lines, wild type one cell stage zebrafish embryos will be injected with DNA at the one cell stage with a nitrogen-driven picoinjector. Constructs injected will be composed of various promoters driving the expression of a reporter protein, typically the gene encoding Green Fluorescent Protein. GFP and other reporter proteins have no effect on zebrafish health, and transgenic fish are indistinguishable from wild type fish. **Animal number:** Generating a transgenic line requires injection of 100-500 embryos with the transgene. These embryos are then grown to adulthood, and their ability to pass on the transgene to their progeny is assessed by natural matings (see above).

mRNA-mediated rescue

Some zebrafish mutants can be rescued to viability by injection of the missing mRNA at the one cell stage using a nitrogen-driven picoinjector. Injections are carried out as described in protocol 7. Injected embryos are indistinguishable from wild type, and grow into fertile healthy adults. **Animal number:** Approximately 600 embryos will be injected each year to maintain the stocks.

mRNA injection

To determine the function of specific proteins in vivo, they will be overexpressed by injecting the corresponding mRNA into embryos at the one-sixteen cell stage using a nitrogen driven picoinjector. The embryos are then raised for a short period of time (6 hours-3 days), and then fixed and processed for whole mount in situ hybridization. **Animal number:** Approximately 200 embryos are injected for each experiment. Between 0 and 2000 embryos will be injected with mRNA each year.

Morpholino injection

Morpholinos are antisense oligonucleotides that bind to RNA in vivo to block either protein translation or splicing. This is our principle method of assessing the function of proteins in development and circadian function. Morpholinos targeting specific mRNAs are injected into zebrafish embryos at the one cell stage using a nitrogen driven picoinjector. Embryos are then raised for a few hours to a few days, fixed, and analyzed using molecular probes. **Animal number:** Approximately 200-500 embryos, or the progeny of 1-12 single pair matings of adult fish, are injected in each experiment. Approximately 20,000 embryos will be injected each year.

Whole mount in situ hybridization

Whole mount in situ hybridization will be used to assay gene expression in embryos, larva, and in adult brains. Embryos and larva are fixed in 4% paraformaldehyde, dehydrated in methanol, and then processed for in situ using established methods. For in situ on adult brains, adult fish are euthanized (see below), the brains are dissected, and then fixed and processed as with the embryos and larva. **Animal number:** Approximately 100-500 embryos are used for a single in situ experiment. Approximately 20,000 embryos, and 100 adult fish will processed for in situ hybridization each year. Some of the embryos will be the same as those injected with mRNA or morpholinos (see above).

In vitro fertilization

In rare cases, in vitro fertilization will be used instead of natural matings. For this procedure, adult fish are anesthetized, and their abdomen is then gently squeezed to release eggs or sperm onto the surface of the body. The eggs and sperm are placed together in a small petri dish. The adult fish are placed into a small isolation tank so that they can recover from the anesthesia. **Animal number:** Approximately, 50 fish each year will be used for in vitro fertilization.

5) Justify the number of animals. *The IACUC is mandated to minimize the number of animals used. Include a statistical justification, if possible. Alternatively, a listing of experimental procedures and the estimated number of animals required for each may be submitted. Prior experience is not adequate justification in itself. Tools for power analysis and other statistical methods for estimating sample size are available at* http://iacuc.cwru.edu/resources/onlineresources.html#statistics.

Because we are studying genetic pathways, it is necessary for us to maintain dozens of different mutant lines that lack specific genes. We also carry several transgenic lines of fish, which express a fluorescent protein in specific tissues, making it easier to follow development by microscopy. We also plan several mutagenesis screens and experiments to generate our own transgenic lines, which will increase the number of fish lines that we are maintaining. Since we need to constantly have fish that available for mating, we must keep both adult and developing fish for each line. Typically, each line will require ~300 fish in the facility. We currently carry approximately 30 lines of mutant and transgenic fish and two wildtype strains. We will likely add 10 additional lines over the time of this protocol. In addition, since the fish live only about 1.5 years, and have maximum breeding capacity between 3-12 months of age, several generations will be raised during the time period of this protocol. We currently have

approximately 9,000 adult fish in our facility. At maximum capacity, our fish facility can hold approximately 20,000 fish. The numbers of embryos used for different kinds of experiments are listed in the previous section.

SEARCHES FOR ALTERNATIVES:

The Animal Welfare Act requires that you search databases for alternatives to using animals and for the procedures and justify why the proposed studies will be performed as described.

You must address the following three points ("the three R's" from "The Principles of Humane Experimental Technique" by W.M.S. Russell and R.L. Burch) with searches and justifications.

- ✓ **REPLACEMENT:** Search for alternatives including in vitro models, in silico methods, invertebrate models, and vertebrate models which will be more informative. Document and describe the search results and justify why the alternatives are not used in place of animals.

- ✓ **REDUCTION:** Search to demonstrate that the proposed studies do not unnecessarily duplicate previous work. Indicate if the animals can be reused for other purposes. Search for statistical methods which could reduce animal numbers. Document and describe the search results and justify why the results will not be used in the proposed studies.

- ✓ **REFINEMENT:** Search for procedures which would cause less pain, or distress, or would result in better animal welfare. Housing, environmental enrichment, animal identification, anesthesia and analgesia and euthanasia procedures can be refined, in addition to things normally thought of as procedures, such as surgeries, tissue or fluid collection, etc. Document and describe the search results and justify why these procedures will not be used for the proposed studies.

DOCUMENT SEARCHES

The Animal Welfare Act requires that you document your justifications with data from **two or more databases**. One source **must** be a set of searches of a relevant database such as Pubmed. To document the searches for each species and procedure, name the database searched, the terms searched, when it was searched, the frequency of searches and the years covered by the most recent search. **Use the table in 7A) to document these searches.** A set of example searches for dogs can be found below, as well as links to example searches for other species.

DOCUMENT CONSULTATIONS

An appropriate, documented consultation can replace a second database in searches. Document the consultation with the consultant's name and qualifications and the date and content of the consult to demonstrate the expert's knowledge of the availability of alternatives in the specific field of study.

DESCRIBE THE RESULTS AND JUSTIFY PROCEDURES AND ANIMAL USE

You must describe the results of the searches, and justify why alternatives found by searches are not used in the place of the proposed procedures and animal use. **Complete the three parts of 7B) to fulfill this requirement.**

HOW TO DESIGN SEARCHES FOR ALTERNATIVES

Use the following questions to guide your search for replacement and reduction alternatives:

- Are there in vitro techniques that may reduce or replace the number of animals used (e.g., chorioallantoic membrane assays, primary cells, cell lines, etc.)?
- Are there lower species or more informative animal models (e.g., invertebrates, fish, microbes, etc.)?
- Are there computer models or simulations which could replace animal use?
- Are there statistical methods which would reduce sample size?

Use the following questions to guide your search for refinement alternatives:

- Are there surgical approaches which are less invasive?
- Are there anesthetics or analgesics which are more effective?
- Are there experimental endpoints which are earlier than those proposed or other ways to shorten post-procedure times?
- Are there other means to lessen the pain or distress of the procedure or improve the welfare of the animal, such as alternative housing or alternative animal identification methods?

Performing a meaningful search for Replacement, Refinement and Reduction is not the same as searching for recent advances in your field of science or medicine. Special search strategies are required to find meaningful results.

PubMed: PubMed uses a specialized vocabulary to structure information, the "Medical Subject Headings" (MeSH Terms). **To perform an effective search for alternatives, it is essential that you use MeSH Terms. Relevant results will not be found without MeSH Terms.** MeSH Terms are specific words and phrases drawn from the NLM's controlled vocabulary. Use the **MeSH Terms Browser** to find the MeSH Terms most appropriate for your study.

Hot Tip! The [MAJR] qualifier identifies a MeSH Term which is a main topic of the article. Use [MAJR] in place of [MeSH] and [ALL] to find the subset of articles focussed on the search terms. Compare the "focussed" and "inclusive" examples below to see the effect of using the [MAJR] qualifier.

The tables on the next two pages contain links to searches of multiple databases for alternatives and refinements for a study involving a diabetic retinopathy dog model and several commonly used procedures in dogs demonstrating the use of MeSH Terms. Construct your own queries with appropriate terms, or use the examples below if they are relevant. Links to example searches for other species:

Mice | Rats | Dogs | Cats | Rabbits | Frogs | Ferrets | Goats | Pigs | Fish | Primates

Use Pubmed Clinical Queries for studies focussed on therapies.

REPLACEMENT AND REDUCTION OF ANIMAL USE SEARCHES:

Example searches for dog diabetic retinopathy. Example searches for other species at:

Mice | Rats | Dogs | Cats | Rabbits | Frogs | Ferrets | Goats | Pigs | Fish | Primates

The goal of these searches is to find models or systems which are more informative, to reduce animal numbers through better statistical methods, to replace species with lower species and to replace animal use with in vitro or computer models, if possible.

	Database		
	PubMed	**Agricola**	**Other**
Gene, condition or disease AND species	***focussed:*** Insulin [MAJR] AND Dogs [MAJR] ***inclusive:*** Insulin [ALL] AND Dogs [ALL]	w=Insulin & (w=Dog + w=Dogs)	
	focussed: "Diabetes Mellitus, Experimental" [MAJR] AND Dogs [MAJR] ***inclusive:*** "Diabetes Mellitus, Experimental" [MeSH] AND Dogs [ALL]	w=Diabetes & (w=Dog + w=Dogs)	
	focussed: "Diabetic Retinopathy" [MAJR] AND Dogs [MAJR] ***inclusive:*** "Diabetic Retinopathy" [MeSH] AND Dogs [ALL]	w=Retinopathy & (w=Dog + w=Dogs)	
	"Diabetes Mellitus, Experimental" [MeSH] AND (“Double-Blind Method” [MeSH] OR “Placebos”[MeSH])		
Genetic Models	"Diabetes Mellitus, Experimental" [MeSH] AND ("Animal Experimentation" [MeSH] OR "Models, Animal" [MeSH] OR "Models, Genetic" [MeSH])		**MGI** Insulin **MGI** Diabetes
	"Diabetic Retinopathy" [MeSH] AND ("Animal Experimentation" [MeSH] OR "Models, Animal" [MeSH] OR "Models, Genetic" [MeSH])		
In Vitro Assays	("Diabetes Mellitus, Experimental" [MeSH] AND "Biological Assay" [MeSH]) NOT ("Models, Animal" [MeSH] OR "Animal Experimentation" [MeSH])		
Models Not Involving Animals	("Diabetes Mellitus, Experimental" [MeSH] AND ("Models, Theoretical" [MeSH] OR "Computer Simulation" [MeSH])) NOT ("Models, Animal" [MeSH] OR "Animal Experimentation" [MeSH])		
Statistical Methods	"Statistics" [MeSH] AND ("Animal Experimentation" [MeSH] OR "Models, Animal" [MeSH])	(w=Statistics + w=Statistical) & ((w=Sample & w=Size) + w=Power)	Statistics Core Hyperstat StatPages.net bibliography crossover designs
Teaching	"Teaching" [MeSH] AND ("Animal Experimentation" [MeSH] OR "Models, Animal" [MeSH])	w=teaching & w=alternatives	**NORINA**

REFINEMENT OF ANIMAL PROCEDURES SEARCHES:

Searches for dogs are below. Searches for other species may be accessed at:

Mice | Rats | Dogs | Cats | Rabbits | Frogs | Ferrets | Goats | Pigs | Fish | Primates

The goal of these searches is to find alternate procedures which cause less pain or distress.

	Database		
	PubMed	**Agricola**	**Other**
Euthanasia	"Euthanasia" [MeSH] AND Dogs [ALL]	w=Euthanasia & (w=Dogs + w=Dog)	Methods pdf Methods
Blood Collection	"Blood Specimen Collection/Methods" [MeSH] AND Dogs [ALL]	(w=Blood & (w=Sampling + w=Collecting)) & (w=Dogs + w=Dog)	
Anesthesia	"Anesthesia/Methods" [MeSH] AND Dogs [ALL]	w=Anesthesia & (w=Dogs + w=Dog)	**altweb** Dogs OR Dog
Analgesia	"Analgesia/methods" [MeSH] AND Dogs [ALL]	w=Analgesia & (w=Dogs + w=Dog)	**altweb** Dogs OR Dog
Neuromuscular Blockade	"Neuromuscular Blockade" [MeSH] AND Dogs [All]	w=Neuromuscular & w=Blockade & (w=Dogs + w=Dog)	
Surgery	"Surgical Procedures, Operative"[MAJR] AND Dogs [MAJR]	w=Surgery & (w=Dogs + w=Dog)	
Substance Administration	"Drug Administration Routes" [MeSH] AND Dogs [ALL]		
Toxicology	"Toxicology" [MeSH] AND ("Animal Experimentation" [MeSH] OR "Models, Animal" [MeSH])	w=Toxicology & w=Alternatives & (w=Dog + w=Dogs)	**Toxnet**
Restraint	"Restraint, Physical/Methods" [MeSH] AND Dogs [ALL]	w=Restraint & (w=Dogs + w=Dog)	
Diet	"Diet" [MeSH] AND Dogs [ALL]	w=Diet & (w=Dogs + w=Dog)	
Intubation	"Intubation/Methods" [MeSH] AND Dogs [ALL]	w=Intubation & (w=Dogs + w=Dog)	
Perfusion	"Perfusion/Methods" [MeSH] AND Dogs [ALL]	w=Perfusion & (w=Dogs + w=Dog)	
Catheterization	"Catheterization/Methods" [MeSH] AND Dogs [ALL]	(w=Catheter + w=Catheterization) & (w=Dog + w=Dogs)	
Housing	"Animal Housing" [MeSH] AND Dogs [ALL]	w=Caging & (w=Dog + w=Dogs)	Dog Housing **Enrichment DB**

6) Describe the searches you performed to determine that the proposed studies do not unnecessarily duplicate previous work by yourself or others (Reduction). *Links to databases to search to meet this requirement are available at*: http://iacuc.cwru.edu/resources/avoiding_duplication.html.

Pubmed searches for (("Zebrafish"[MAJR] AND "Embryo"[MAJR]) AND "Circadian rhythm"[MAJR]); "zebrafish"[MeSH] and "pineal gland"[MeSH]

7) Document searches of two or more databases for Replacement, and Reduction of animal use and for Refinement of procedures performed. Describe the search results and justify why they are not used in place of the proposed procedures and animal use.

- **Document Searches.** Complete all cells of the following table for all species and all procedures. Duplicate the table for additional searches. **More than one database must be searched.**

Species	Danio rerio	Danio rerio	Danio rerio	Danio rerio	Danio rerio
Procedure	microinjection	euthanasia	alternative animal models	in vitro assays	statistical methods
Database	Pubmed	http://depts.washington.edu/compmed/iacuc/	Pubmed	Pubmed	Pubmed
Search Strategy/Key words	"microinjection"[MeSH] AND "zebrafish"[MeSH]	read website	(("circadian rhythm"[MESH]) AND (("Animal Experimentation"[MeSH] OR "Models, Animal"[MeSH]) OR "Models, Genetic"[MeSH]))	(("Circadian rhythm"[MESH] AND "Biological Assay"[MeSH]) NOT ("Models, Animal"[MeSH] OR "Animal Experimentation"[MeSH]))	("Statistics"[MESH] AND "zebrafish"[MESH] AND ("Animal Experimentation"[MESH] OR "Models, Animal"[MeSH]))
Frequency of Searches	every two weeks	once a year	once a year	once a year	once a year
Last Search date	5/14/05	5/14/05	5/14/05	5/14/05	5/14/05
Years Covered Last Search	2004-5	2005	2004-5	2004-2005	all

Species	Danio rerio	Danio rerio	Danio rerio	Danio rerio	Danio rerio
Procedure	teaching	euthanasia	anesthesia	anesthesia	surgical procedures
Database	Google: http://www.zfin.org; http://www.sdbonline.org/; http://worms.z	Agricola	Agricola	Pubmed	Pubmed

	oology.wisc.edu/embryology_main.htmhttp://depts.washington.edu/fishscop/Boss/pages/movie_pages/landmarks/landmarks.html; http://www.twocw.net/mit/Biology/7-02Introduction-to-Experimental-BiologyFall2001/RelatedResources/; http://www.cincinnatichildrens.org/research/div/dev-biology/fac-labs/bartman-lab/movies.htm				
Search Strategy/Key words	searched for "movies and zebrafish"	w=Euthanasia & w=Fish	w=Anesthesia & w=Fish	"Anesthesia/Methods" [MeSH] AND ("Zebrafish" [MeSH] OR "Fishes" [MeSH])	("Surgical Procedures, Operative/Methods"[MeSH] AND ("Zebrafish"[MeSH] OR "Fishes"[MeSH]))
Frequency of Searches	twice a year	once a year	once a year	once a year	once a year
Last Search date	5/14/05	5/14/05	5/14/05	5/14/05	5/14/05
Years Covered Last Search	all	2005	2004-5	2004-2005	all

If a consultation is meant to replace one database in the searches, complete the following table.

Consultant's Name	
Consultant's Qualifications	
Date of Consult	
Content of Consult	

- Describe the alternatives found by the searches and consultations documented in part A, if any, and justify why these alternatives will not be used in the proposed studies.

    i) Describe the Replacement alternatives found, and justify why they will not be used. *Example*

of an appropriate response: "The purpose to these studies is to identify the neural circuits involved apnea induced by laryngeal stimulation. No computer models or simulations were found by searches. We do not understand the circuits sufficiently to model them yet. Searches found alternatives involving organ culture and cell culture of isolated neurons. However, organ culture and cell lines cannot replicate the complete neural circuit. Search results for lower species models returned aquatic species, and these are inappropriate because they do not breathe air. Use of this species is necessary to obtain the highest quality and most significant data with greatest relevance to human health and our understanding of this basic scientific problem. Almost all prior studies have been performed in this species, and the model is well developed with the most tools available to extract the most meaningful results."

ii) Describe the Reduction alternatives found, and justify why they will not be used. *Examples of appropriate responses, the first for small numbers of animals: "Three animals will be used for each experimental condition. This is the absolute minimum number of animals which can be used to establish the validity and reproducibility of the results."* **OR** *"Fifteen animals will be used for each experimental endpoint. A power calculation was performed with an expected mean for controls of 6.5, experimental mean of 8, sigma of 2 for samples with expected equivalent variance, in a 2 sided test, with alpha of 0.05 and power of 0.8 calculated using the power calculator online at <http://www.health.ucalgary.ca/~rollin/stats/ssize/n1.html>. These calculations gave a sample size of 14 animals, and we included one extra animal because of uncertainty in the estimated input values."* **OR** *"In order to obtain 10 homozygous double mutant male mice for each experiment, doubly heterozygous males and females will be mated, and an expected total of about 80 offspring must be produced (1/4 of offspring of the desired genotype, and 1/2 of those of the correct sex). The mutant strains will be maintained by a breeding scheme.....This is the minimal number of mice needed to maintain the strains and obtain the experimental animals."*

iii) Describe the Refinement alternatives found, and justify why they will not be used. *Example of an appropriate response: "These procedures will cause moderate to serious pain which will be relieved with anesthetic during the procedure and analgesics for pain after the procedure. Searches returned only anesthetics and analgesics which have unacceptably strong depressive effects. The surgical method we have chosen has been described as the least invasive, least painful and least distressful method to date. Searches returned only older, less refined techniques which required longer, more traumatic surgeries."*

i) Replacement: Our purpose is to study the vertebrate nervous system, so the use of non-vertebrate animals is not possible. The zebrafish has become widely accepted throughout the world as a useful vertebrate model system. The embryos are transparent and develop rapidly outside the mother, making possible to viewing differentiation of many tissues in the live organism. Access to the developing embryos makes it possible to do experimental manipulations (cell transplantation, injection of different molecules, cell labeling) that reveal mechanisms underlying development. Adult fish can be mutagenized, and their progeny screened for mutations that affect function or development of the nervous system. Fish are small and easy to maintain and breed in large numbers in laboratory conditions. None of the other vertebrate model systems have all of these characteristics, which are needed for our research. Searches for alternative animal models found only mammalian systems and

chicks (which are higher vertebrates than zebrafish) and non-vertebrates. Searches for alternative methods did find in vitro assays, and computational methods for studying circadian rhythms. However, the field is not advanced enough for these to replace the ongoing work in animal models. We are planning to enrich (not replace) our in vivo work with in vitro assays, and two grants will be submitted for in vitro work in the next two months. **ii) Reduction:** Our adult fish almost entirely used as mating stock, so they are not directly used in experiments, and we keep only the adult fish that are needed to maintain the stocks. Typically, we raise 60 fish in each new stock, and 2/3 of these will be carrying the given mutation. This gives us 40 fish, or up to 20 mating pairs. However, the sex of zebrafish is determined by unknown environmental factors, and so there is often an uneven mix between males and females, and we may get as few as five mating pairs for each new stock. For this reason, we typically have three stocks of 60 for each line, two adult tanks and one tank of immature fish to replace the others when they get too old. We also use the smallest number of embryos as possible for each experiment. Our in vivo assays do not produce quantitative data, so it is not possible to do statistical analysis Typically, we use only a small number of embryos (10) per time point. We need this many to ensure that our results are reproducible, and to have enough embryos that we can get adequate picture to document the data. **iii) Refinement:** The searches of Pubmed and Agricola did not identify any techniques for zebrafish other than the ones we are currently using. Although the University of Washington IACUC website is not officially a database, I knew from Nan Kleinman that they were doing some work on zebrafish euthanasia. I found additional documentation for our method of euthanasia on this website. In addition, I am continuing to compile resources that will help new students in the laboratory learn fundamental skills in our laboratory. For example, I used searches of developmental biology websites to identify new movies of zebrafish development that will help teach the stages of zebrafish development and zebrafish anatomy without using live fish.

8) Can these animals be used for other purposes after the study? *Reuse of animals is encouraged. If your animals can be reused in some manner, please state how and by whom.*

We almost always use the fish in our laboratory for multiple purposes. Adult fish are used mainly for natural matings, and can be used as often as once a week for this purpose. A typical adult fish in our facility might be set up to mate 20-40 times during its natural lifetime. Natural matings not only produce embryos for our experiments, but also maintains the health of the adult fish. In another example, adult fish that carry a specific mutation are usually identified by setting them up in natural matings, and examining the phenotype of their progeny. Embryos produced during this process can be fixed and used for whole mount in situ hybridization, used to start a new stock, or to teach new students how to properly stage growing embryos. We also have started a collaboration with Christine Beatties laboratory to share mutagenized fish, so we can each screen the same fish for phenotypes in our areas of interest. This significantly reduces the number of fish we would need to mutagenize.

VI. Animal Use Checklist

Please complete the checklist and any required attachments.

Yes/No	
no	Will you be collecting body fluids prior to euthanasia?

	If *Yes*, complete Attachment A: Antemortem Fluid Collection.
no	Will you be using animals to produce antibodies? If *Yes*, complete Attachment B: Immunizations.
yes	Will anesthetics, analgesics, sedatives, paralytics or tranquilizers be used? If *Yes*, complete Attachment C: Anesthesia, Analgesia Or Paralysis.
yes	Will surgery be performed on the animals and the animals allowed to recover? If *Yes*, complete Attachment D: Survival Surgery.
no	Will surgery be performed on the animals and the animals *not* allowed to recover? If *Yes*, complete Attachment E: Non-Survival Surgery.
yes	Will animals be subjected to Pain Class E procedures? If *Yes*, complete Attachment F: Justification Of Pain Class E Animal Use.
no	Will animals be subjected to restraint other than regulation caging? If *Yes*, complete Attachment G: Animal Restraint.
yes	Will infectious agents, hazardous chemicals, recombinant DNA (creation of knockout or transgenic animals) or radioactive materials be introduced into animals? Will animals which present a biohazard be imported? If *Yes*, complete Attachment H: Infectious Agents, Biohazards And Recombinant DNA.
no	Will tumor cells (including hybridomas) be introduced into animals? If *Yes*, complete Attachment I: Tumors In Animals.
no	Will chemicals, non-infectious biological substances, cells (nontumorous) or tissues be administered to animals other than for anesthesia or production of antibodies? If *Yes*, complete Attachment J: Exogenous Substance Administration.
no	Will animals be irradiated? If *Yes*, complete Attachment K: Irradiation.
no	Will animals be housed for more than 12 hours in locations other than those listed in Part VII? If *Yes*, complete Attachment L: Alternative Housing.
no	Will diet be restricted or modified or the environment manipulated? If *Yes*, complete Attachment M: Manipulation of Environment.
no	Will there be operant conditioning or other behavioral tests? If *Yes*, complete Attachment N: Behavioral Tests.
no	Will procedures not fitting the above descriptions be performed on the animals? If *Yes*, complete Attachment O: Other Procedures.

no	Will animals be studied in the field? If *Yes*, complete Attachment P: Field Studies.
no	Will you obtain rodents from private colonies? All shipments must be approved prior to shipment by the CWRU veterinarian. Nonstandard vendor forms are available for download on the web at: http://iacuc.cwru.edu/forms/nonstandardvendor.doc

VII. Pain Classification

Classify individual procedures as C, D or E in the chart below. Investigators should assign procedures to pain classes honestly. There is no penalty to the investigator for placing a procedure in a higher pain class.

Class C:

Pain or distress will be absent or minimal and momentary

This category includes but is not limited to

- *Breeding.*
- *Gavage.*
- *Tissue collection after euthanasia.*
- *Most injections, if the injection does not introduce an agent which causes pain or distress.*
- *Blood collection from a peripheral vein.*
- *The use of Freund's complete adjuvant in immunizations in accord with IACUC policy* http://iacuc.cwru.edu/policy/freunds.html.
- *Nutritional studies if they do not lead to debilitation of the animal.*
- *Hypoxic or hypobaric chambers.*
- *Mutants with little or no pain or debilitation.*
- *Subclinical infections.*
- *Procedures which cause only minor discomfort.*

Class D:

Pain or distress will be present and appropriate anesthetics, analgesics or tranquilizers will be provided.

This category includes but is not limited to

- *Terminal tissue or organ harvest under anesthesia.*
- *Pithing or exsanguination under anesthesia.*
- *Surgeries such as biopsy, gonadectomy, exposure of blood vessels, chronic catheter implantation, bronchoalveolar lavage, laparotomy or laparoscopy when performed with appropriate anesthesia and analgesia.*
- *Surgical invasion of body cavities, orthopedic procedures, dentistry or other hard or soft tissue damage when performed with appropriate anesthesia and analgesia.*
- *Blood collection by invasive procedures such as intracardiac or periorbital collection from species without a true orbital sinus such as rats and guinea pigs when performed with appropriate anesthesia.*
- *Administration of chemicals or agents which produce chronic pain or distress which will be relieved with analgesics.*
- *Mutants with debilitating effects which are alleviated by analgesia or appropriate intervention.*
- *Clinical infection alleviated by appropriate treatment.*
- *Blood collection from the periorbital sinus of mice when performed with appropriate anesthesia.*
- *Tumor studies in rodents within the IACUC-recommended limits* http://iacuc.cwru.edu/policy/tumor_inoculation.html.

Class E:

Pain or distress will be induced and will not be relieved because relief would interfere with procedures, results or interpretation of the results.

This category includes but is not limited to

- *Administration of chemicals or agents which produce pain or distress which is not relieved with analgesics.*
- *Mutants with chronic pain or debilitation which is not relieved with analgesics or by appropriate intervention.*
- *Procedures producing pain or distress unrelieved by analgesics such as toxicity studies, microbial virulence testing, radiation sickness studies and research on stress, shock or pain.*
- *Negative conditioning via electric shocks or other methods of stress which would cause pain or distress in humans.*
- *Prolonged restraint without appropriate measures to alleviate distress.*
- *Clinical infection without treatment.*
- *The administration of Freund's complete adjuvant by means not in accord with IACUC policy* http://iacuc.cwru.edu/policy/freunds.html.
- *Studies where the death of an animal is an experimental endpoint.*

Investigators should consider that pain relief may be incomplete despite best efforts to provide analgesia. In such cases, it may be appropriate to categorize the procedure as Class E. Where appropriate, the experience of humans subject to the same conditions can be used as a guide to classification.

USDA Regulations and Guidelines Pertaining to Pain Classification:
http://iacuc.cwru.edu/resources/policy11.pdf

List the estimated number of animals in each pain class for the complete duration of the protocol, using the guidelines above.

		Number In Pain Class			
Species, Strain and Procedure		**C**	**D***	**E*†**	**Total**
Zebrafish, *Danio rerio* natural breeding (B for breeding)	purchased	**20**			**20**
	bred	**7000**			**7000**
Zebrafish, Fin clipping, Isolation of gametes for in vitro fertil.	purchased				
	bred		**70**		**70**
Zebrafish, Mutagenesis	purchased				
	bred			**20**	**20**
	purchased				
	bred				
	purchased				
	bred				
	purchased				
	bred				
	purchased				

	bred				
	purchased				
	bred				
	purchased				
	bred				
	purchased				
	bred				

* If animals are present in Class D or E, consultation with a lab animal veterinarian is required.
† If animals are present in Class E, complete Attachment F: Justification for Pain Class E Animal Use.

For Class D and E animal procedures, identify the laboratory animal veterinarian consulted and the date they were consulted.

On 5/17/05 I consulted with Dr. Nan Kleinman about each of these procedures.

VIII. Housing Of Animals And Location Of Procedures

1) Where Will The Animals Be Housed*?

Main ARC Facility – Health Science Animal Facility (HSAF)
Metro Health Medical Center/Rammelkamp
Transgenic Mouse Facility/Wearn
Veterans' Administration
Athymic (Nude) Mouse Facility/CRC
Biomedical Engineering /Wickenden
ABSL3/P 3
Ultra-Barrier Facility
x Other. If checked, complete Attachment L.

*It is recommended that social animals be housed in groups whenever possible.

2) Are there special requirements for housing (for example, microisolators for rodents, special conditions for immunocompromised animals, biohazard or infectious agent containment)**?**

Fish need to be kept in a fresh water aquatic facility, which is not available at the ARC. Fish will be kept in a ~500 tank, aquatic facility built by Aquatic Habitats in 126 Millis. Fish will be kept in the facility for their entire lives (~1.5 years). Standard Operating Procedures for the maintaining a zebrafish colony are described in detail in "The zebrafish book" available at http://www.zfin.org and as a hard in my laboratory.
A fresh water aquatic facility with ~500 individual tanks (1L, 2.75L, and 9L) has been built in 126 Millis. The facility has a recirculating water system. Building tap water is first purified by a reverse osmosis system, and then conductivity (salt concentration) and pH are adjusted by an automatic dosing system. Water quality (pH, temperature, conductivity) is constantly measured by an electronic monitor. To prevent the spread of disease, water passes through only one tank, and then is pumped through a carbon filter, particle filters, and a UV sterilizer before it returns to a tank. In addition to the main facility, we have a quarantine rack that has its own independent water system. Water quality is monitored in this rack by a dedicated portable pH/conductivity meter, and maintained by Allisan Aquilina-Beck, a fish technician with six years experience running and maintaining fish facilities. Water levels in the main system and quarantine rack are maintained by an automatic fill system controlled by float valve switch. Ammonia and nitrite are monitored manually. If parameters are outside of normal ranges, water is changed and manipulated to correct the problem. Water parameters are recorded every morning.

All fish are fed twice daily, am and pm. The am feed is composed of live food raised within the aquatic facility: brine shrimp for adults and juveniles and paramecium for babies (<2 weeks). The afternoon feed is composed of dry flake fish food for adults and juveniles and paramecium again for babies. When feedings are completed, they are logged.

Health of fish is monitored daily by observation during the feeding process. If a fish appears sluggish and skinny, it will be moved to a quarantine tank to be monitored and fed. If no improvement is seen the fish will be euthanized and recorded. If this behavior is seen in numerous fish, or if physical wounds, fungus, parasites and the like are observed, fish will be moved onto the quarantine rack and administered a formaldehyde treatment 25ppm. This treatment will continue every other day along with water changes. If this occurs in the main facility, all fish will be treated since the water recirculates within the facility.

3) Where will animal procedures be performed? Give the Building and Room Number. (If procedures will be performed in multiple places, please list each.)

Millis RM 126C

IX. Euthanasia

The IACUC follows the recommendations of the AVMA Panel on Euthanasia. This guide to acceptable methods of euthanasia is available for download http://iacuc.cwru.edu/resources/euthanasia.pdf*.The document is readable and easy to navigate. We recommend that you examine the document to find the best approved methods for your needs. Justification is required for methods not in accord with these recommendations. For guidelines on euthanasia of neonatal rodents, see* http://iacuc.cwru.edu/policy/fetal_rodent_euthanasia.html*. Guidelines to the principles of humane euthanasia:* http://iacuc.cwru.edu/guide/guide4.html#anasia*.*

1) Describe the method of euthanasia.

Chemical Methods:

Carbon dioxide. Describe the method.

Anesthetic agent. If using an anesthetic agent, provide the following information:

Drug:

Dosage:

Route of administration:

Physical Methods:

Exsanguination under anesthesia. Describe:

Cervical dislocation under anesthesia or after CO2. Describe:

Cervical dislocation without anesthesia. Describe:

Decapitation without anesthesia. Describe:

Provide scientific justification for decapitation without anesthesia.

x Other Methods:

Describe other methods in detail for each species. Justify the methods if they are not in accord with the AVMA recommendations.

Zebrafish will be euthanized by immersion for 20 minutes in a cold water bath. The 2000 Report of the American Veterinary Medical Association Panel on Euthanasia does not approve this method for euthanisia of fish species in general, but does not address the specific case of tropical species, which have little or no ability to adapt to the cold. This deficiency was addressed in 2002 by the University of Washington IACUC committee:

> "Because tropical fish species, (i.e. zebrafish, medaka, and platyfish), have minimal to no physiologic adaptation mechanism for adjusting to cold (4˚C) water, cooling to 4˚C should be considered an acceptable method of euthanasia since the rapid decrease in temperature from 26˚C (or higher) to 4˚C induces rapid loss of consciousness and is lethal to these species."

The full text of this report can be found at: http://depts.washington.edu/compmed/iacuc/policies/fish_euthanasia.html.

Therefore, a cold water bath fulfills the requirements of the Panel on Euthanasia that a method of euthanasia cause rapid loss of consciousness, and minimize pain and distres.

2) How will the carcasses be disposed of? *(The ARC provides for disposal of animal carcasses in room EB09A in the HSAF.)*

They will be collected in Millis 126, frozen, and then incinerated at the ARC

X. Animal Identification

Identification of animals should be according to approved methods. Ear punches, tattoos, ear tags, and implanted microchips are acceptable for rodent identification. For IACUC policy on toe clipping, see http://iacuc.cwru.edu/policy/toe_clipping.html. For general guidelines on animal identification, see http://iacuc.cwru.edu/guide/guide3b.html#popman.

How will individual animals be identified?

Animals are not individually identified. Different stocks of fish, including wildtype strains and strains carrying specific mutations or transgenes, are identified by a label attached to their tank with birth date, stock name and number, the number of fish housed and the name of the person in the laboratory responsible for the stock. When the fish are removed from their tank as for breeding, the temporary tank is always labeled with the number of the tank of origin and the strain and sex of the fish.

XI. Environmental Enrichment

Enrichment is required for dogs and primates.
Information on enrichment: http://iacuc.cwru.edu/enrichment/enrichment.html

Dogs

Provide a plan for exercise and socialization for dogs. If you are seeking exemption from the institution's requirement to provide dogs with the opportunity for exercise or human contact, provide justification. *Dogs housed in cages or runs providing less than twice the minimum required floor space must be provided with daily access to an exercise pen during routine cage/pen cleaning. Dogs housed alone in a room must receive daily positive human contact.*

Primates

Provide a plan for enrichment for primates. If you seek exemption from the requirement to provide enrichment, provide a scientific justification.

x **Other Species (specify):** zebrafish (Danio rerio)

Choose one of the following options for environmental enrichment of other species:

1. ARC-provided environmental enrichment program

The ARC will provide environmental enrichment to other species: Nestlets will be provided to singly-housed mice and nylabones provided to singly-housed rats. Environmental enrichment of at least one of the following will be provided to other species on a random/rotating basis: Alfalfa blocks, pineapple rings or plastic balls to rabbits and guinea pigs, rubber or rawhide chews or plastic balls to ferrets and cats.

x **2. No ARC provided enrichment: Investigator will provide environmental enrichment.**

Special care instructions forms and cage, room or rack labels must be provided for cages not subject to ARC-provided environmental enrichment. Please describe plan for enrichment that you will provide.

Fish are housed in clear tanks with anywhere from 2-40 fish. They can interact with one another within the same tank and can see fish in neighboring tanks. Fish are set up regularly in natural matings.

3. No ARC provided enrichment: No environmental enrichment.

Special care instruction forms and cage, rack or room labels must be provided for cages not subject to ARC-provided environmental enrichment. Provide a scientific justification if animals must be exempt from environmental enrichment.

XII. Personnel

Identify all personnel handling animals in this study, and identify the procedures they will perform, and their training or level of experience in performing the proposed procedures.
Include the principal investigator, co-investigators, research associates, postdoctoral fellows, students, research associates, research assistants and technicians.

A Facility Access Health and Training (FAHT) Form must be submitted for each individual listed, if a form has not previously been submitted for that individual a FAHT form can be found on the IACUC website.

Name And Degree	Position	Procedures To Be Performed By This Person	Experience Or Training In These Procedures	Signature
J. Liang	PI	All written	8 years experience	
A. Beck	Research Assistant	All written	6 years experience	
E. Greer	PhD student	All written	3 years experience	
R. Noche	PhD student	All written	3 years experience	
Chunrui Tan	Student	Fish feeder	2 years experience	
Syed Hasnul Syed Mahamud	Student	Fish feeder	2 years experience	
Jessica Kingsberg	Student	Fish feeder	1 year experience	

XIII. Occupational Health And Safety

1) Will all personnel who will have contact with animals in this project participate in the Occupational Health Program? http://iacuc.cwru.edu/policy/occupational_health.html.

x Yes No. If *No*, explain why not.

2) Are there any additional measures such as special vaccines or additional health screening which could benefit research, husbandry, or veterinary staff in this project? Vaccination for tetanus need not be mentioned.

no

XIV. Transportation

Transportation of animals must conform to all institutional policies and federal regulations. The "Guide for the Care and Use of Animals" http://iacuc.cwru.edu/guide/guide4.html#apt *is a good source of information on the regulations governing transport of animals. If animals will be transported on public roads or out of state, describe efforts to comply with USDA regulations* http://iacuc.cwru.edu/resources/cfr.html*. If animals will be transported within or between buildings, appropriate containment must be used. (Appropriate containment prevents pathogen spread and inadvertent observation. Examples of appropriate containment include cages of rodents placed inside a cardboard box or under a drape, and draped anesthetized animals on a cart.)*

If you will transport animals, describe the manner of transportation.

Adult fish are transported in specialized fish bags purchased from Aquatic Habitats. These bags are filled with air and sealed. They are then placed in a Styrofoam lined cardboard box and padded so as to reduce vibration from transportation. Fish will not be in transit longer than 24 hrs. Adult fish and embryos transported between buildings on campus will be carried in a cardboard box.

XV. Conditioning And Quarantine

Conditioning is required for mammals other than rodents. Purpose-bred rabbits, ferrets, cats, dogs and hoofed stock require a minimum of 7 days of conditioning. Random-source cats and dogs require a minimum of 14 days. Primates may require in excess of 90 days depending on source and use. The IACUC policies on conditioning and quarantine of animals are available as a pdf file for download at: http://iacuc.cwru.edu/policy/cqm.pdf.

Describe plans for conditioning and/or quarantine for your animals. If the required conditioning and quarantine is to be waived, provide scientific justification.

Fish received from an outside source are first introduced into our facility on a designated quarantine rack. This is a self-contained recirculating system holding 60 tanks. Fish are kept here up to two weeks in order to monitor their health. Water quality is checked daily and food is administered twice a day. Once fish are considered healthy they are moved to the main system. This is a larger recirculating water system. Monitoring of water quality, food and fish health are administered daily throughout the system.

XVI. Suspended Caging

The use of suspended caging for rodents is discouraged by the IACUC.

Provide scientific justification for the use of suspended caging.

XVII. Investigator Input on the Form and Review Process

Please use the space below to provide constructive criticism on how the form and the review process could be changed to serve you better.

-- end of Core --

Attachment A: Fluid Collection

table of contents (attachments are to be completed only as required by the checklist)

Please describe the procedures for fluid (blood, urine, bile, lymph, etc.) collection prior to death. IACUC policies on the collection of blood are available at http://iacuc.cwru.edu/policy/blood_collection.html. NIH guidelines for the collection of blood from rats and mice can be downloaded from http://iacuc.cwru.edu/policy/nihpolicies/nihsurvivalbleeding.pdf.

Describe procedures for fluid collection, using a separate attachment for each procedure or species.

1) Species and Procedure:

2) Fluid:

3) Volume per collection:

4) Frequency of collection:

5) Total number of collections:

6) Site and method of collection:

7) Will the animals be anesthetized or sedated during the procedure? Yes or No.

If *Yes*, complete Attachment C.

8) Describe the method of restraint.

-- end of Attachment A --

Attachment B: Immunizations

table of contents (attachments are to be completed only as required by the checklist)

IACUC guidelines for immunizations and the use of complete Freund's adjuvant are available at http://iacuc.cwru.edu/policy/freunds.html. For example, footpad injections are strongly discouraged, complete Freund's adjuvant beyond the initial injection is strongly discouraged, and attention to potential contaminants that may lead to inflammation is required.

Describe the immunization procedure, using a separate attachment for each species or route of administration.

1) Species:

2) Antigen(s):

3) Adjuvant for initial injection:

4) Adjuvant for subsequent injections:

5) Injection site(s):

6) Volume per injection site:

7) Number of sites:

8) Preparation of injection site (e.g. clipping, disinfection):

9) Total volume of adjuvant and antigen:

10) Frequency of immunization:

11) Will the animals be anesthetized or sedated during the immunization procedure?

☐ No ☐ Yes (If *Yes*, complete Attachment C)

12) Fluid or tissue collected:

☐ Blood (Complete Attachment A)

☐ Ascites fluid (Complete Attachment A)

☐ Tissue (Specify):

-- end of Attachment B --

Attachment C: Anesthesia, Analgesia Or Paralysis

table of contents (attachments are to be completed only as required by the checklist)

For CWRU Guidelines for appropriate anesthesia and analgesia for different animal species, see http://iacuc.cwru.edu/policy/analganesth.html. For IACUC guidelines on the safe use of ethyl ether as an anesthetic, see http://iacuc.cwru.edu/policy/ethyl_ether.html. For general guidelines to anesthesia and analgesia, see http://iacuc.cwru.edu/guide/guide4.html#paa. Investigators are strongly encouraged to consult with an ARC veterinarian about the most appropriate methods of anesthesia, analgesia and the appropriate use of tranquilizers and paralytics. The ARC phone number is 368-3490.

Describe the conditions of anesthesia, using a separate Attachment for each species or procedure.

1) Species and Procedure: Zebrafish anesthesia

2) Drugs used for restraint, tranquilization, sedation.

Drug(s): MS222/Tricane/3-amino benzoic acidethylester

Dosage(s): 0.16mg/ml of fish water

Route(s) of Administration: Absorption through skin

3) Drugs used for anesthesia.

Pre-Anesthetic:

Drug(s): MS222/Tricane/3-amino benzoic acidethylester

Dosage(s): 0.16mg/ml of fish water

Route(s) of Administration: Absorption through skin

Induction:

Drug(s):

Dosage(s):

Route(s) of Administration:

Maintenance:

Drug(s):

Dosage(s):

Route(s) of Administration:

4) Expected duration of anesthesia:

x <30 minutes

	30 - 60 minutes
	1 - 2 hours
	2 - 4 hours
	> 4 hours, specify:

5) How will depth of anesthesia be monitored?

Fish will slow down or stop moving, however heart rate will continue at a slow pace and will be monitored visually throughout the process.

6) If analgesics will be used, but the method of their use is not described elsewhere, please do so here, giving drug, dose, route and frequency.

7) Will a paralytic agent be used?

x	No		Yes. If *Yes*, complete the following:
	Drug(s):		
	Dosage:		
	Route of administration:		

What is the purpose of using a paralytic agent?

Describe how ventilation will be maintained and how pain will be assessed.

Justify the use of a paralytic.

-- end of Attachment C --

Attachment D: Survival Surgery

table of contents (attachments are to be completed only as required by the checklist)

It is required that you consult with a veterinarian trained in Laboratory Animal Medicine. ARC veterinarians may be contacted at 368-3490.

NIH guidelines for survival surgery on rodents: http://iacuc.cwru.edu/policy/nihpolicies/surguide.htm.
NIH guidelines for oocyte collection from Xenopus: http://iacuc.cwru.edu/policy/nihpolicies/oocyte.htm.
IACUC guidelines for surgery on rodents: http://iacuc.cwru.edu/policy/rodent_surgery_guide.html
IACUC guidelines for surgery on non-rodents: http://iacuc.cwru.edu/policy/survsurgnonrodents.html *General guidelines to surgery*: http://iacuc.cwru.edu/guide/guide4.html#surg

Describe survival surgery, using a separate attachment for each procedure and species.

1) Species and Procedure: Zebrafish, fin clips

2) Location of Procedure: Millis Rm 126c

3) How many animals will undergo this procedure per year? 20

4) Will more than one survival surgery be performed on an animal? [ ] Yes [x] No

If *Yes*, provide scientific justification for multiple survival surgeries. *USDA guidelines for justification of multiple survival surgery are available at* http://iacuc.cwru.edu/policy/policy14.pdf.

5) Are some animals likely to require an **elective** subsequent surgical intervention to correct or modify the original procedure? [ ] Yes [x] No

If Yes, describe the type and necessity of surgical repair anticipated and estimate the percentage of animals needing reoperation. The IACUC Policy for Elective Surgical Repair and submission form is available at http://iacuc.cwru.edu/policy
NOTE: ARC Veterinary Staff must be notified in advance of all surgical procedures including emergency repairs via the submission of a **Procedure Data Sheet.**

6) Describe the presurgical procedure in detail.

If food will be withheld, give duration. **N/A**

If water will be withheld, give duration. **N/A**

If analgesics will be given, describe route and dosage.

N/A

7) Describe the aseptic procedures (fur clip, disinfection, sterilization of instruments, maintenance of asepsis between surgeries).

Fish will be administered anesthesia as described above, moved to a clean Petri dish and the tip of the caudal fin will be clipped off using a sterile scalpel. The fish will be moved to a clean water tank until it recovers from the anesthesia. At this point, the fish will be moved into a quarantine tank where it's health and recovery will be monitored. Once the fish has recovered and the fin has regenerated, the fish will be moved back to its original tank in the main facility.

8) Describe the surgical procedures, including a description of access to the anatomic site.

See above

9) Expected duration of surgery:

- [x] <30 minutes
- [ ] 30 - 60 minutes
- [ ] 1 - 2 hours
- [ ] 2 - 4 hours
- [ ] > 4 hours, specify:

10) List major support equipment used or available for use (e.g., anesthesia machines, electrocautery, suction, fluoroscope, ventilator, heating pad, CO2 analyzer, defibrillator, EKG monitor).

N/A

11) Administration of antibiotics or other drugs (specify):

Drug:	MS222
Dosage:	0.16mg/ml
Route:	Absorbed through skin
Frequency:	one time, 5-10 minutes

12) Post-operative care:

Observation frequency and duration: One month

Analgesics

Drug:	
Dosage:	
Route:	
Frequency:	

Antibiotics

Drug:

Dosage:

Route:

Frequency:

13) Describe other supportive care in detail, including fluids or special diets and the frequency and duration of treatment.

The fish will be fed twice daily and monitored daily for infection

14) Who will provide post-operative care?

The fish feeder/research assistant

15) How long will the animals be maintained after surgery?

At least one year

16) If the surgery will normally result in an impairment of the physical or physiological function of the animal, or if analgesia is expected to be incomplete despite best efforts, describe the expected severity and effect on the animals' welfare.

The clipping is so minor that this surgery will not impair the fish

17) What unintended complications may occur as a result of this surgical procedure (e.g., hemorrhage, wound infection, physical impairment, etc.)?

Wound infection

18) Describe how complications will be managed and the criteria for termination of the experiment by euthanasia. *(Examples of appropriate criteria that should be considered include a weight loss limit as a percentage of initial or expected body weight, allowable duration of anorexia, the presence of health problems refractory to medical intervention, and severe psychological disturbances.)*

Fish that lose weight or appear sick or skinny will be isolated, and given extra brine shrimp. Those that show signs of infection will be given a salt treatment. Extra salt in the water is a noninvasive way to treat fungal infections.

19) For cannulae, acrylic implants, venous catheters and other devices which will extend through the skin for longer than 12 hours, explain what wound management measures will be taken to minimize infections at the site of penetration.

--end attachment D--

Attachment D: Survival Surgery

table of contents (attachments are to be completed only as required by the checklist)

It is required that you consult with a veterinarian trained in Laboratory Animal Medicine. ARC veterinarians may be contacted at 368-3490.

NIH guidelines for survival surgery on rodents: http://iacuc.cwru.edu/policy/nihpolicies/surguide.htm.
NIH guidelines for oocyte collection from Xenopus: http://iacuc.cwru.edu/policy/nihpolicies/oocyte.htm.
IACUC guidelines for surgery on rodents: http://iacuc.cwru.edu/policy/rodent_surgery_guide.html
IACUC guidelines for surgery on non-rodents: http://iacuc.cwru.edu/policy/survsurgnonrodents.html *General guidelines to surgery*: http://iacuc.cwru.edu/guide/guide4.html#surg

Describe survival surgery, using a separate attachment for each procedure and species.

1) Species and Procedure: Zebrafish, collection of sperm and eggs for in vitro fertilization

2) Location of Procedure: Millis Rm 126c

3) How many animals will undergo this procedure per year? 50

4) Will more than one survival surgery be performed on an animal? [X] Yes [ ] No

If *Yes*, provide scientific justification for multiple survival surgeries. *USDA guidelines for justification of multiple survival surgery are available at* http://iacuc.cwru.edu/policy/policy14.pdf.

This is a non-invasive procedure that does not hurt the animal. Fish that are used for sperm and egg collection can be used for this process several times, although we do not use a single fish more that once a month.

5) Are some animals likely to require an **elective** subsequent surgical intervention to correct or modify the original procedure? [ ] Yes [x] No

If Yes, describe the type and necessity of surgical repair anticipated and estimate the percentage of animals needing reoperation. The IACUC Policy for Elective Surgical Repair and submission form is available at http://iacuc.cwru.edu/policy
NOTE: ARC Veterinary Staff must be notified in advance of all surgical procedures including emergency repairs via the submission of a **Procedure Data Sheet.**

6) Describe the presurgical procedure in detail.

If food will be withheld, give duration.	**N/A**
If water will be withheld, give duration.	**N/A**
If analgesics will be given, describe route and dosage.	

N/A

7) Describe the aseptic procedures (fur clip, disinfection, sterilization of instruments, maintenance of asepsis between surgeries).

N/A

8) Describe the surgical procedures, including a description of access to the anatomic site.

Fish will be administered anesthesia as described above, moved to a wet sponge that will hold the fish ventral side up. The abdomen of the fish will then be gently squeezed to expel the sperm or eggs onto the surface of the fish, where they will be collected. Note that this is a completely non-invasive procedure. Collecting the eggs or sperm takes only a few seconds, and the fish are not harmed. After the sperm or eggs are collected, the fish will be moved to a clean water tank until it recovers from the anesthesia.

9) Expected duration of surgery:

x	<30 minutes
	30 - 60 minutes
	1 - 2 hours
	2 - 4 hours
	> 4 hours, specify:

10) List major support equipment used or available for use (e.g., anesthesia machines, electrocautery, suction, fluoroscope, ventilator, heating pad, CO2 analyzer, defibrillator, EKG monitor).

N/A

11) Administration of antibiotics or other drugs (specify):

Drug:	MS222
Dosage:	0.16mg/ml
Route:	Absorbed through skin
Frequency:	fish are treated one time for 5-10 minutes

12) Post-operative care:

Observation frequency and duration:	One month
Analgesics	

Drug:
Dosage:
Route:
Frequency:

Antibiotics

Drug:
Dosage:
Route:
Frequency:

13) Describe other supportive care in detail, including fluids or special diets and the frequency and duration of treatment.

The fish will be fed twice daily and monitored daily for infection

14) Who will provide post-operative care?

The fish feeder/research assistant

15) How long will the animals be maintained after surgery?

At least one year

16) If the surgery will normally result in an impairment of the physical or physiological function of the animal, or if analgesia is expected to be incomplete despite best efforts, describe the expected severity and effect on the animals' welfare.

N/A

17) What unintended complications may occur as a result of this surgical procedure (e.g., hemorrhage, wound infection, physical impairment, etc.)?

none

18) Describe how complications will be managed and the criteria for termination of the experiment by euthanasia. *(Examples of appropriate criteria that should be considered include a weight loss limit as a percentage of initial or expected body weight, allowable duration of anorexia, the presence of health problems refractory to medical intervention, and severe psychological disturbances.)*

Fish that lose weight or appear sick or skinny will be isolated, and given extra brine shrimp. Those that show signs of infection will be given a salt treatment. Extra salt in the water is a noninvasive way to treat fungal infections.

19) For cannulae, acrylic implants, venous catheters and other devices which will extend through the skin for longer than 12 hours, explain what wound management measures will be taken to minimize infections at the site of penetration.

Attachment E: Non-Survival Surgery

table of contents (attachments are to be completed only as required by the checklist)

In non-survival surgery, the animal is euthanized at the end of the procedure without recovery from anesthesia. The procedures for anesthesia must be described in Attachment C.

Describe non-survival surgery, using a separate attachment for each procedure and species.

1) Species and Procedure:

2) Location of Procedure:

3) Describe the presurgical procedure.

If food will be withheld, give the duration.

If water will be withheld, give the duration.

If anesthetics will be loaded, give drug, route and dosage.

Describe the aseptic preparation.

4) Describe the surgical procedures, including a description of access to the anatomic site.

n/a

5) How long will the animal be maintained under anesthesia prior to euthanasia?

6) How will humane euthanasia be enacted, and how will death be determined?

-- end of Attachment E --

Attachment F: Justification For Pain Class E Animal Use

table of contents (attachments are to be completed only as required by the checklist)

Procedures which may cause more than momentary or slight pain must, in their planning, involve consultation with a veterinarian trained in Laboratory Animal Medicine. ARC veterinarians may be contacted at 368-3490.

The definition of experimental endpoints are particularly important for Class E Animal Use. The NIH provides guidelines for the selection of humane experimental endpoints: http://iacuc.cwru.edu/policy/nihpolicies/endpoint.htm.

1) If pain relief will be withheld from animals in pain or distress, provide scientific justification. *Provide the scientific rationale for this decision and provide references. This information is required in our annual USDA report and may be quoted directly from this protocol form.*

Genetic screens are one of the most powerful ways to identify new genes and pathways involved in pineal development and circadian rhythm. ENU mutagenesis is the only current way to generate mutagenized fish for these genetic treatments. The ENU treatment requires three 1 hour treatments. Because fish use gills, they cannot be anesthetized for this period of time because they will not be swimming, and they will die. This treatment sometimes causes distress for the animals, and occasionally they die during the process. To minimize the stress, light and sound levels are kept very low during the treatment, and if the fish are exhibiting signs of serious distress (they stop swimming and sink to the bottom of the tank) they will be removed to a recovery tank.

2) Describe the expected clinical signs of pain or distress. *For example, signs of extreme distress in rodents include hunched posture, disheveled coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, respiratory problems, and solid tumor growth. Indicate the expected severity and duration, the frequency the animal will be monitored and when the pain will be eliminated or managed by euthanasia, drugs or withdrawal of painful stimulus.*

During the treatment, signs of distress include lethargic swimming, or failure to swim. If this occurs before the treatment is finished, fish will be removed to a recovery tank.
There is also a potential that the ENU-treated fish will become sick with opportunistic infection or with tumors. They will be monitored at least twice a day. Fish that lose weight or appear sick or skinny will be isolated, and given extra brine shrimp.
If fish become sick as a result of the ENU treatment, the will first be treated as described above. If this fails, they will be euthanized. Fish will also be euthanized if they show tumor growth greater than 10% of body weight. Signs of illness include inability to swim properly, failure to feed, and discoloration of the skin or scales.

3) If death will be the experimental endpoint, you must justify why an alternate endpoint (such as weight loss, clinical signs, tumor size, etc.) prior to death cannot be used.

N/A

4) List the number of animals of each species to be used in Class E procedures for each year.

	Number Of Animals			
Species and Procedure	**Year 1**	**Year 2**	**Year 3**	**Total**
Zebrafish mutagenesis		**20**		**20**

-- end of Attachment F --

Attachment G: Animal Restraint

table of contents (attachments are to be completed only as required by the checklist)

Short periods of restraint such as those required for routine veterinary or husbandry procedures do not require documentation or IACUC approval.

- What is the maximum length of time any one animal would be restrained within a 24-hour period?

2) Method of restraint:

Manual.

Chemical (i.e., tranquilizers and/or anesthetics agents. Complete Attachment C).

Restraint device or cage. Describe the device or cage.

3) Animals should be gradually trained to accept extended restraint periods. Please describe your training program.

4) Justify the need for prolonged restraint, and address why alternatives to prolonged restraint are inadequate.

-- end of Attachment G --

Attachment H: Infectious Agents, Biohazards, Recombinant DNA

table of contents (attachments are to be completed only as required by the checklist)

If the project requires approval for any of the agents below, a copy of the approved safety form from the appropriate safety committee must be provided before activation of the protocol, with the exception of transgenic and knockout mice, as work can proceed on these while the application is pending.

1) Identify the agent in the appropriate category. (Pathogen)

	Infectious agents. Identify:	

For forms, http://www.cwru.edu/finadmin/does/web/Forms/PDFdocs/IACUCPathProt.pdf

X	Hazardous chemicals, including chemical carcinogens. (Carcinogen) Identify:	N-ethyl-N-nitrosourea (ENU)

For forms, contact G. David McCoy 368-3233, gdm@po.cwru.edu.

X	Recombinant DNA. (IBC) Describe:	zebrafish promoter coupled to green fluorescent protein, a non-toxic reporter protein. Note that "Generation of transgenic flies or fish unless the source of DNA is from a classified pathogen" are exempt experiments not requiring registration with the IBC (see http://ora.ra.cwru.edu/orc_rdna_ibc.asp#Experiments)

Production of transgenic and knockout mice needs to be approved for Recombinant DNA, in accord with NIH policy. For forms, http://ora.ra.cwru.edu/maim.ibc

	Importation of Biohazardous Animals. (Non-Standard Vendor) Describe:	

The importation of animals which present a biohazard must be approved by the CWRU IBC. For forms, http://ora.ra.cwru.edu/main_institutional_biosafety_committee_page.htm

	Radioactive Materials. Describe:	

For forms, http://www.cwru.edu/finadmin/does/web/RadSafety/application.pdf

2) Specify the containment methods to be followed in protecting other research animals and personnel from any of the agents listed above. Describe the procedures required for the safe handling and disposal of contaminated animals, caging, bedding, food and materials associated with this study. Describe methods for removal of radioactive waste and monitoring of radioactivity, if applicable.

ENU mutagenesis: For more detail on ENU mutagenesis, see attached protocol. After the protocol is complete, treated fish are washed extensively over several days to remove the carcinogen. They will be placed into the quarantine rack, which will be cleared of all other fish stocks. Netting of the ENU fish

will be done with separate nets that are set aside for the mutagenized fish, and personnel will wear appropriate protective equipment (gloves, lab coat) when handling fish.
Transgenesis: The transgenic fish will be expressing Green Fluorescent Protein, which is not hazardous.

3) Describe the expected physical or physiological consequences to the animals due to administration of pathogens, hazardous chemicals, carcinogens, transgenes, or mutation(s).

ENU mutagenesis: During the treatment, signs of distress include lethargic swimming, or failure to swim. If this occurs before the treatment is finished, fish will be removed to a recovery tank.

Transgenesis: There are no consequences to making a transgenic line that expresses Green Fluorescent Protein

4) Describe any special care or monitoring that the animals will require.

ENU mutagenesis: There is a potential that the ENU-treated fish will become sick with opportunistic infection or with tumors. They will be monitored at least twice a day. Fish that lose weight or appear sick or skinny will be isolated, and given extra brine shrimp.

Transgenesis: No special care is required.

5) How long will the animals be maintained after introduction of the agent?

The treatment lasts one week. The fish will be maintained for up to one year following the treatment.

6) Describe the criteria for interventional euthanasia. *(For example, signs of extreme distress in rodents include hunched posture, disheveled coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, respiratory problems, and solid tumor growth.)*

If fish become sick as a result of the ENU treatment, the will first be treated as described above. If this fails, they will be euthanized. Fish will also be euthanized if they show tumor growth greater than 10% of body weight. Signs of illness include inability to swim properly, failure to feed, and discoloration of the skin or scales.

-- end of Attachment H --

Attachment I: Tumors In Animals

table of contents (attachments are to be completed only as required by the checklist)

IACUC guidelines for tumor inoculation: http://iacuc.cwru.edu/policy/tumor_inoculation.html

The NIH Office of Animal Care and Use (OACU) and the CWRU IACUC discourage the use of the ascites method for propagation of monoclonal antibodies. If the ascites method must be used, you must provide a justification (question 6, below), and procedures should be in accord with the NIH guidelines: http://iacuc.cwru.edu/policy/nihpolicies/ascites.htm
Resources and information on in vitro production of monoclonal antibodies and ascites production are available at: http://iacuc.cwru.edu/policy/mabs.html, http://www.nal.usda.gov/awic/pubs/antibody/, http://grants1.nih.gov/grants/olaw/references/dc98-01.htm

1) Host animal (species and strain):

2) Is this a human primary tumor cell? Yes No

Human primary cell lines require a pathogen safety form.

3) Is the tumor derived from a rodent or has the tumor been passaged through rodents? Yes No

Tumor cell lines derived from or passaged through rodents must be tested by Rat or Mouse Antibody Production or PCR to detect rodent infectious agents (MAP or RAP) before being implanted into rodents. Tissue culture Mycoplasma testing does not meet the testing requirements.

4) If derived from rodents or humans, has the material been tested for pathogens? Yes No

A copy of RAP, MAP or PCR test results must be attached to this protocol.

5) Describe criteria for interventional euthanasia (see the guidelines at the links above).

6) If the size of the tumor will exceed 10% of the body weight of the animal provide scientific justification.

7) If the ascites method is used for the production of monoclonal antibodies, justify why *in vitro* production would not be adequate.

Describe the priming, inoculation(s), number of taps and monitoring of animals during ascites production. It is highly recommended that the NIH guidelines http://iacuc.cwru.edu/policy/nihpolicies/ascites.htm be followed.

-- end of Attachment I --

Attachment J: Administration of Exogenous Substances or Tissues

table of contents (attachments are to be completed only as required by the checklist)

For tumor studies and ascites production, please use Attachment I instead.

Use a separate Attachment for each recipient species

1) Recipient species:

2) Identify the substance and its source. Describe whether the substance is biological and give its source (species, preparation, synthesis, purification, etc as appropriate). If the substance is a proprietary compound or agent that cannot be explicitly described, address the issues listed in Attachment J, Item 3 (Proprietary Substances). If the substance is of biological origin, state what steps have been taken to render it free of adventitious infectious agents to man and the recipient species or provide documentation that the substance has been tested (via PCR, MAP test, RAP test, etc.). Animals given biologic material with an unknown infectious potential will be maintained in quarantine for the duration of the study.

3) Proprietary Substances:

a) Provide written assurance that the substance, at the levels/doses to be used in the animal protocols, is not toxic or otherwise harmful to animals/humans involved in the proposed research. This assurance should briefly describe how the substance has been subjected to standard *in vitro* tests for toxicity or mutagenicity.

b) If evaluation of *in vivo* toxicity or potential harmfulness of the proprietary substance is the goal of the proposed animal experimentation, indicate how the protocol will estimate the maximum tolerated dose (MTD) and no effect dose level (NOEL) for that substance in the animal subjects.]

4) Administration.

Dosage:

Volume:

Route:

Frequency:

Animals will be anesthetized or sedated for administration ☐ Yes ☐ No

If *Yes*, complete Attachment C

5) How long will animals be maintained after administration of the substance, and what are the experimental endpoints?

6) Will physical or physiological effect(s) (e.g., inflammation, decreased blood pressure, increased heart rate, etc.) likely result from this treatment? If *Yes*, describe in detail expected physical or physiological effects, including expected severity, duration and frequency. For introduction of cells and tissues, what will be done to prevent graft rejection?

7) Describe the plan for monitoring the animals, including frequency and length of observation, and the criteria for termination of the experiment through interventional euthanasia. *(Examples of appropriate criteria that should be considered include a weight loss limit as a percentage of initial or expected body weight, allowable durations of anorexia, allowable tumor size or total tumor burden expressed as a percentage of body weight, the presence of health problems refractory to medical intervention, and severe psychological disturbances. Other criteria appropriate for the species under consideration should also be considered.)*

-- end of Attachment J --

Attachment K: Irradiation

table of contents (attachments are to be completed only as required by the checklist)

Complete a separate Attachment for each procedure or species.

1) Describe the method of irradiation of the animals.

Species:

Radiation Source:

Dose:

Frequency of Irradiation:

Anatomical site irradiated:

2) Expected physical and physiological effects:

3) Describe the experimental endpoints, and how long the animals will be maintained after irradiation.

4) Give the criteria for interventional euthanasia of animals too sick to continue in the study.

5) Describe the plan for monitoring the animals, including the length and frequency of observation, and clinical signs which will be monitored.

-- end of Attachment K --

Attachment L: Alternative Housing

table of contents (attachments are to be completed only as required by the checklist)

All locations where animals are kept longer than **12 hours** *must be approved by the IACUC. All locations for housing must meet legal requirements for the housing of animals. The IACUC must inspect these housing areas prior to use, and at each Semiannual Inspection. The USDA regulations on the housing of animals are available at* http://iacuc.cwru.edu/resources/cfr.html.*General guidelines to animal housing are available at* http://iacuc.cwru.edu/guide/guide3a.html#aehm

1) Location of alternative housing.

Building: Millis Room number: 126B

2) Justify your need for alternative housing.

This room contains a state of the art aquatic facility for raising and maintaining zebrafish. There is no other housing for zebrafish on campus.

3) Maximum period that animals will be housed here.

They will be housed in this facility their whole lived, typically 2-3 years.

Please attach a copy of your Standard Operating Procedures for extramural housing.

-- end of Attachment L --

Attachment M: Manipulation of Diet or Environment

table of contents (attachments are to be completed only as required by the checklist)

Describe dietary restrictions or manipulations, and environmental manipulations, using a separate attachment for each species and procedure. *General guidelines to dietary restriction are available at:* http://iacuc.cwru.edu/guide/guide2.html#mcua.

Species and Procedure:

1) Describe the dietary manipulations or manipulation of the environment. Describe relevant parameters including duration, nutrients removed or supplemented, weight criteria, endpoints, etc.) Describe alterations to lighting, temperature or other environmental variables or conditions and their duration.

2) What physical or physiological effect(s) will result from this treatment and what is their expected duration?

3) If pain, distress or significant symptomatology are possible, list the criteria to be used to determine when euthanasia is to be performed. *(For example, signs of extreme distress in rodents include hunched posture, disheveled coat, reduced food consumption, emaciation, inactivity, difficulty in ambulation, respiratory problems, and solid tumor growth.)*

-- end of Attachment M --

Attachment N: Behavioral Tests

table of contents (attachments are to be completed only as required by the checklist)

NIH guidelines for manipulation of diet in behavioral studies
http://iacuc.cwru.edu/policy/nihpolicies/dietctrl.htm.

Describe Behavioral Tests, using a separate attachment for each species or procedure.

Species and Procedure:

Complete the appropriate sections below.

Conditioning

1) What is the purpose of the conditioning?

2) Describe the reinforcement techniques which will be used.

- No reinforcement.
- Food reward. Describe:
- Electrical shock. Give strength and duration:
- Food deprivation. State duration:
- Water deprivation. State duration:
- Other. Give strength and duration:

3) What criteria will be used to monitor the health and welfare of the animals?

Other Behavioral Training And Testing

1) What is the purpose of the training and testing?

2) Describe the procedure, detailing any aversive stimuli or conditions, including their strength and duration. If aversive stimuli or conditions will be used, describe how the health and welfare of the animals will be monitored.

-- end of Attachment N --

Attachment O: Other Procedures

table of contents (attachments are to be completed only as required by the checklist)

For procedures not fitting the preceding categories, give the following information.

Complete a separate Attachment for each species or procedure.

1) Species and Procedure:

2) Describe the procedure in detail.

3) How long will the animals be maintained after the procedure?

4) Describe the plan for monitoring the animals after the procedure, including frequency and length of observation, and clinical signs which will be monitored.

5) Describe the experimental endpoints.

6) What are the expected physiological or clinical effects of these procedures?

7) Describe the criteria for interventional euthanasia of animals too sick to continue in the study.

-- end of Attachment O --

Attachment P: Field Studies

table of contents (attachments are to be completed only as required by the checklist)

If animals in the wild will be used, identify the species, describe how they will be observed, any interactions with the animals, whether the animals will be disturbed or affected, and any special procedures anticipated. Indicate if Federal permits are required and whether they have been obtained. Information on regulations governing field studies: http://iacuc.cwru.edu/guide/guide1.html#finv.

-- end of Attachment P --