IACUC Application (Version 1.1)

1.0 General Information

***Please enter the full title of your study:**

Marine Toxicology in Marine Mammals

***Please enter the Short Study Title you would like to use to reference the study:**

Marine Mammal Toxicology
* This field allows you to enter an abbreviated version of the Study Title to quickly identify this study.

2.0 Add Department(s)

2.1 List the departments associated with this study. Add the Principal Investigator's department as the PRIMARY DEPARTMENT. For research conducted at a Norton facility add: Norton Healthcare. For research conducted at a Jewish Hospital/KyOne facility (e.g. Frazier, St Mary's, etc) add: Ky One Health. For research conducted at University Hospital /James Graham Brown Cancer Center add: University Hospital:

Primary Dept?	Department Name
◉	**U of L** - *42* - Pharmacology

3.0 Assign key study personnel (KSP) access to the project

3.1 *Please add a Principal Investigator for the study:

Wise, John P

3.2 In this section, please add any project personnel that needs to have access to the submission or will need to approve the submission. In the case of the Undergraduate Research Symposium, your mentor and co-authors would be included in this section.

A) Additional Investigators

Wise, Sandra S
Co-Investigator

B) Research Support Staff

Croom-Perez, Tayler
Research Associate
Lopez Montalvo, Carlos A
Research Associate
Lu, Haiyan
Research Associate
Speer, Rachel M
Research Associate
Toyoda, Jennifer H

Research Associate
Young, Jamie L, M.S.
Research Associate

3.3 ***Please add a Study Contact. The Study Contact(s) will receive all important system notifications along with the Principal Investigator. If applicable, please add Kentucky One Health, Norton Healthcare or UMC Research as a study Contact. Adding someone here does not add them as study personnel.**

Wise, John P
Wise, Sandra S

The Study Contact(s) will receive all important system notifications along with the Principal Investigator. If applicable, please add Kentucky One Health, Norton Healthcare or UMC Research as a study Contact.

3.4 Please select the Designated Approvers:

Add the name of the individuals authorized to approve and sign off on this protocol.

4.0 IACUC Form Type

4.1 Please indicate the type of form you are submitting

- (•) Live Animals
- ( ) Tissue Only

- **Live Animals** - Use this form if you will be using any live animals at UofL, including description of Core Animal Laboratories.
- **Tissue Only** - Use this form if you will be using ***fresh*** or ***frozen*** (not fixed) tissues, organs, or carcasses obtained from animals outside of UofL or from animals assigned to, and euthanized by, PIs with other IACUC *Proposals*. If you plan to handle live animals in any way, a *Live Animal* form must be used. [Link to Policy]

5.0 Emergency Contacts

5.1 Indicate the Key Study Personnel who will act as emergency contacts

Study Personnel	Phone Numbers
Wise, John P, Ph.D.	During Work Hours 5058528524 After Hours 2076328308
Wise, Sandra S	During Work Hours 2073185863 After Hours 2073185863

6.0 Welcome Page

6.1

Welcome to IACUC @ Louisville Replacement Reduction Refinement Responsibility Do I need an IACUC protocol? For best viewing of form materials, it is recommended that you expand your window as much as possible.

7.0 Proposal Purpose / 3 Year Renewal

7.1 Proposal Purpose (Check all that apply)

- [x] Research using live animals
- [x] Teaching and Training
- [ ] Core Animal Laboratory Proposal

Is this a 3-Year Renewal?

(•) Yes ( ) No

7.2 Previous Proposal number:

16506

7.3 Scientific Review and Funding Source(s)

Scientific review has been, or will be, performed by an internal or external review panel before experimentation begins. Select all that apply:

- [x] Federal Agency
- [ ] State Agency
- [ ] Private Foundation
- [ ] U of L Review Panel
- [ ] Industry Sponsor
- [ ] Department Chair or Designee
- [ ] Other

List any funding sources applicable to this Proposal

Grant No.	Sponsor	Title
Not Yet Available		

8.0 Species to be used in this Proposal (Only ONE Allowed)

8.1 Select species in drop down list below. Note: If the species you would like to use is not listed, please contact the IACUC Office (IACUC@Louisville.edu, 852-7307) so that it may be added to the form.

Select Species to be used in the Proposal (for Field Study, select "Wild Caught Species")

Wild Caught Species

8.2 Justification of Selected Species

Give rationale for the selection of this species. In all cases, a "lower" species should be given primary consideration.

This project obtains a skin biopsy from whales to better understand ocean pollution and the effects of climate change. As marine mammals intergate exposure routes from the air, water and diet they best reflect humans in the environment. Moreover, the data are also used to support the conservation of each marine mammal species by providing species specific data that cannot be accomplished from a lower species. It has been the observation of many that the marine mammals are not notably disturbed by the sampling. Animals are neither captured nor sacrificed in these efforts. Traditional methods to obtain baseline toxicological data from whale have relied mainly on postmortem studies of stranded animals, making it difficult to judge the pathological significance of tissue contamination and the extent to which living populations are being affected. While sloughed skin from whales has been used successfully in genetic studies, only samples containing blubber can be used in analyses of organic pollutant levels and only living tissue can be used to culture cells to develop *in vitro* approaches. Biopysing is the least invasive method available to gain this insight. The International Whaling Commission has recommended biopsy sampling techniques as important tools for obtaining information valuable to the management and conservation of cetaceans. Responsibly conducted biopsy sampling is not likely to have any long-term or even short-term detrimental effects on the individuals or populations (International Whaling Commission 1991). Under these circumstances the proposed method is considered desirable and necessary for data collection.

The proposed activities are designed to maximize the information obtained while minimizing disturbance to the animals. The primary goal of our research is to better understand toxicant loading in various whales while increasing our knowledge of population dynamics, behavioral and feeding dynamics, and ultimately by analyzing samples. We will also use the samples we collect to develop whale cell lines to be used as tools to study the effects of environmental pollutants and the mechanism of poisoning by these pollutants. To develop these requires fresh tissue from the skin blubber interface and rapid access to a laboratory.

8.3 List strains, lines, stocks, breeds, *etc*. in the table below. - NOTE: it is acceptable to group multiple strains/lines on a single row AS LONG AS the description of potential adverse phenotypes is the same for all strains/lines included in that row. For example, you may be using a number of transgenic lines. You are not required to list every line as long as the expected phenotypes are the same.

General Information	Strain Origin	Decription of Strain and Adverse Phenotypes (if any)
Strain or Lab Name Sperm Whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Blue whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Bryde's whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name	( ) Commercial Vendor	

Dwarf sperm whale Is strain irreplaceable? (•) Yes ( ) No	( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name False kller Whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Fin whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Gray whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Humpback whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Killer whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Minke whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name	( ) Commercial Vendor ( )	

Long finned pilot whale Is strain irreplaceable? (•) Yes ( ) No	( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Pygmy sperm whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Sei whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Southern right whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Short finned pilot whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A
Strain or Lab Name Beaked whale Is strain irreplaceable? (•) Yes ( ) No	( ) Commercial Vendor ( ) Import from Colleague ( ) Existing colonies bred at UofL ( ) New genetically-modified line created at UofL (•) Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) N/A

8.4 Are any of the strains/lines above genetically-modified (transgenic, knock-out, knock-in, etc.)?

( ) Yes (•) No

8.7 Are you willing to share tissues from animals or transfer live animals to other colleagues at U o f L?

(•) Yes ( ) No

8.8 Related Proposals Are the experiments similar or related to those described in another proposal for a different species? For example, multiple species may be needed to satisfy regulatory requirements.

○ Yes ◉ No

9.0 Lay Project Summary (250 words max)

9.1 Provide a non-technical summary of the proposed research, using language that a person not trained in biomedical sciences can understand. Describe the significance of the project and the reasons for which it has been proposed. This description should allow the reader to weigh the potential human or other animal health benefits against animal welfare concerns.

Lay Summary (250 words maximum):

This study builds on previous work conducted by the Wise Laboratory. Marine mammals are sentinels for the health of the world's oceans, because they reflect the threats of illegal hunting, habitat destruction, interference from vessels, and toxic pollution. Our goals are to document the extent and frequency of pollutants adn DNA damage in marine mammals and develop cell ines to study the impact of pollutant on marine mammal cells. This work requires collecting a skin/blubber samples from live whales. All biopsy samples will be analyzed for the presence of contaminants (e.g. metals) and DNA, RNA and protein will be isolated in order to determine: 1) which classes of pollutants are elevated around the world; 2) which pollutants pose the largest risk to whales; 3) which responses made by whales to pollutants are similar to responses made by humans; and 4) the locations of pollution hotspots around the world.

10.0 Technical Summary (800 words max)

10.1 Technical Summary Describe specific aim(s) and the long-term goals of the project.

Metals are environmental pollutants and are known to be genotoxic in rodents and humans. Recently, we demonstrated that metals are a concern for whales as well. Thus, the goal of this research is to determine the potential impacts of metal pollution on whales. We will consider metal accumulation in whale skin, DNA damage in whale skin and the ability of metals to damage DNA in cultured whale cells. These endpoints will be compared from multiple individuals of different ages and gender and over time. Specifically, we will investigate the following hypothesis: **Environmental metal pollutants accumulate in whales and damage whale DNA.**

We will test this hypothesis through the following two specific aims:

Specific Aim #1: Determine Tissue Levels of Metals in Whales

Currently, the extent of metal bioaccumulation in whales is poorly understood. We have found significant site specific variations. For example, we sampled s from the Gulf of Maine in 2010 and found particularly high levels of Cr and Ni. The Cr levels were 2.6-times higher than the mean level found for another baleen whale inhabiting the same waters. By contrast, in bowhead whale skin tissue collected in Barrow, Alaska, we found a mean Cr level 40-times lower (0.45 ppm) than the mean Cr level in s (18.2 ppm). These data suggest variability is species and location. Time trends are unknown. Accordingly, this aim will investigate metal levels in whales with particular attention paid to Cr and Ni. Twenty-six elements will be measured including: Aluminum, antimony, arsenic, barium, beryllium, cadmium, Cr, cobalt, copper, gold, iron, lead, lithium, magnesium, manganese, mercury, molybdenum, Ni, selenium, silver, strontium, tin, titanium, uranium, vanadium and zinc. We will measure these levels over a period of 10 years of the project to determine the trends over time. This approach will give us a perspective on differences over time and across individuals, age and gender.

Specific Aim #2. Determine DNA Damage Levels in Whale Tissue and Evaluate the Genotoxicity of
Cr and Ni in Whale Cell Lines

Primary whale cell lines can be established from the cells at the interface of the skin and blubber. We have optimized protocols to develop these cell lines from whales. We will use these cell lines in two ways. First, by isolating whale chromosomes from these cells, we can measure the amount of DNA damage in whale skin tissue. These data will give us an indication of how much DNA damage the whales are experiencing. By comparing the amount of damage in individual whales, we can ascertain if some whales have more damage than others. By comparing the amount of damage in each year of the project, we can assess if damage levels are changing over time. Our second use of the cell lines will be to determine the cytotoxic (cell death) and genotoxic (DNA damage) responses to metals in them using state-of-the-art toxicological techniques.

Cytotoxicity is important because it shows biological activity and chronic production of cell death can lead to fibrosis, scarring and decreased organ function. In addition, determining a cytotoxic range will guide the other specific aims by providing a relative context for the genotoxicity and carcinogenicity and ensure that positive results are not at concentrations that are supratoxic (i.e. doses that cause greater than 99% cell death). Some level of toxicity will occur as DNA damage itself can lead to cell death, so we will study doses of low (< 10%) medium (~50%) and high (75-99%) cytotoxicity to ensure we have considered a full range.

Genotoxicity is important because all life depends on its DNA. DNA damage is well understood to be a short-term test for a myriad of diseases and negative health outcomes including both cancer and reproductive toxicity. DNA damage is a required hazard characterization by the U.S. Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) as part of their risk assessments. We measure of DNA damage because chromosomal rearrangements are commonly found in cancers and reproductive and developmental disorders and are a required hazard characterization for the EPA and the FDA and are often cited in the workplace health standards for the Occupational Safety and Health Administration (OSHA). The effects of metals in whale cells have generally not been studied, beyond our initial reports, but they are known to damage DNA in rodents and humans. It may seem reasonable to extrapolate from humans or rodents to marine mammals, but it has been shown with marine mammals that there are such significant differences in potency among species and a level that is highly toxic to one species may not be toxic to another. For example, the concentrations of cadmium in the kidney cortex of a bowhead would kill most land dwelling animals, but the bowheads seem unaffected. It may even seem reasonable to extrapolate from right whale cells to other whales, however, we have found that Cr does not break chromosomes in bowhead whale cells. Thus, its effects vary based on species. It is essential to know the differences to better assess the risk to the population.

11.0 Justification for Animal Use

11.1 Justification for Animal Use

Describe why the Proposal requires the use of animals, as opposed to *in vitro* or *in silico* approaches. (250 words or less)

The primary goal of our research is to better understand toxicant loading in various marine mammal while increasing our knowledge of population dynamics, behavioral and feeding dynamics, and ultimately by analyzing samples. Current. *in vitro* and *in silico* approaches cannot accurately predict how much pollutant has accumlated in a marine mammal nor can it predict how much they have been exposed to. Biopysing is the least invasive method avialable to gain this insight. Though it should be noted we do develop *in vitro* models from the biopsies. The International Whaling Commission has recommended biopsy sampling techniques as important tools for obtaining information valuable to the management and conservation of cetaceans. Responsibly conducted biopsy sampling is not likely to have any long-term or even short-term detrimental effects on the individuals or populations (International Whaling Commission 1991). Under these circumstances the proposed method is considered desirable and necessary for data collection. The proposed activities are designed to maximize the information obtained while minimizing disturbance to the animals. We note, we will also use the samples we collect to develop marine mammal cell lines to be used as *in vitro* tools to study the effects of environmental pollutants and the mechanism of poisoning by these pollutants. To develop these cell lines requires fresh tissue from the skin blubber interface and rapid access to a laboratory.

12.0 Assurance of Non-Duplication

12.1 Assurance of Non-Duplication

Provide a *written assurance* that the proposed activities do not unnecessarily duplicate previous or ongoing experiments. Describe methods and sources (journals, abstracts, *etc*.) to support this assurance. Include the date the search was performed, years included in the search, and keywords used. [Examples can be found in the help button to the right. Links to Databases: PubMed; Google Scholar]

Database Info	Keywords and Description of Results
PubMed 10/12/2018	List Keywords whale, dolphin, marine mammal, toxicology, metal Describe Results There are sporadic reports of metals in marine

Years Searched ALL years in database	mammals. None that evaluate similar locations over time and none on metals from our study sites. None that evaluate a population over time. Our study appears to be the only prospective study monitoring free ranging animals for our field sites, possibly anywhere, which is not surprising given the difficulty in obtaining samples.
Google Scholar 10/11/2018 Years Searched All years in database	List Keywords whale, dolphin, marine mammal, toxicology, metal Describe Results There are sporadic reports of metals in marine mammals. None that evaluate similar locations over time and none on metals from our study sites. None that evaluate a population over time. Our study appears to be the only prospective study monitoring free ranging animals for our field sites, possibly anywhere, which is not surprising given the difficulty in obtaining samples.

13.0 Experimental Groups

13.1 Describe Experimental Groups in Table Below

Provide a description of each experimental group in enough detail that reviewers can understand what happens to each animal assigned to that group. - Group Name or Number: May be a user defined number or brief descriptive name. *Example:* heart transplant; dietary restriction. - Pain Class: Class 0 - Animals will be acquired/held, but not used or manipulated in any way. - Class I - Animals w experience no pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. - Class II - There is a potential for pain or distress which is minimized or eliminated by anesthetics, analgesics, and/or tranquilizers. Examples include induction of cancer/tumors, biopsy, endoscopy, vascular cut-down, footpad injections, use of adjuvants, implantation of chronic catheters, as well as survival and non-survival surgery. - Class III - Animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. Examples include exposure to agents or radiation levels that cause serious illness, research involving significant stress, or procedures involving prolonged restraint. A written justification (including supporting sources, journals, abstracts, etc.) for withholding pain-relieving agents must be provided in a following section. - Treatment/Description: Devise a brief descriptive title for each procedure and describe treatments each animal in this group will receive, including the time period between procedures. For studies in which the exact sequence or number of procedures cannot be determined, include a range of potential time periods and note the maximum potential procedures to be performed on the animals in that group. *Example:* This group will receive heart transplant followed by stem cell treatments. The stem cel will be given IV by tail vein injection 10 days after heart transplant surgery. In later sections you will describe "heart transplant" (Survival Surgery), "stem cell (IV) injection," etc. in the "*Procedures Table*" below. Note: You may also inc in each group a small number of variables as long as it is very clear from the description what will happen to the animals in these groups and the sample size used. Example: Following an acclimation period of 14 days, animals will receive treatment with XJ-47 in the drinking water at 3 levels (0, 15, and 30 mg/ml) for 30 days. At this point, we will perform intrasplenic implantation of WKW-95 (or control group) and follow groups of animals for an additional 30, 60, or 90 days until euthanasia and tissue collection. 10 animals/group x 3 dose groups x 2 implantation groups x 3 time points = 180. - Number of Animals in This Group: The number of animals needed in the group is generally the sample size ("n"). If multiple variables were included in the "Treatment/Description," then this number may be a multiple of the sample size.

Group Name or Number and Pain Classification	Treatment/Description	Number of Animals in This Group
	We biopsy all whales we come upon regardless of age or length as even the calves are large enough to handle a biopsy dart. Animals are approached at slow speed to within 20 meters of the research vessel or from smaller inflatable boats. Once within range, a biopsy is taken utilizing a biopsy dart	

Group Name or Number whale Pain Class (See definition above) Class I	and crossbow. A single biopsy will be taken from an individual animal. Notes and digital documentation on the behavior of the animals will be made from the moment they are sighted, during the approach and darting, and after the darting. Images are immediately reviewed for distinctive markings and pigmentation patterns to assist the darter in avoiding re-sampling the same animal. We believe this research does not expose the subject animals to more than momentary or slight pain or distress as evidenced by observations of greater startle responses from darts that missed than the observations of pain responses to the darts that were successful in retrieving biopsies.	2160

13.2 Will You Maintain a Breeding Colony?

○ Yes ◉ No

13.4 Total Number of Animals Requested **Remember to include those from Breeding colony if relevant.**

2160

13.5 Animal Number Justification ***Provide specific justification for the number of animals to be used in each group i.e., the sample size*. This must address statistical significance as it relates to experimental design. Please be as detailed as possible. Statistical power analysis or calculation given a known or expected error/failure rate and difference between groups is preferred, but experience with the model may be acceptable. [IACUC Fact Sheet] For teaching or training, the number of "students" per animal and expected number of training exercises may suffice. Note: Details of treatments, procedures, and other experimental variables should be included in the "Treatment/Description" column in the table above.**

We are permitted by National Marine Fisheries Service (NMFS) for 80 samples per year for eahc of nine whale species per year (80 samples X 9 species = 720 sampled animals). This number per species results from a combination of the likelihood of encountering such species and the sample size desired to detect population structure or differences in contaminant burden. Research permits and associated CITES permits are obtained using the guidelines set forth in our NMFS permits from various jurisdictions for research activities carried out in their respective territorial waters. We intend to take only one biopsy sample from each whale encountered using a maximum of only two attempts per animal. Our previous success rate for biopsy sampling was greater than 75%. Our researchers' accumulated experience gives us confidence to anticipate the same success rate, or better.

We have been permitted for more than 80 samples for humback whale (160), sperm whales (400), killer whales (100), dolphins (160), pilot whales (280) beaked whales (140) and southern right whales (200) per year . We estimate these samples by looking at the minimum number of samples needed to detect differences in the values of stable isotopes between years. In previous analysis (unpublished) we detected an isotopic difference between years of 0.9≈ (ppm). If we accept this as our margin of error and want to collect samples to detect possible differences of similar magnitude between years with a 95% confidence we need at least 150 - 200 samples.

14.0 Field Studies

14.1 Does proposal involve field studies?

(•) Yes ( ) No

14.2 Notes

- *This section pertains to the capture of wild animals*. A *Proposal* must be completed for any study that involves an invasive procedure, harms, or materially alters or influences the behavior or activities of an animal under study. Observational studies on free-living wild animals in their natural habitat are exempt from IACUC oversight.
- All procedures to be performed should be described in the "Procedures Table" section of the *Proposal* form, including "Non-Surgical Procedures" (*e.g.,* blood or other tissue collection, animal identification/marking) and "Survival Surgery" (*e.g.,* telemetry device implantation).
- Method(s) of euthanasia or release should be described in "Euthanasia or Other Disposition" section of the form and should also include the method of euthanasia that will be used for animals injured during capture procedures, even if not anticipated.

Click on the link below to be redirected to AAALAC International's Reference Resources, which contains the several species-specific guidelines for conducting field studies under the "Species Specific" section.

Link to relevant AAALAC Reference Resources

14.3 Field Studies: Species **List the target (desired) and non-target (incidental capture; not the focus of the study) species involved. Include an estimate of the numbers of animals involved in the three-year approval of this *Proposal*.**

Target/Non-Target	Common Name	Scientific Name	Approx. Number to be collected
Target Species	sperm whale	Physeter macrocephalus	400
Target Species	blue whale	Balenoptera musculus	80
Target Species	Bryde's whale	Balenoptera edeni	80
Target Species	dwarf sperm whale	Kogia simus	80
Target Species	false killer whale	Caperea marginata	80
Target Species	fin whale	Balenoptera physalus	80
Target Species	gray whale	Eschrictius robustus	80
Target Species	humpback whale	Megoptera novaeangliae	160
Target Species	killer whale	Orcinus orca	100
Target Species	minke whale	Balenoptera acutorostrata	80

Target Species	long finned pilot whale	Globicephala melaena	140
Target Species	pygmy sperm whale	Kogia breviceps	80
Target Species	sei whale	Balenoptera borealis	80
Target Species	southern right whale	Eubalena australensis	200
Target Species	short finned pilot whale	Globicephala macrorhynchus	140
Target Species	beaked whale	Mesoplodon sp.	140
Target Species	Dolphin	all	160

14.4 Field Studies: Location

Describe with as much detail as possible the specific site(s) where the field studies will be conducted.

Current field sites include the Gulf of Maine, Gulf of Mexico, the Gulf of California, the Bay of Biscay, the waters around Spain, Iceland, Columbia, Antarctica, and Norway and the water of the Florida and California coasts

14.5 Field Studies: Capture and Handling

Describe the method(s) of animal capture and handling. Include details such as the frequency of checking traps and precautions for preventing capture of non-target species. For each capture method, estimate the expected injury and mortality (if any). For studies involving re-capture, describe the expected time period between captures.

No animal capture or handling are proposed.

14.6 Field Studies: Animal Holding/Housing

Specify the duration of time animals will be held in captivity prior to euthanasia or release. Note that retaining animals for greater than 12 hours may be considered “Overnight”, "Temporary" or "Satellite Housing”; justification and other consideration for such areas must be included in the Study Sites section of the *Proposal* form.

Animals will not be held or housed.

14.7 Field Studies: Animal Transportation

If live animals are to be transported from the capture site, describe the transportation method (private vehicles, road, air, etc.), transport enclosures, estimated time in transit, and animal care in transit.

Animals will not be transported.

14.8 Field Studies: Population Impact

Describe any anticipated impact on local populations of target and non-target species.

No negative impacts are anticipated on the local population.

14.9 Field Studies: Permits

Please provide permit numbers for local, state, federal, and/or international regulatory agencies.

Permit No.	Local (specify)	State (specify)	Federal	International (specify)

18636			National Marine Fisheries Service	
21329			National Marine Fisheries Service	

If permits are not required, please explain:

14.10 Field Studies: Occupational Health Considerations

Principal investigators conducting field research should be knowledgeable about relevant zoonotic diseases and associated safety issues (e.g., traumatic injuries, use of venomous species). Please confirm your acknowledgement in the following checkbox and describe training of personnel and other methods used to ensure personnel safety.

Describe training of personnel and other methods used to ensure personnel safety.

All personnel are trained in occupational health and safety at the University of Louisville. We do not capture or hold the animals, thus exposure is limited to the small biopsy that is retrieved. This sample is handled in a BSL-2 manner with gloves and manipulation of it is miminal. All perosnnel are advised in the risk of poential soonotic diseases and will have the species sampled listed in their animal cintact healrh survey.

- [x] As Principal Investigator (or designee), I acknowledge my responsibility in discussing potential zoonotic diseases associated with these studies with all personnel. I will ensure that Periodic Animal Contact Health Surveys for all participants in this Proposal include the species used herein so that Campus Health Services may perform an individual medical risk assessment.

15.0 Procedures

15.1 Procedures Checklist

Indicate the types of procedures used in this proposal (check all that apply). Definitions: *Minor*: Any surgical intervention that does not expose a body cavity and causes little or no physical impairment. Example: laparoscopy; wound suturing; peripheral vessel cannulation; percutaneous biopsy; routine farm-animal procedures such as dehorning, castration; prolapse repair; and most procedures done on an "outpatient" basis in veterinary clinical practice. *Major:* Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for inducing permanent physical or physiologic impairment; and/or any procedure associated with orthopedics or extensive tissue dissection or transection. - For *Multiple Survival Surgical Procedures, i.e.*, surgical procedures that will be performed under separate anesthetic periods from which the animal will recover from each anesthetic period, describe each surgical procedure separately (there must be two separate procedures in the "Procedures Table"). - Be sure to save often!

- [ ] None: Animals will be acquired/held and/or bred, but not used or manipulated in any way (exceptionally rare)
- [ ] Genotyping
- [ ] Individual Animal Identification
- [x] Non-Surgical: may require anesthesia, but do not involve a surgical incision. Examples include test article administration, behavioral assessment, tissue collection (prior to euthanasia), imaging, irradiation, etc.
- [ ] Surgery - Non-Survival: a surgical procedure, performed under anesthesia, from which the animal does not recover from the anesthetic (also known as terminal or acute surgery)
- [ ] Surgery - Survival: a surgical intervention from which the animal is expected to recover from the anesthesia
- [ ] Surgery - Multiple: TWO or more survival surgical procedures (major or minor) between which the animal recovers from anesthesia

15.4 Procedures

Note when anesthetics or analgesics will be used, DO NOT provide dose, route, frequency, *etc*., as these must be included in a later section.

	Description. Provide sufficient detail such that the reviewer can understand exactly what will occur

Procedure Type and Name (descriptive title used in EXPERIMENTAL GROUP section above)	and the potential impact of the procedure on the health and well-being of the animal. For surgical procedures include incision site, all tissue manipulations, temporary wound closures, etc. Indcate when anesthetics or analgesics will be used, but DO NOT include doses - these will be described in a later section.
Non-Surgical Procedure Name Skin biopsy	We will sample the whales by use of a NMFS approved biopsy dart and crossbow. The crossbow is a Barnett International RC-150 compound crossbow, with a maximum range of 37 m. The arrows are produced for cetacean biopsy sampling by Finn Larsen (CETA-DART). The arrows are 16 inches long with a carbon-graphite shaft, with a foam floatation collar just posterior to the tip and a flared tail that allow the darts to be spotted and picked up at the surface with a dip net, thus eliminating the use of a line retrieving system that may cause additional disturbance. The biopsy tip consists of a circular razor blade up to 40 mm long by 8 mm in diameter with three internal prongs to retain the skin/blubber sample. A conical float at the tip end adds buoyancy to the arrow after it bounces off the whale. This float also acts as a stop collar to prevent the dart from penetrating beyond the external skin/blubber layer of the animals. For the larger cetacean species, like P. macrocephalus, B. musculus, and E. australis, a 40 mm biopsy tip with an 8 mm diameter will be used. For the smaller species and calves more than six months of age, including O. orca, Delphinus delphins, and Z. cavirostris, a 25 mm tip with an 8 mm diameter will be used. After use the tips are thoroughly cleaned and then sterilized with ethanol. A single biopsy sample will be taken from individuals. The target area is the flank or back at about the middle of the body (Brown et al 1991). The darts take a sample that is 2-3cm long by less than 1cm in diameter. Notes and digital documentation on the behavior of the animals will be made from the moment they are sighted, during the approach and darting, and after the darting. Images are immediately reviewed for distinctive markings and pigmentation patterns to assist the darter in avoiding re-sampling the same animal. Each biopsy sample will be divided into 2-5 sub-samples and placed into containers labeled with unique serial number codes. These serial codes are alphanumeric with an alphabetic area code as well as the date of collection and the number of the sample in that area, and samples will continue to be catalogued in this manner. Detailed information for each sample is entered into a database including: date, location (latitude and longitude), species, estimated size or age class, sex (when possible), collector, observed reaction of the animal, and other pertinent remarks. Each sample obtained from a whale will be handled according to well-established toxicological protocols established by the National Center for Toxicological Research (NCTR) and the National Institute of Environmental Health

	Sciences (NIEHS). Sterile conditions are adhered to and after a sample is documented, it is either frozen or fixed in solution in preparation for the following three types of analyses. 1) The skin is used for genetic testing, and 2) for cell cultures, and 3) the blubber is tested for direct contamination. Samples not used for cell cultures are frozen immediately in a -20°C or -80°C freezer on the boat until its return to shore. The skin will be stored in plastic and the blubber in hexane-washed glass vials. Samples are shipped or hand delivered to The Wise Laboratory in the U. S. where they will be tested for contaminant burden using various methods, such as gas chromatography and inductively coupled mass spectrometry.

Project Participants

Provide name(s) (in order of greatest involvement) of individual(s) participating in or involved with this *Proposal* (experimental procedures, animal observation or care, *etc*.) and describe their role in the proposed study. PI and Co-PIs should also be included. The experience described for each person should match their role in the studies described in this *Proposal*. If not, please indicate how they will obtain sufficient training.

Name	Role (List Specific Procedures to be performed)	Experience
Wise, John P, Ph.D.	Sighting of whales, Skin biopsy and manipulation of skin biopsy after it is obtained. Trains new personnel in the proper techniques and procedures for sampling marine mammals and in handling marine mammal biopsies and their dissection and preservation.	See Online Training Log
Wise, Sandra S	Sighting of whales and manipulation of skin biopsy after it is obtained. Trains new personnel in the proper techniques and procedures for in handling marine mammal biopsies and their dissection and preservation.	See Online Training Log
Young, Jamie L, Ph.D.	Sighting of whales, Skin biopsy and manipulation of skin biopsy after it is obtained.Trains new personnel in the proper techniques and procedures for sampling marine mammals and in handling marine mammal biopsies and their dissection and preservation.	See Online Training Log
	Sighting of whales and manipulation of skin biopsy after it is obtained.Trains	

Speer, Rachel M	new personnel in the proper techniques and procedures for in handling marine mammal biopsies and their dissection and preservation.	See Online Training Log
Toyoda, Jennifer H	Sighting of whales and manipulation of skin biopsy after it is obtained	See Online Training Log
Lu, Haiyan	Sighting of whales and manipulation of skin biopsy after it is obtained	See Online Training Log
Croom-Perez, Tayler	Sighting of whales and manipulation of skin biopsy after it is obtained	See Online Training Log
Lopez Montalvo, Carlos A	Sighting of whales, Skin biopsy and manipulation of skin biopsy after it is obtained.Trains new personnel in the proper techniques and procedures for sampling marine mammals and in handling marine mammal biopsies and their dissection and preservation.	See Online Training Log

If there are any non-UofL personnel who will be handling animals or performing any procedures as part of this Proposal, please provide their names, the functions they will perform, and the relevant experience /training they have in performing those functions.

Name	**Procedures Performed and Experience**
John Pierce Wise, Jr.	Functions: Sighting of whales, skin biopsy and manipulation of skin biopsy after it is obtained. Trains new personnel in the proper techniques and procedures for sampling marine mammals and in handling marine mammal biopsies and their dissection and preservation. Experience: Dr. Wise has over eight years of experience in sighting and biopsying marine mammals. He has trained numerous staff and scientists in the proper techniques and procedures for sampling marine mammals. Dr. Wise also has over 8 years of experience in handling marine mammal biopsies and their dissection and preservation. He has trained numerous staff and scientists in the proper techniques and procedures for handling these samples.
Derek Walker	Functions: Sighting of whales and skin biopsy. Trains new personnel in the proper techniques and procedures for sampling marine mammals Experience: Mr. Walker has over eight years of experience in sighting and biopsying marine mammals. He has trained numerous staff and scientists in the proper techniques and procedures for sampling marine mammals.

Mark Martin Bras	Functions: Sighting of whales, skin biopsy and manipulation of skin biopsy after it is obtained. Experience: Mr. Martin has over four years of experience in sighting and biopsying marine mammals. Mr. Martin also has over 4 years of experience in handling marine mammal biopsies and their dissection and preservation.
Catherine Wise	Functions: Sighting of whales, skin biopsy and manipulation of skin biopsy after it is obtained. Trains new personnel in the proper techniques and procedures for sampling marine mammals and in handling marine mammal biopsies and their dissection and preservation. Experience: Catherine Wise has over eight years of experience in sighting marine mammals and has been trained for biopsying marine mammals. Ms. Wise also has over 8 years of experience in handling marine mammal biopsies and their dissection and preservation. He has trained numerous staff and scientists in the proper techniques and procedures for handling these samples.
James Wise	Functions: Sighting of whales, skin biopsy and manipulation of skin biopsy after it is obtained. Trains new personnel in the proper techniques and procedures for in handling marine mammal biopsies and their dissection and preservation. Experience: James Wise has over eight years of experience in sighting marine mammals and has been trained for biopsying marine mammals. Ms. Wise also has over 8 years of experience in handling marine mammal biopsies and their dissection and preservation.
Russell Lowers	Functions: Sighting of whales and skin biopsy. Experience: Mr. Lowers has extensive field work experience working with wildlife all over the world. He is a licenced commercial sea captain. He also has extensive bow hunting experience. We plan to train him in the sighting and biopsying of marine mammals in the summer of 2019

☑ As Principal Investigator (or designee), I attest that the Key Study Personnel selected for this study have, or will obtain, the necessary experience, training, and are proficient, or will be proficient, in performing all of the procedures listed above.

16.0 Anesthetics, Analgesics, and Other Therapeutic Agents

16.1 List ALL pre-anesthetic, anesthetic, analgesic, tranquilizing agents, surgical support fluids, antibiotics, and other veterinary medical therapeutics to be used (even if their use has been described elsewhere in this *Proposal*). Examples include not only peri-operative drugs,

but also such drugs as insulin for diabetic animals, or hot/cold packs.

Analgesics "PRN" or "as needed"

The USDA Research Facility Inspection Guide states that "PRN" or "as needed" frequency of administration is not acceptable unless there are detailed instructions and criteria for determining administration of the drug. Non-pharmacological methods, such as hydrotherapy and hot/cold packs, should also be described. Availability of experienced personnel, especially at night and on weekends, should also be assured in protocol review.

IM = intramuscular, IP = intraperitoneal, IV = intravenous, PO = per os (by mouth), SC = subcutanous

Be sure to save often!

Click "Save and Continue" button at top right of screen. (You will move to the next section. You can return to "Anesthetics, Analgesics, and Other Therepeutics" by clicking the appropriate section on the left. This annoying feature will be fixed in future releases!)

Name and Purpose (check labels)	Dose (mg/kg) and Route (check labels).	Frequency (e.g., twice daily) and Duration (e.g., once, three weeks) - check labels
No records have been added		

In the text box below, provide additional information on the use of the agents listed above. Examples may include use of certain analgesics pre-emptively, clarifying different anesthetic regimens for specific procedures, anesthetics used in combination, decision making process used to determine frequency or duration, etc.

None

17.0 Anesthesia and Anesthetic Monitoring

17.1 Will animals be anesthetized for any reason OTHER THAN Euthanasia?

○ Yes ◉ No

18.0 Surgical Preparation and Support

18.1 Will animals undergo surgical procedures?

○ Yes ◉ No

19.0 Privately-Owned Animals

19.1 Privately-Owned Animals

Does this Proposal include the use of any privately-owned animals?

○ Yes ◉ No

20.0 Non-Standard Housing, Food and Water (or Other Special Considerations)

20.1 Indicate which of the following, if any, pertain to this proposal

☐ Animals require special housing conditions (e.g., individual housing, special caging).
☐ Animals receive special food
☐ Animals receive special drinking water

- [ ] Animals will experience food or drinking water restriction or regulation
- [ ] Use of non-sterile or expired medical materials (disposable surgical supplies)
- [ ] Animals will be physically restrained for prolonged periods of time. Brief manual restraint for the purpose of performing routine clinical or experimental procedures (< 15 minutes for rodents, <30 minutes other mammals) need not be described unless the procedures will cause pain or distress.

21.0 Collaborating Institutions

21.1 Does this project involve the use of animals at any other institution? *Example:* We perform a surgery at U of L and a colleague at another instution performs a very specialized procedure. Note: This does not include collaborations in which you import a new strain from a collaborator.

See **IACUC Policy**

- ( ) Yes
- (•) No

22.0 Biological Agents

22.1 Indicate which Types of Biological Agents that will be administerd to animals. - *Examples: Mammalian cell lines; bacteria; other microbes; viruses; materials of human or non-human primate origin (e.g. antibodies etc.); toxins of biological origin (e.g., Complete Freund's Adjuvant, pertussis toxin).* These tables will be reviewed by the Biological Safety Office to determine the need for IBC Registration and/or applicable SASPs. - Select ALL that Apply

- [x] Not Applicable: No Biological materials will be used in live animals
- [ ] Microbial Agents or Parasites (bacteria, viruses, protozoa, etc.)
- [ ] Cells or Tissues (cell lines, primary tisues or cells, etc.)
- [ ] Other Biological Material (antibodies, rDNA, toxins of biological origin such as Complete Freund's Adjuvant, pertussis toxin, etc.)

23.0 Chemical Agents

23.1 Will animals be exposed *in vivo* to ANY other chemical agent not included in the "Anesthetics, Analgesics, and Other Therapeutic Agents" table above? This includes investigational drugs, test articles, or any other chemical agent introduced into the animal other than biological or radiological materials.

- ( ) Yes
- (•) No

24.0 Radiation or Other Physical Hazards

24.1 Will animals be exposed to radiation (e.g., isotopes, lasers, irradiators) or other physical hazards (e.g., loud noises)?

- [ ] Radioactive Material
- [ ] Cs-137 irradiator
- [ ] X-Ray radiation
- [ ] Other (magnet, lasers, noise, etc.)

25.0 Agent Administration and Return to RRF

25.1 Will live animals be returned to the RRF after exposure to hazardous substances (biological, chemical, or radioactive)?

- [x] Not Applicable. No hazardous agents used or animals are not returned to the RRF
- [ ] Biological Exposure then return to RRF
- [ ] Chemical Exposure then return to RRF
- [ ] Radioactive Material Exposure then return to RRF

26.0 Euthanasia or Other Disposition

26.1 In the space below, please describe all euthanasia techniques employed. Include agent, dose, and route of administration. For chemical methods of euthanasia (*e.g.*, CO_2, pentobarbital), provide a description of how death will be ensured (*e.g.*, cervical dislocation, vital organ removal).

Noe euthanasia will be used.

26.9 For animals that will not undergo euthanasia at the end of these studies, provide a description of their final disposition. If this includes assignment to another *Proposal*, identify the other *Proposal* (if known) and estimate the minimum time period before using the animal(s) in subsequent procedures.

We anticipate the animals will resume normal behaviors and be unaffected.
Biopsy sampling is a widely used method that has been demonstrated to be effective without causing any significant damage to whales (Whitehead et al, 1990). In addition to the published reports, the lack of effect is believed to be true predominately from two other lines of evidence. The first line of evidence is the work on sperm whales by Ocean Alliance during the voyage of the Odyssey. We tracked animals for as long as a week after biopsying, the biopsy mark is typically evident and seen repeatedly after biopsying over the next few days. We have never in the course of 15 years work seen evidence of a biopsy causing infection. In some instances, individual and pods of whales were encountered more than once, the whales exhibited no evidence of altered behaviors to the presence of the boat or infections on their backs indicating no effect.

The second line of evidence is from North Atlantic and South Atlantic right whale studies. The North Atlantic right what studies is led by our close collaborator Scott Kraus at the New England Aquarium and the South Atlantic right whale studies are led by Ocean Alliance. These are two populations where individual animals are identified and studied from year to year. Each population has been studied for months at a time for more than 35 years with familial and genetic relationships studied by observation and genetics from biopsies. There is no evidence of any sort-term or long-term effect of the biopsies and no evidence of infection as the whales show normal behaviors and even continue to swim up to the boat after sampling. In the case of the South Atlantic right whales this includes allowing their calves to swim up to the boat (we have not asked Dr. Kraus if the North Atlantic right whale calves swim up to the boat).

27.0 Non-Pharmaceutical-Grade Agents

27.1 Are ANY of the agents, substances, drugs, test articles, etc. to be used in live animals chemical grade, that is, not pharmaceutical grade?

○ Yes ◉ No

28.0 Non RRF Study Site(s)

28.1 Will animals be transported to and used in rooms outside of the RRF?

○ Yes ◉ No

29.0 Consideration of Alternatives

29.1 Indicate HIGHEST Pain Classification of Procedures in this protocol.

- ○ Class 0 - Animals will be acquired/held, but not used or manipulated in any way.
- ◉ Class I - Studies in which animals will experience no pain or distress greater than that produced by routine injections or venipuncture and will therefore receive no pain-relieving agents.
- ○ Class II - Studies in which there is a potential for pain or distress which is minimized or eliminated by anesthetics, analgesics, and/or tranquilizers. Examples include biopsy, endoscopy, vascular cut-down, footpad injections, use of adjuvants, implantation of chronic catheters, as well as survival and non-survival surgery.
- ○ Class III - Studies in which animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. Examples include

exposure to agents or radiation levels that cause serious illness, research involving significant stress, or procedures involving prolonged restraint. A written justification (including supporting sources, journals, abstracts, etc.) for withholding pain-relieving agents must be provided in a following section.

29.5 **Humane Endpoints** **For some Class I and *all* Class II and III procedures, there is a potential for adverse effects. Humane endpoints are objective signs indicating a pain/distress level that warrants intervention (usually euthanasia), regardless of experimental timelines. These may be specific for each procedure or may be general for an experimental group or the entire *Proposal*. Often, basic "sick animal" signs such as inappetance or lethargy lasting over 24-48 hours or weight loss exceeding 10% are used. Other signs/criteria may be more appropriate for this study. [IACUC Policy and Pain Scoring Sheet Templates] - Make sure that your response**

1. **Precisely defines the humane endpoint, including assessment criteria**
2. **Describes the frequency of animal observation**
3. **Describes the response required upon reaching the humane endpoint**

Experimental Group or Procedure	Endpoint and Assessment Criteria	Frequency of Observation	Response
whales	observed sampling site and behavior subsequent to sampling	As long as the animal is visible	Individual animals may show brief and variable behavioral responses to darting: tail flicking, forceful blowing and swimming away. However, whales normally resume their normal behavior after the boat approach is complete (e.g., Whitehead et al. 1990, Brown et al. 1991, Weinrich et al. 1991). We incorporate Weinrich et al.'s (1991) classification of responses to biopsy sampling by documenting four potential levels of reaction: 1) no reaction (no detectable behavioral change); 2) low-level reaction (slight, mild behavioral change, e. g. flinch or fast dive, short duration); 3) moderate (forceful behavioral change, e. g. breach, short duration); 4) strong (succession of forceful activities). During previous voyages, sperm whales' average response to being sampled by darting was one on a scale of one to four (1 being the lowest). The response to close approaches and

			/or focal follows was less than one where there was no significant change in behavior at all. Brief behavioral (e.g., quick tail slashes and diving) and physiological (e.g. breathing rate) changes, lasting seconds to several minutes, may occur in a subgroup of individuals upon approach and darting and are believed to be necessary temporary disturbance for this research. This type of behavior was observed less than 10% of the time. A greater reaction was observed from darts that missed suggesting that the animals that did react were exhibiting a startle response and not a pain response.

30.0 Other Information for IACUC Review

30.1 Is there any additional information that may assist the IACUC in their review, *e.g.*, request for exemptions to IACUC policies not described elsewhere in this Proposal?

(•) Yes ( ) No

Provide additional information for IACUC review in the space below.

This application is a renewal of our current protocl which expires in March 2019.

Others may assist in the preparation for and the collection of biopsies from the animals described in this protocol. However, personnel that are not specifically listed as Key Study Personnel for this protocol will not handle, touch, or otherwise manipulate the animals

31.0 End of Form

31.1 STOP To Submit Proposal click "Save & Continue," and complete the Initial Review Submission Packet Otherwise - Log Out or return to the sections you wish to revise.

UNIVERSITY OF LOUISVILLE.

George M. Pantalos, Ph.D.
Chair
Institutional Animal Care and Use Committee (IACUC)

October 29, 2020

Dr. John P Wise, Ph.D.
Department: U of L - 42 - Pharmacology
Protocol Number: IACUC 18407
Expiration Date: 10/29/2021
Protocol Title: Marine Toxicology in Marine Mammals

Dear Dr. Wise,

The University of Louisville Institutional Animal Care and Use Committee (IACUC) has reviewed and approved an annual review of your proposal to use laboratory animals in research and teaching as listed above.

IACUC approval to use laboratory animals is granted for a period of three (3) years subject to annual review. Although continued approval may be granted annually, a new application must be submitted at the end of the three years and will be subject to a *de novo review*. During the approval period, it is the responsibility of the Principal Investigator to notify the IACUC of any change in the proposal (e.g., animal species/number, personnel, procedures, project classification, funding source(s), study site, and/or use of hazardous materials) via a modification in iRIS. The proposal may not be transferred to any other project or investigator without IACUC approval and the Principal Investigator may not use the IACUC approval number to conduct another project. Any adverse effects observed during the course of this activity **must** be reported to the IACUC.

All individuals involved with the use of laboratory animals in this project should be knowledgeable of the contents of the *Guide for the Care and Use of Laboratory Animals* (*Guide*), National Research Council (NRC), National Academy Press, 2011. A copy of the *Guide* can be obtained from the IACUC office (HSC, MDR, Room 015). All individuals associated with this project must comply with IACUC training and occupational health and safety requirements. Any individuals associated with this project in the future must also receive training and fulfill health and safety requirements.

Sincerely,

George M. Pantalos
IACUC Chair

University of Louisville, Louisville, KY 40292
P: 502.852.7307 **F**: 502.852.7943

UNIVERSITY OF LOUISVILLE.

IACUC Training Specialist
Institutional Animal Care and Use Committee (IACUC)

July 06, 2020

Dr. John P Wise, Ph.D.
Department: U of L - 42 - Pharmacology
Protocol Number: IACUC 18407
Expiration Date: 10/29/2021
Protocol Title: Marine Toxicology in Marine Mammals

Dear Dr. Wise,

The University of Louisville Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the personnel addition of John Wise to your proposal to use laboratory animals in research and teaching as listed above. Please make sure any new participants review your animal use proposal and are familiar with approved procedures before beginning animal work. Principal Investigators are responsible for ensuring that training is obtained and documented in the Training Log ***prior*** to a Participant performing any animal-related procedures unsupervised. Participants conducting procedure for which the Log does not record an appropriate level of training may lose animal use privileges.

All individuals involved with the use of laboratory animals in this project should be knowledgeable of the contents of the Guide for the Care and Use of Laboratory Animals (Guide), National Research Council (NRC), National Academy Press, 2011. A copy of the Guide can be obtained from the IACUC office (HSC, MDR, Room 015). All individuals associated with this project must comply with IACUC training and occupational health and safety requirements. Any individuals associated with this project in the future must also receive training and fulfill health and safety requirements.

Sincerely,

Stacy R. Cantrell, MBA, BS, CMAR, RLATg
IACUC Training Specialist

UNIVERSITY OF LOUISVILLE.

David Magnuson, Ph.D.
Vice Chair
Institutional Animal Care and Use Committee (IACUC)

October 04, 2019

Dr. John P Wise
Department: U of L - 42 - Pharmacology
Protocol Number: IACUC 18407
Expiration Date: 10/29/2021
Protocol Title: Marine Toxicology in Marine Mammals

Dear Dr. Wise,

The University of Louisville Institutional Animal Care and Use Committee (IACUC) has reviewed and approved an annual review of your proposal to use laboratory animals in research and teaching as listed above.

IACUC approval to use laboratory animals is granted for a period of three (3) years subject to annual review. Although continued approval may be granted annually, a new application must be submitted at the end of the three years. During the approval period, it is the responsibility of the Principal Investigator to notify the IACUC of *any* change in the protocol (e.g., animal species/number, personnel, procedures, project classification, funding source(s), study site, and/or use of hazardous materials). The protocol may not be transferred to any other project or investigator without IACUC approval and the Principal Investigator may not use the IACUC approval number to conduct another project. Any adverse effects observed during the course of this activity must be reported to the IACUC.

All individuals involved with the use of laboratory animals in this project should be knowledgeable of the contents of the Guide for the Care and Use of Laboratory Animals (Guide), National Research Council (NRC), National Academy Press, 2011. A copy of the Guide can be obtained from the IACUC office (HSC, MDR, Room 015). All individuals associated with this project must comply with IACUC training and occupational health and safety requirements. Any individuals associated with this project in the future must also receive training and fulfill health and safety requirements.

Sincerely,

David Magnuson
IACUC Vice Chair

University of Louisville, Louisville, KY 40292
P: 502.852.7307 **F**: 502.852.7943

UNIVERSITY OF LOUISVILLE.

George M. Pantalos, Ph.D.
Chair
Institutional Animal Care and Use Committee (IACUC)

10/30/2018

Dr. John P Wise
Department: U of L - 42 - Pharmacology
Proposal Number: IACUC 18407
Expiration Date: 10/29/2021
Proposal Title: Marine Toxicology in Marine Mammals

Dear Dr. Wise,

The University of Louisville Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your Proposal to use animals in research and teaching as listed above.

IACUC approval to use laboratory animals is granted for a period of three (3) years subject to annual review. Although continued approval may be granted annually, a new application must be submitted at the end of the three years. During the approval period, it is the responsibility of the Principal Investigator to notify the IACUC of *any* change in the Proposal (e.g., animal species/number, personnel, procedures, project classification, funding source(s), study site, and/or use of hazardous materials). The protocol may not be transferred to any other project or investigator without IACUC approval and the Principal Investigator may not use the IACUC approval number to conduct another project. Any adverse effects observed during the course of this activity must be reported to the IACUC.

All individuals involved with the use of laboratory animals in this project should be knowledgeable of the contents of the Guide for the Care and Use of Laboratory Animals (Guide), National Research Council (NRC), National Academy Press, 2011. A copy of the Guide can be obtained from the IACUC office (HSC, MDR, Room 015). All individuals associated with this project must comply with IACUC training and occupational health and safety requirements. Any individuals associated with this project in the future must also receive training and fulfill health and safety requirements.

Sincerely,

George M. Pantalos
IACUC Chair

University of Louisville, Louisville, KY 40292
P: 502.852.7307 **F**: 502.852.7943