TITLE: Creating an Animal Study Proposal – for Investigators

PURPOSE:
The purpose of this SOP is to serve as a guide for research staff members who are creating an Animal Study Proposal (ASP) in the Internet Animal Study Proposal (IASP). Information required for ASPs is based on the Animal Welfare Act, The Guide for the Care and Use of Laboratory Animals and NIH requirements as listed in NIH Manual 3040-2.

GENERAL INFORMATION:
All NINDS/NIDCD/NCCIH ASPs are created and maintained in the IASP. Tips and hints about navigating while working in the IASP can be found in SOP 8720-PI. The NINDS/NIDCD Animal Health Care Section (AHCS) website contains valuable information which is very helpful when developing an ASP or about other subjects related to use of animals after the ASP has been approved. The Animal Care and Use (ACUC) part of the website covers information about ASP creation, policies and guidelines, and training requirements. The AHCS part of the website contains vital information about animal ordering, transfer of animals, and technical requests. (https://ahcs.ninds.nih.gov/)

PROCEDURES:
Creating an ASP – each section of the NIH ASP requires specific information. Each Section (A-P) should contain the appropriate information. There is a row of lettered tabs at the top of the page when you click on the “Create a Protocol” button. These tabs correspond to the sections of the ASP. The tab “Supplements” contains a link to templates for documents requested in various sections of the ASP. To receive access to the IASP, please refer to the information in the second paragraph at: http://ahcs.ninds.nih.gov/ACUC_pages/asp.html

A. Section A: Administrative Data
1. Project tab: Enter the name of your institute, your division, laboratory, or branch name, the title of your ASP, and the type of submission in the appropriate box. Use the pull down boxes where available. If your laboratory name has changed or is not in the pull down box, contact the ACUC Coordinators to have your laboratory name added or changed.

2. Principal Investigator (PI) tab: Find your name in the pull down box and click on the name. This will populate the box with the information which has been entered in the personnel database. If you do not find your name in the pull down box, contact the ACUC coordinators so they can add you to the database.

3. Co-investigators tab: Find the name of the person in the pull down box, click on the name to choose it and then click on “add” to bring up the information from the personnel database. If the name of a new investigator is not in the pull down menu, please send the name to the ACUC Coordinators so they can add the person to the personnel database. If any of the Co-Investigators will be serving as the Co-PI, click on the CoPI box underneath and to the left of that investigator’s name. Save this section before going to the next Section.

4. A personnel training form must be completed for the principal investigator and for each co-investigator listed in Section A. Open the template in the supplement section and save it to your computer. When the form is completed, please print it
and have both the PI and co-investigator sign the form. Scan the signed form and upload it into the supplement section of the ASP. Information on training requirements can be found at: http://ahcs.ninds.nih.gov/ACUC_pages/train.html

B. Section B: Animal Requirements

This section opens to a general tab where you designate the number of years you are requesting for the ASP and the types of procedures that will be performed in the ASP. We recommend asking for 3 years, even if you expect your work to be completed before then. If you are doing both in vitro and in vivo, please check both boxes.

1. To choose a species, use the pull down list on the general page and click on the species name and then click on “Add a New Species”. A new tab will open which contains boxes for information about the species, size, stock/strain, sources (vendor name and/or in house, if breeding), holding and procedure locations, and the number of animals you estimate will be required to meet your experimental goals. Save the information using the save button at the top of the page.

2. To add another species, go back to the general tab and repeat B1. for the new species.

C. Section C: Transportation

The NIH Animal Research Advisory Committee (ARAC) and each animal facility/building have specific rules for the transport of animals within a building or to other buildings on campus. This information should be clearly stated in this section. Save the information before going to another Section.

1. Animals to be moved from the animal facility to a lab or to a Core facility must be in approved transport containers.

2. When moving animals within a building, there are specific elevators that must be used to move animals between floors. Name the elevators to be used.

3. The movement of animals between buildings must be approved by both the sending and receiving facilities. Please include whether or not the animals will be returned to their original location.


D. Section D: Study Objectives

This section should be written in simple, non-technical language so that a non-scientist can read it and have a good sense of the goal(s) of the ASP, human or animal health issues that relate to the goal and an understanding of what procedures the animal will/may undergo. Any technical words that must be included should be defined. If this is a renewal, it should also include a description of how the upcoming work will build on the previous work. Save this Section before going to another Section.

1. The technical and scientific details of the specific goal and a description of procedures should be described in the beginning of Section F.

2. The non-affiliated, non-scientific members of the ACUC review this section to ensure it is understandable to the layperson. Use language that would be understandable by an average high school graduate. Simple terms such as gene are fine; the specific name of the gene means little to the reviewers of the section and would be better in the beginning of Section F.

E. Section E: Rationale for Animal Use
1. Section E.1 should describe why animals must be used for this study. Why can’t cell culture or computer modeling be used? Justify the *use of live animals* in this sub-section.

2. Section E.2; describe why the *species chosen is appropriate* to explore the goals of the ASP. If more than one species is selected, please provide the information for each species and explain why 2 (or more) species are needed to answer specific questions in the experimental objectives.

3. Section E.3 is where the investigator *justifies the number of animals requested*. The first paragraph which starts with “Based on information...” should be left in this section. The animal numbers should be equivalent in Sections B = E.3 = H = information in supplemental documents (e.g. flow charts and breeding charts). A summary of the animal use from the other Sections and supplement forms mentioned above is very helpful for the investigator creating the ASP and for the reviewers of the ASP. Refer to information in breeding charts or other Sections rather than duplicate the information here.

F. **Section F: Description of Experimental Design and Animal Procedures**

This section provides information about the technical details and the procedures that will be performed under this ASP. See section P Supplemental Documents, below for further information about supplemental documents which might be required for the ASP.

1. The nine procedures listed at the beginning of the section should be used as prompts regarding the type of information requested to describe procedures that will be performed under the ASP.

2. The NINDS/NIDCD/NCCIH ACUC would like descriptions of all procedures that are to be performed, but they would like the narrative in the section to be written so that the time flow of the experiment is evident. Don’t just describe each procedure; provide enough information so that the flow of the experiment is evident and one can follow the sequence of events which could be experienced by a single animal. If there are multiple paths that animals may follow, describe them and indicate how many animals are estimated to follow each path.

3. In addition to a description of each procedure and when it occurs in the experimental design, please complete the following supplemental documents, sign them if a signature is required and upload into the supplemental document section. This section can be quite long, so preparing it in a word document and then pasting into the text box is recommended.

i. Information on ACUC policies and guidelines are available at [http://ahcs.ninds.nih.gov/ACUC_pages/pg.html](http://ahcs.ninds.nih.gov/ACUC_pages/pg.html). Please use these documents as a guide when writing Section F. Information about breeding, genotyping, surgery, euthanasia and other common ASP procedures can be found here.

ii. On the same page of the NINDS/NIDCD/NCCIH website, there are guidelines which provide information on suggested anesthesia and doses, multiple survival surgery and requirements for *in vitro* charts.

iii. Provide a flow chart which includes a time line for the procedures the animals would experience during the experiments. Provide group sizes and indicate when in time a procedure occurs in relation to the rest of the experiment (e.g. injection will occur 5 days after cannula implantation). Include the endpoint of the experiment and state what happens to the animal at the endpoint (e.g. euthanized for tissue harvest). If there are several experimental paradigms within the ASP, please provide a flow chart for each arm of the experiment.

iv. If a portion of the animal use is limited to euthanasia and tissue harvest, please complete and upload an *in vitro* chart into the supplemental documents section.

v. Provide an intervention and endpoint chart which lists any sign/symptom that could occur due to the experimental procedures or animal phenotype. For each
sign/symptom, describe how the sign/symptom should be assessed, and what treatment action or endpoint would be appropriate for this sign/symptom.

vi. For any drugs used, please include whether or not they are pharmaceutical grade and if not, why they’re being used (e.g. – pharmaceutical grade not available or the non-pharmaceutical grade has a higher purity). Provide an experimental drug chart for ASPs involving the use of multiple drugs in the animals.

vii. If imaging in the Mouse Imaging Facility (MIF) or any other part of the Nuclear Magnetic Resonance (NMR) Center is planned, an NMR form must be completed and uploaded as a supplemental document.

G. **Section G: Survival Surgery**

Complete this section to describe major survival surgery procedures. Major survival surgery is defined as any surgical intervention that penetrates or exposes a body cavity or has the potential for producing a permanent handicap in an animal.

1. Section G.1 should describe the entire surgical procedure. It should be written sequentially so that the reader can follow the procedure from animal preparation to full recovery.

i. NINDS/NIDCD/NCCIH ACUC policy limits survival surgery to Monday-Wednesday, unless prior approval has been obtained from the veterinarian. If approval is granted, the investigator will be responsible for weekend health assessments and treatments. Please state that this policy will be followed. See policy at [http://ahcs.ninds.nih.gov/ACUC_pages/pg_015_surv_surg_scheduling.html](http://ahcs.ninds.nih.gov/ACUC_pages/pg_015_surv_surg_scheduling.html)

ii. For rodent surgery, state that Aseptic Technique will be used and the surgeon will wear a clean lab coat and gloves. Gloves will be changed between animal preparation and the actual surgery. Consult with the veterinary staff for non-rodent surgeries.

iii. Include details such as the sterilization of surgical instruments, how the animal is anesthetized (including the dose/concentration of the anesthetic and if given by injection, the needle gauge used), shaved and the surgical site prepared. If a stereotaxic instrument is used, please state that lidocaine will be put in the ear canals prior to placement in the stereotaxic unit, unless using ear bars with cups. Include a statement that sterile ophthalmic ointment will be put on the eyes to protect them from drying out.

iv. Describe the procedure step by step. Include details such as incision site and size; if a craniotomy is to be performed describe how the burr hole is made, the instrument used to make the burr hole and the size of the hole. Provide information on how the incision is closed.

2. Section G.2 lists the names of the people who will be performing survival surgery and their qualifications. All investigators performing or listed on an ASP that contains survival surgery must take the Aseptic Training Course before the ASP can be approved. [http://ahcs.ninds.nih.gov/ACUC_pages/train_aseptic.html](http://ahcs.ninds.nih.gov/ACUC_pages/train_aseptic.html)

3. Section G.3 should state the building and room number where the survival surgery will be performed.

4. Section G.4 contains all information on post-surgical care of the animal.

i. The ACUC recommends that all animals receive an injection of warmed saline containing ketoprofen or other analgesic before recovery from anesthesia. The analgesic is usually given for 2 days after surgery in addition to the dose at the completion of surgery.

ii. Dependent on the facility housing the post-surgical animals, a technical request for post-surgical monitoring or other means of alerting the facility to the health status of the animal should be described. In NINDS-NIDCD managed facilities, a
yellow post-operative surgical record is submitted with each cage or animal. An orange watch card is placed in the cage card holder when the cage is returned to the holding room.

iii. Provide information on who is responsible to removal of suture or wound clips and when they should be removed.

5. Section G.5 asks if a major survival surgery has been performed on the animal prior to it being placed on this study. If yes, please provide scientific justification for performing more than one major survival surgery on the animal.

6. Section G.6 asks if multiple survival surgery will be performed on an animal while on this study. If yes, provide scientific justification for multiple survival surgery and state the number of days between surgeries. 


H. **Section H: Pain or Distress Category**

1. Animal numbers are assigned to the appropriate pain/distress category based on the procedures performed and in some cases the age at which the procedure is performed.

   i. Column C: animals receiving IP or SC injections, tail snip at ≤ P10, perfusion, breeding, and CO2 narcotization followed by tissue harvest or anesthesia used for restraint are examples of Column C procedures.

   ii. Column D: animals anesthetized for surgery, tail snip at ≥ P11, receiving analgesia to treat pain/distress caused by an experimental procedure or phenotypic expression causing pain/distress.

   iii. Column E: animals experiencing pain/distress who are not treated with analgesics would be considered Column E animals. A Column E form describing the cause of the pain/distress and a scientific justification for withholding analgesia must be completed and uploaded into the supplement section of the IASP.

2. If the ASP has animals listed as Column D or E, the investigator is required to search two databases for alternatives to the painful/distressful procedures which result in the animals being placed in these pain categories. The database search should be done within 2-3 months from when the protocol is submitted. The databases searched should be scientific databases, not Google. Suggested websites include:

   ALTWEB:  http://altweb.jhsph.edu/

   AGRICOLA:  http://agricola.nal.usda.gov/

   Web of Science:  http://isiknowledge.com/?DestApp=WOS


3. The narrative section should discuss what you found during the database search and why you can or cannot use the alternatives the database search produced.

I. **Section I: Anesthesia, Analgesia, Tranquilization**

In this section, please describe each kind of analgesia that will be used and include the dose (mg/kg), volume of injectate, route of administration (if by injection state needle gauge), and the schedule of administration.

1. If there are multiple types of anesthesia, please describe each separately (make a new paragraph for each method) and state which procedure goes with which anesthetic.

2. For inhalant anesthesia, please state the percentage of anesthetic and the carrier(s) for both induction and maintenance. For maintenance, please
describe how the animal is receiving the maintenance dose (nosecone, intubation or other method).

3. If a reversal agent is used, please state this, including the name of the reversal agent, dose etc, when describing the use of the specific anesthesia.

J. **Section J**: Method of Euthanasia or Disposition at the End of Study

Describe the proposed method(s) for euthanasia. If multiple methods are described in the ASP, please put each method in a separate paragraph. For each method, describe which cohorts of animals are euthanized with that method and indicate the method of carcass disposal.

1. If using CO2 followed by a secondary method to ensure death, follow and include the information from the OACU guidelines for euthanasia: Adult Rodents:


2. For euthanasia of rodent neonates or pups, please review the ACUC policy:


3. When perfusion is used as a method of euthanasia, it should be described here instead of Section F.

4. For methods that are "conditionally acceptable" by the AVMA Panel Report on Euthanasia (e.g. cervical dislocation without anesthesia) a scientific justification for use of this method must be supplied. In addition, all investigators performing euthanasia by this method must be named in this section and fulfill the training and certification required by the ACUC:


K. **Section K**: Hazardous Agents

The use of hazardous agents requires the approval of an NIH safety specialist. Recombinant DNA or potential pathogens must be approved by the NIH Biosafety Committee and the approved registration documents must be attached as supplements to the ASP. Please contact your institute’s Division of Occupational Health and Safety (DOHS) representative (https://www.ors.od.nih.gov/sr/dohs/safety/laboratory/Pages/safety_health_specialists.aspx) for instructions on completing this section.

A description of the practices and procedures required for safe handling of hazardous agents should be described in this section. The NIH Biosafety or Radiation Safety will provide you with the appropriate language for the agents listed in your ASP and tell you what ABSL to use.

L. **Section L** – This section should be completed if cell lines or material derived from rodents are to be used. See the NINDS/NIDCD/NCCIH policy about the testing of biological materials at [http://ahcs.ninds.nih.gov/ACUC_pages/pg_002_culture_testing.html](http://ahcs.ninds.nih.gov/ACUC_pages/pg_002_culture_testing.html).

M. **Section M** – This section should list all special handling or husbandry information and should specify which experimental group which will require the specialized handling. Exceptions to housing requirements (e.g. physical restraint, cage density, food restriction, or lack of standard enrichment) as stated in the Guide to the Care and Use of Laboratory Animals must be justified in this section.

N. **Section N** – The principal investigator must sign this section after reading the text and uploading all supplemental documents. After submitting the protocol, open the document in preview mode. Print the signature page, sign and return it to the ACUC office either in person or scan and send by email.
O. **Sections O through P** – These sections contain the signatures of the laboratory chief or the Scientific Direction if the PI is a Lab Chief. In addition, there are signature areas for safety (health and radiation), facility managers and vets, the Attending Veterinarian and the Chair of the ACUC. The ACUC coordinators will obtain these signatures for you after the ASP has been approved.

P. **Supplemental Forms** – Depending on the experimental procedures described in the ASP, some or all of the supplemental documents may be required. Templates for these documents may be found on the supplement screen in the IASP or at: [http://ahcs.ninds.nih.gov/ACUC_pages/forms.html](http://ahcs.ninds.nih.gov/ACUC_pages/forms.html)

1. **Flow Chart** – this form should outline the procedures for each arm of an experimental paradigm. The chart(s) should allow the reader to follow each step of the procedure(s) and have a sense of the sequence and time line of the experiment. For some protocols, multiple flow charts will be required so each arm of the experiment is clearly presented.

2. **Breeding Chart** – if breeding is requested in the ASP, please provide a chart of the breeding scheme(s). Indicate the genetic type for the scheme (homozygous, heterozygous etc) and provide information which shows the time line, the estimated number of pups, the disposition of the pups, the frequency of replacement of the breeders, and the estimated number of animals born. All animals born are counted in the total estimate of animals for the ASP. Do not count embryos taken from pregnant dams.

3. **In vitro chart** – should provide information on which arm of the ASP generates the animals for the specific test, the number of animal/s/time period for each in vitro testing scheme, the number of investigators who would be performing these tests, and a description of the in vitro test to be performed. For multiple in vitro tests, please describe each in a separate row.

4. **Intervention and Endpoint Form** – any potential animal health issue should be listed on this form along with information on assessing the problem and explicit information on treatments and endpoints.

5. **Analgesia Form** – lists the three major drug classes for analgesia; local anesthetics, OPIOIDS and NSAIDS. The investigator should mark which class of drug can be used in their study and the maximum time the drug may be used. This document requires the signature of the PI.

6. **Experimental Drugs Form** – all drugs (other than those used for analgesia or anesthesia) used in the study should be listed on this chart. The information required for each drug includes;
   i. The name of the drug or compound code if the drug has not yet been named.
   ii. The concentration of the solute and the name of the vehicle used to solubilize the drug to be used (in mg/ml).
   iii. The dose of the drug (in mg/kg) and the volume of solution that will be given.
   iv. The site and route of administration.
   v. Any side-effects known to be related to the use of the drug. If possible side-effects are unknown, please state that here.

7. **NMR form** – any ASP describing imaging at the MIF or other parts of the NMR must complete and upload this form. This document requires the PI signature. It also requires an IC Veterinarian and the Scientific Directors signature. The ACUC coordinators will obtain these signatures.

8. **Column E Form** – any ASP that has Column E animals listed or has the potential for Column E must complete and upload a column E form. This document requires the PI signature.
9. **Disposition Form** – all ASPs require a completed disposition form. There should be one form for each species unless there are different points of contact or different criteria for euthanasia and post-mortem handling of the carcass. Make sure that all facilities related to the holding and experimental procedures are listed on each form. This form requires the PI signature.

10. **Personnel Education and Training Form**: a form is required for each investigator listed on the ASP. Make sure that all questions are answered. The form must be signed by both the PI and the investigator listed on the form.

**Recombinant DNA Form**: the safety documents should be uploaded for all biological agents requiring these forms. If the forms have been submitted to safety and are under review when you submit the ASP, please upload them once approved by the Institutional Biosafety Committee (IBC) during the next ASP revision.

**REFERENCES:**

4. SOP 8720-PI Working in the Internet Animal Study Proposal – For Investigators
7. NINDS/NIDCD AHCS - provides information about aspects of animal care after the ASP has been approved: [http://ahcs.ninds.nih.gov/AHCS_pages/index.html](http://ahcs.ninds.nih.gov/AHCS_pages/index.html)


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Attachments: None

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Updates and/or Changes:
10/25/16:

- Updated hyperlinks
- Section A3. Added more details regarding the co-investigator tab
- Section D. Added verbiage in section D to address renewals
- Section F3vi. Added instructions for use of non-pharmaceutical grade drugs
- Simplified section K instructions
- Added more details regarding completion of section N