Protocol Number#: ---------- As assigned by IACUC; leave blank for new submissions

International Center for Chemical and Biological Sciences Animal Study Protocol

Institutional Animal Care and Use Committee Phone: ----------

Principal Investigator	**Dr. Asmat Salim**		
Academic Position	Associate Professor		
Institution & Lab #.	Dr. Panjwani Center for Molecular Medicine and Drug Research, Lab # P-132		
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Research Project Title	**Therapeutic Potential of Preconditioned Mesenchymal Stem Cells in Rat Burn Wounds**			
Funding (if any)	**Source/Agency**			
	Grant Number			
	Start Date	1-08-2018	**End Date**	1-08-2020

Check only one:

☒ **New Protocol**

☐ **Renewal** ASP number to be replaced:_____

The undersigned accepts responsibility for compliance with all regulations and laws pertaining to animal care and use.

Principal Investigator: _____ Date: 16-8-2018

1. PAIN / DISTRESS CATEGORY CHECKLIST

1.1. Species. Enter the **total** number of animals to be used in each pain category. In this table, animals should be placed only in the highest pain category they encounter. The number of animals used should be for a **2 year period**. USE A SEPARATE ROW FOR EACH SPECIES. Different strains of the same species should not be listed in this table. LIST INDIVIDUAL STRAINS IN TABLE II.

TABLE I - USDA Pain/Distress Category (see appendix I)

Species	B	C	D	E	Total # of Animals
1. Rat			100		100

3.					
4.					

TABLE II – Identification of Background Strain(s) (*e.g.,* BALB/c) **or Stock(s)** (*e.g.*, SD, Wistar,). Do not specify numbers of animals. Add additional rows to the chart if needed.

Species	Strain
Rat	Wistar

1.2. Procedures and Pain Categories. Check all the procedures performed on animals, for each species and/or strain number as indicated in Tables I and II, above.
For selecting pain categories see Appendix I
Pain scale for each category: 1= Lowest, 4= Highest

Pain Category B. Teaching, research experiments or tests that involve no pain:

1	2	3	4	
✓	✓	✓	✓	
☐	☐	☐	☐	Breeding Colony (no genotyping)
☐	☒	☐	☐	Housing Only

Pain Category C. Teaching, research experiments or tests that involve minimum pain or distress:

1	2	3	4	
✓	✓	✓	✓	
☐	☐	☐	☐	Alert Animals (behavioral observation or brief restraint)
☐	☐	☐	☐	Anesthetize and Release (e.g., for restraint only; imaging procedures)
☐	☐	☐	☐	Identification (tattoo, ear tag, ear notching, etc.)
☐	☐	☐	☐	Change in Environmental Parameters (light cycle, temperature, etc.)
☐	☒	☐	☐	Chemical Agents
☒	☐	☐	☐	Euthanasia Followed by Tissue/Organ Harvest
☐	☐	☐	☐	Food or Water Restriction
☐	☐	☐	☐	Forced Exercise
☐	☐	☐	☐	Gavage
☐	☐	☐	☐	Microbiological Agents
☐	☐	☐	☐	Non-Surgical Collection of Body Fluids (blood, urine, etc.)
☐	☒	☐	☐	Observation Only (no physical contact with animals)
☐	☒	☐	☐	Simple Injections (i.e., IP, IV, etc.)
☐	☐	☐	☐	Exposure to Ionizing Radiation
☐	☐	☐	☐	Use as Parasitic Hosts
☐	☐	☐	☐	Tail Biopsy for Genotyping (mice less than or equal to 24 days old)
☐	☐	☐	☐	Noxious Stimuli That Causes No More than Brief Pain from Which There Is an Escape

Pain Category D. Teaching, research experiments, or tests that involve pain or distress, and for which appropriate anesthetic, analgesics or tranquilizing drugs will be used:

1	2	3	4	
✓	✓	✓	✓	
☐	☐	☐	☐	Anesthetize and Release (e.g., for retro-orbital and cardiac injections)
☐	☐	☐	☐	Antibody Production: Polyclonal (non-ascites, no footpad)
☐	☐	☐	☐	Antibody Production: Ascites
☐	☒	☐	☐	Burns
☐	☐	☐	☐	Controlled Toxic Substance, Toxic Drug or Alcohol
☐	☐	☐	☐	Footpad Injections (antibody production or microorganism injections)
☐	☐	☐	☐	Induction of Illness (including the administration of toxic substances)
☐	☐	☐	☐	Lavage
☐	☒	☐	☐	Minor Survival Surgery
☐	☐	☐	☐	Major Survival Surgery
☐	☐	☐	☐	Minor Multiple (or Combination of Minor and Major) Survival Surgeries
☐	☐	☐	☐	Major Multiple Survival Surgeries
☐	☐	☐	☐	Non-Survival Surgery
☐	☐	☐	☐	Physical Trauma
☐	☐	☐	☐	Tail Clip (Mice over 24 days of age)
☐	☐	☐	☐	Tumor Induction or Implantation
☐	☐	☐	☐	Unusual Restraint

Pain Category E. Teaching, research experiments, surgery, or tests that involve pain or distress, and for which appropriate anesthetic, analgesics, or tranquilizing drugs are not used because they would adversely affect the results or interpretation of data:

1	2	3	4	
✓	✓	✓	✓	
☐	☐	☐	☐	Death as an Endpoint
☐	☐	☐	☐	Lethal Dosage Studies
☐	☐	☐	☐	Pain Study or Other Noxious Stimuli from Which There Is No Escape
☐	☐	☐	☐	Functional Deficit (e.g., paralysis)
☐	☐	☐	☐	Transgenic/Mutants That Have Functional Deficits
☐	☐	☐	☐	Induction of Arthritis
☐	☐	☐	☐	Other – Please specify:

2. EXPERIMENTAL SUMMARY

2.1. Summary. Briefly describe your work *using language without technical jargon* that can be **EASILY** understood by the public at large. Briefly explain (in 1-3 sentences each):

a. The purpose (goal) of this research.

A synergistic approach, based on the use of stem cells and chemical compound involved in wound healing will help in the treatment of chronic and severe burn wounds and will reduce the risk of death. Therefore, therapeutic stem cells conditioned with natural compounds involved in

regeneration and differentiation into skin cells during wound healing, may provide a potential therapy for stimulated and accelerated regeneration of damaged cutaneous tissue

b. The intended use of animals in this research

We will be using rat to make burn wound model, to treat burn wounds.

c. How your research is linked to the advancement of a larger body of knowledge

Scientific advancements in stem cell technology, tissue engineering and cell transplantation will provide the basis for the introduction of new treatment technologies for chronic and burn wounds. This research will produce novel treatment for burn wounds. This will also provide opportunity of treating other chronic wounds.

2.2. Aims and Significance. Briefly describe the scientific aims and significance of your experiments. For funded projects, the grant abstract or specific aims section(s) may be used.

In Aim (1), we will develop burn wound animal model to test the stem cell therapy *in vivo* by transplanting the conditioned stem cells in burn wounds. Finally, in Aim (2), we will observe the healing efficiency of the burn wounds treated with conditioned stem cells and the effect on the expression of proteins released during wound healing.

2.3. Research Plan and Procedures. Describe the experimental design of this project. Clarity in this section will greatly facilitate the review of your protocol and will likely reduce the need for revisions.

- Include information about all procedures checked in Section 1.2.
- Include the endpoints for all experimental procedures.
- Indicate the number of animals required for each procedure. A flow chart supplementing the text is often useful.
- Do not include details of in-vitro procedures.
- Do not replicate detailed information required in later sections of this protocol (e.g., Genetically Modified Animals, Special Review Items, Animal Numbers Justification and especially, Surgical Procedures).

Project Details:

- Groups for in *vivo* burn model

Control group	Treatment group (compound 1)	Treatment group (compound 2)
10 rats	20 rats	20 rats

- 50 Rats will be required for MSCs isolation.
- Time intervals for tissue harvesting will be as follows:
  a. 0 day (hemostasis)
  b. 3rd day (inflammation)
  c. 6th day (granulation tissue formation)
  d. 9th day (epithelialization)
  e. 14th day (contraction/ complete healing of wound)

- Since burn model is made in normal rat therefore gender specification is not necessary, male or female both gender can be used.
- Rats with an optimal range of body weight (200-250 g) ang 4 months of age will be used in this study.
- Conditioned MSCs with natural compounds and normal MSCs will be injected, immediately after producing burn wound to analyze the protein expression in both normal and treated MSCs

3. PERSONNEL

(As a general rule, personnel who will not **directly** handle the animals or their unfixed tissues/by-products should not be added here.)

3.1 Principal Investigator: Dr. Asmat Salim
Years of experience with each species listed on this ASP: 10 years
If this person has no experience with the species, provide:
The name of the person who will provide special training and their own experience/ qualifications:

By checking this box, you affirm that required training has been completed: ☒ Yes
Additional animal care and use training:

Procedures to be performed:

3.2 Name: Shazmeen Aslam
Title: Junior Research Fellow
Key Personnel (will receive notifications about this ASP): ☒ Yes
Authorized to order animals: ☒ Yes
Years of experience with each species listed on this ASP: 1 month
If this person has no experience with the species, provide:
The name of the person who will provide special training and their own experience/ qualifications:

By checking this box, you affirm that required training has been completed: ☒ Yes
Additional animal care and use training:

Procedures to be performed: In Vivo Burn Wound Model

3.3 Name: Midhat Batool
Title: Junior Research Fellow
Key Personnel (will receive notifications about this ASP): ☒ Yes
Authorized to order animals: ☒ Yes
Years of experience with each species listed on this ASP: 01 Year
If this person has no experience with the species, provide:
The name of the person who will provide special training and their own experience/ qualifications:

By checking this box, you affirm that required training has been completed: ☒ Yes
Additional animal care and use training:

Procedures to be performed: In Vivo Burn Wound Model

3.4 Name: Dr. Irfan Khan

Title: Assistant Professor
Key Personnel (will receive notifications about this ASP): ☒ Yes
Authorized to order animals: ☒ Yes
Years of experience with each species listed on this ASP: 08 Years
If this person has no experience with the species, provide:
The name of the person who will provide special training and their own experience/ qualifications:

By checking this box, you affirm that required training has been completed: ☒ Yes
Additional animal care and use training:

Procedures to be performed: In Vivo Burn Wound Model

3.5 Name:
Title:
Key Personnel (will receive notifications about this ASP): ☐ Yes
Authorized to order animals: ☐ Yes
Years experience with each species listed on this ASP:
If this person has no experience with the species, provide:
The name of the person who will provide special training and their own experience/ qualifications:

By checking this box, you affirm that required training has been completed: ☐ Yes
Additional animal care and use training:

4. HOUSING AND HUSBANDRY

4.1. Indicate where your animals will be used (i.e., laboratory location, Animal house core facilities, observations).

				Time in this Area		
Species	**Building**	**Room #**	**Planned Procedures in this Area**	**< 12 hrs ✓**	**> 12 hrs < 24 hrs ✓**	**> 24 hrs ✓**
Rats	Animal House Core Facility	Experimental Room	Burn wound Model	✓		

PLEASE NOTE: Once animals have been removed from the animal house they may not be returned to the animal house. Special permission will be required to return animals back in animal house.)

5. EUTHANASIA

5.1. Describe methods of euthanasia. Cervical dislocation or decapitation *without anesthesia* requires scientific justification and description of training and proficiency of personnel performing these procedures

Species	Primary Method	Secondary Method	Justification (as necessary)
Rats	Overdose of Anesthesia (Pentobrabitol)	Decapitation	These procedures are referred by IACUC Guideline.

5.2. If drugs will be employed during the euthanasia procedure, provide the following information.
Paralytics should never be used to perform euthanasia.

Species	Name of Drug	Dosage	Route
N/A	N/A	N/A	N/A

6. IMMUNIZATION

1. Will the animals be immunized? ☐ Yes ☒ No (If "No," proceed to Section 7)

2. Will the animals be immunized to produce antibodies? ☐ Yes ☒ No

Are you using adjuvants? ☐ Yes If "Yes," specify the following: ☒ No

Type of adjuvant	
Dose of adjuvant Initial injection:	
Subsequent injections:	
Route and Location of injections	
Number of injection sites and volume per injection site	
Number of immunizations	

3. Have you considered commercial sources? ☐ Yes ☐ No

4. A justification is needed for the use of Complete Freunds Adjuvant (CFA). Note specifically whether other adjuvants such as TiterMax, Ribi, or Aluminum compounds have been considered as an alternative to CFA.

5. Are you performing footpad injections? ☐ Yes ☐ No
If Yes, provide a justification for the use of footpad injections instead of other methods of immunization. The *Guidelines* recommend injecting only one footpad per animal. If your experiments require multiple footpad injections, you must justify the procedures and provide assurance that the animals will be housed on soft bedding with easy access to food and water (special arrangements need to be made in advance with animal house staff).

7. OCCUPATIONAL HEALTH and SAFETY ASPECTS of the ANIMAL STUDY PROTOCOL
Answer "n/a" for any question that does not apply to your research work; *do not leave any sections blank*

Use of Hazardous Agents/Hazardous Procedures

7.1 Chemical Agents. List all hazardous chemical agents to be administered.

Chemical Agents
Xylazine
Ketamine
Sodium Pentobarbital
Diclofenac Sodium Penicillin and Streptomycin

7.2 Inhalational Anesthetic Agents. List all inhalational anesthetic agents used to anesthetize or euthanize animals. Identify the method of scavenging for the waste anesthetic to prevent exposure to the person(s) administering the gas. Insert extra rows as needed.

Inhalational Anesthetic Agents	Describe Scavenging Technology	Date when vaporizer was last certificated
NIL	NIL	
NIL	NIL	
NIL	NIL	
NIL	NIL	

7.3 Infectious Agents. List all infectious agents to be administered. Identify the necessary Biosafety Level. Insert extra rows as needed.

Infectious Agents	Necessary Biosafety Level
NIL	
NIL	
NIL	
NIL	

7.4 Use of Radioactivity. Indicate if the gamma irradiator or radioisotopes are to be used in this protocol.

Irradiator ☐ Yes ☒ No
ORS will verify that you are authorized to use the irradiator during the protocol review process.

Radioisotopes ☐ Yes ☐ No. If yes:

Which radioisotopes are used? _____
What is the dose of each? _____

8. Hazard Controls

8.1 Controls for Use of Chemical or Biological Agents

1. How will you ensure that carcinogens, reproductive toxins, and substances of high acute toxicity are used in a "designated" area?
N/A

2. For either chemical or biological hazards, how will you communicate the potential for exposure to animal house employees and any other personnel in contact with the animals (e.g., signage, training)?
N/A

3. What protection is required for workers (specify for both research and animal house personnel) subject to possible contamination or exposure to the animals? This may include engineering controls, personal protective equipment (PPE), and/or special work practices.

Use of Personal Protective Equipment throughout the duration of animal work

4. What is the likelihood of chemical or biological contamination of the cage or the bedding?
N/A

5. On what basis have you made this evaluation? Provide current literature references and research citations to assess whether the agents are fully metabolized by an animal. Can shedding be expected and if so, for how long?
N/A

6. Does bedding need to be collected as contaminated, i.e., as hazardous chemical or medical waste? If so, inform ORS on your campus that such waste will be generated and arrange for appropriate collection and disposal.
N/A

7. Describe how animals are safely contained during transport in cases when live animals, given a hazard, are required to be removed from the animal facility for terminal procedures.
N/A

8. Describe if teratogenic agent will be given to animals if yes how you will ensure those chemicals will be used in "designated" area and what protection is required for workers and environment?
N/A

9. ADMINISTRATION OF SUBSTANCES

Tables must be *completed for all substances administered to animals*, including those listed in Sections 7.

Use of pharmaceutical grade materials is recommended. Specify non-pharmaceutical grade substances and explain why they must be used (e.g., pharmaceutical grade is not available, higher chemical purity needed).

SUBSTANCE AND DOSE:	60mg/kg ketamine (50mg/ml) 7mg/kg xylazine (100mg/ml)
Route of administration (e.g., IV, Inhaled)	Intra-peritoneal (IP)
Site(s) of injection (if applicable)	Dorsal Surface near left hind limb
Number of injections (if applicable)	Single Dose
Desired effect of the agent or substance	Anesthesia
Length of survival time after administration	45 minutes
Will the animal experience pain, distress or other adverse effects as a result of the treatment? ☐ Yes ☒ No If "Yes," describe the adverse effects and procedures you will use to alleviate the associated pain, distress or illness, and how animals will be monitored.	
Is this a pharmaceutical grade substance? ☒ Yes ☐ No If "No," please explain why it must be used.	

SUBSTANCE AND DOSE:	Sodium pentobarbital (150mg/kg of body weight)
Route of administration (e.g. IV, Inhaled)	IP
Site(s) of injection (if applicable)	-
Number of injections (if applicable)	Single Dose
Desired effect of the agent or substance	Euthanasia
Length of survival time after administration	-
Will the animal experience pain, distress or other adverse effects as a result of the treatment? ☐ Yes ☒ No If "Yes," describe the adverse effects and procedures you will use to alleviate the associated pain, distress or illness, and how animals will be monitored.	
Is this a pharmaceutical grade substance? ☒ Yes ☐ No If "No," please explain why it must be used.	

SUBSTANCE AND DOSE:	Mesenchymal Stem Cells Transplant
Route of administration (e.g. IV, Inhaled)	At the site of injury
Site(s) of injection (if applicable)	Burn Wounds
Number of injections (if applicable)	2-3

Desired effect of the agent or substance	To improve wound healing mechanism
Length of survival time after administration	Normal Survival depending on the healing effect
Will the animal experience pain, distress or other adverse effects as a result of the treatment? ☐ Yes ☒ No If "Yes," describe the adverse effects and procedures you will use to alleviate the associated pain, distress or illness, and how animals will be monitored.	
Is this a pharmaceutical grade substance? ☐ Yes ☒ No If "No," please explain why it must be used. It must be used for research purpose.	

10. SURGERY

Will surgery be performed? ☒ Yes ☐ No (If "No," proceed to Section M)

If "Yes," list all personnel performing surgical procedures:

Ms. Shazmeen Aslam and Dr. Irfan Khan

10.1. Non-survival surgeries (animals will not recover from anesthesia)

a. Location of surgery (building and room):
Experimental room of Animal resource facility

b. Describe anesthetic regimen, including dose, route of administration, monitoring and additional dosing anesthetics if necessary:
Sodium pentobarbital (150mg/kg of body weight) will be used for euthanasia and Ketamine 60mg and xylazine 7mg per Kg of body weight will be used

c. Describe procedures in detail, including expected duration:
Intra-peritoneal injections of sodium pentobarbital will be given until breathing stops
Ketamine 60mg and xylazine 7mg per Kg of body weight will be used for survival surgery

d. Specify primary and secondary methods of euthanasia:
Primary Method: Sodium pentobarbital over dose.
Secondary Method: Decapitation.

10.2. Survival surgeries (animals will recover from surgery/anesthesia). Insert rows as needed for additional surgical procedures.

Surgical Procedure	Major ✓	Minor ✓	Location of Surgery	Location of Housing After Surgery
2nd degree Burn wounds		✓	Animal house core facility	Animal house (surgery room)

Major surgery: Penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (e.g., laparotomy, amputation of limb).
Minor surgery: Does not expose body cavity and causes little or no physical impairment (e.g., cut-downs, needle aspirations).

10.3 Provide the following for *each* surgical procedure:

a. Reason for survival surgery, i.e., why must animals recover from surgery?
Animal must recover from surgery so that the extent of burn wounds can be determined and effect of stem cell therapy in healing can be studied.

b. Anesthetic regimen, including dose, route of administration, monitoring and additional dosing of anesthetics (if necessary).
Rats will be anesthetized by intraperitoneal injecting ketamine hydrochloride and xylazine hydrochloride at optimized doses of 60mg/kg and 7mg/kg, respectively

c. Vital signs monitored and frequency of recording. *At a minimum recording should be done every 15 minutes*.
Vital signs will be monitored continuously during the surgery as well as pre and post -surgery.

d. Description of pre-surgical procedures (e.g., surgical site prep).
Rats of ~220 g will be used for this procedure and will be anesthetized.

e. Detailed description of surgical procedures.
After hair removal, burn wounds will be produced on the ventral surface by touching hot copper rod without exerting pressure for 20 sec.

f. Duration of the procedure. Provide justification for procedures lasting more than 6 hours (intubation to extubating).
20-35 minutes.

g. Do you anticipate any repair surgeries? If so, describe briefly.
Yes, the process of wound healing will be evaluated with stem cells transplantation

h. Will a paralytic agent be used? If so, specify the agent and indicate what monitoring will be provided while the animal is paralyzed.

Not used.

i. Plan for post-operative care and monitoring of the animals. Include all aspects of post-operative care, time points or intervals for monitoring and the name(s) of personnel responsible for animal monitoring.
Responsible personnel: Ms. Shazmeen Aslam and Dr. Irfan Khan.
Animal will lay rested on heating pad and observed after surgery until it regains consciousness.
Diclofenac Sodium [pain killer] will be given subcutaneously.
Penicillin and Streptomycin will be given subcutaneously.
Animals will be observed daily to check for any visible signs of discomfort, infections or any other

j. Describe any impairment that can be expected from the surgery (i.e., lasting functional deficits), any post-operative complications that may develop, and your plans to handle them.
None Expected

k. What is the expected duration of survival after surgery?
One month
l. Pain management protocol: What criteria will be used to assess post-operative pain, who will monitor and treat the animals, and what analgesic (including dose, route and interval) will be applied? You should administer analgesia with the induction of anesthesia, if possible, and then during the 48 hours after major procedure. A one-time dose of analgesic (to several doses) during the 24 hours after minor procedures is needed; if this will not be done, explain why.
Responsible person: Ms. Shazmeen Aslam and Dr. Irfan Khan.
Analgesic: Diclofenac sodium
Route: Subcutaneous
Monitoring will be done at regular intervals, checking for visible signs of discomfort.

10.4. Multiple Major Survival Surgical Procedures. Will animals undergo more than one major survival surgical procedure?

☐ Yes ☒ No

If "Yes," you must provide a scientific justification. In addition, if the interval between surgeries is less than 30 days this must be noted and justified.

10.5. Multiple Minor (or Combination of Minor and Major) Survival Surgical Procedures. Will animals undergo more than one minor (or minor plus major) surgical procedure?

☐ Yes ☒ No

If "Yes," briefly list procedures and the number of surgeries.

11. SPECIAL REVIEW ITEMS

1. Will you study the effects of trauma or physical injury (i.e., a permanent physical injury beyond which is produced from standard operative procedures)?	☐ Yes	☒ No
2. Will your study involve the effects of burns?	☒ Yes	☐ No
3. Will you be studying pain?	☐ Yes	☒ No
4. Will you be using electrical stimulus without anesthesia?	☐ Yes	☒ No
5. Will your study involve tumor growth or transplantation?	☐ Yes	☒ No

6. Will you be studying death as an endpoint (i.e., LD 50 studies)? This does not include studies involving euthanasia. ☐ Yes ☒ No

If you answered "Yes" to any of the above questions you must address the following items:

a. Names of personnel performing the procedure.
Ms. Shazmeen Aslam and Dr. Irfan Khan

b. Description of the procedure in detail, including the personnel performing the procedure.
Personnel name: Ms. Shazmeen Aslam
Rats of ~220 g will be used for this procedure and will be anesthetized followed by hair removal, burn wounds will be produced on the ventral surface by touching hot copper rod without exerting pressure for 20 sec.

c. Proposed procedures to avoid or alleviate the pain. State the anesthetic or analgesic agents and dosages to be used for each species. Provide a justification if you do not intend to use anesthetic or analgesic.
Ketamine 60mg and xylazine 7mg per Kg of body weight will be used for anesthesia and Diclofenac sodium will be used as analgesic.

d. Who will monitor the animals after the procedure and how often?
Ms. Shazmeen Aslam, at intervals of 2-3 days.

e. What potential impairment can be expected from the procedure? What can be done, if anything, to alleviate the impairment?
None expected

g. What is the duration of survival after the procedure? Endpoints for this procedure should be clearly defined.
15 days to 1 month, then tissue will be isolated and after treatment the survival is expected long term after wound healing.

7. Will you be performing decapitation or cervical dislocation without anesthesia? ☐ Yes ☒ No

If "Yes", address the following:

a. Individuals who will perform decapitation or cervical dislocation without anesthesia:

b. Who will train these individuals and how will their competency be assessed?

8. Will your study involve animals with functional deficits, other than those described previously for genetically modified animals?
☐ Yes ☒ No **If "Yes", address these items:**

a. Description of the expected deficit:

b. Proposed procedures to avoid or alleviate the deficit:

c. Who will monitor the animals while experiencing the deficit and how often?

d. What is the duration of survival? Endpoints should be clearly defined.

9. Will your study involve experiments on alert animals or involve behavioral control, prolonged restraint, forced exercise or analysis of behavior?

☐ Yes ☒ No **If "Yes," provide details of the experiment**, including means to achieve behavioral control (such as use of food and/or fluid restriction) and measures to ensure the welfare of an animal. Provide the duration of these procedures.

10. Will your study have effects on diet and/or other environmental changes?

a. Food restriction	☐ Yes	☒ No
b. Water restriction	☐ Yes	☒ No
c. Special diets or drinking water	☐ Yes	☒ No
d. Temperature changes	☐ Yes	☒ No
e. Changes in the light cycle	☐ Yes	☒ No
f. Special caging (e.g. metabolic caging)	☐ Yes	☒ No
g. Other (please specify):	☐ Yes	☒ No

If you answered "Yes" to any of these questions, you must address the following. *Before initiating these procedures, you must coordinate with the animal house manager.*

(i) Detailed description of the experiment:

(ii) Effect on the animal's health (be specific):

(iii) Duration of the experiment:

(iv) Observation schedule:

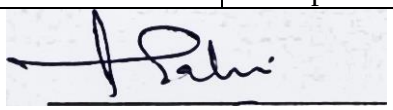
(v) Monitoring criteria:

12. DUPLICATION OF RESULTS

The ICCBS Animal Care and Use Committee requires following assurance from you:

These activities do not unnecessarily duplicate previous experiments, whether my own or those of another investigator

Appendix: I			
USDA Category B	**USDA Category C**	**USDA Category D**	**USDA Category E**
No pain or distress	Slight or momentary pain or distress or no pain or distress	Pain or distress appropriately relieved by analgesia, tranquilization or anesthesia.	Unrelieved pain or distress
Examples	Examples	Examples	Examples



PI Signatures

1. Animals (breeders, offspring that cannot be used because of improper genotype[1] and/or gender, or other animals) being maintained without any research manipulation, prior to euthanasia or transfer to another protocol. 2. Observation of animal behavior in the wild without manipulating the animal or its environment. 3. Physical restraint and preventative medical procedures such as routine vaccination 4. Routine husbandry procedures	1. Holding, weighing or transporting animals (relatively short distances under non-stressful conditions) 2. Injections (nonirritating), blood collection or catheterization of superficial vessels 3. Collection of body fluids or tissues post mortem 4. Tattooing animals 5. Ear punching of rodents 6. Routine physical examinations 7. Observation of animal behavior 8. Studies, which do not result in clinical signs of pain and/or distress 9. AVMA approved humane euthanasia procedures 10. Routine agricultural husbandry procedures 11. Observational studies and live trapping (traps must provide adequate shelter/food and be checked frequently to ensure survival)	1. Potentially stressful transportation of animals that requires tranquillization 2. Survival/terminal surgical procedures 3. Retro-orbital blood collection under anesthesia 4. Tail biopsy in mice $\geq$ 21 days old 5. Exposure of blood vessels for catheter implantation 6. Exsanguination and/or perfusion under anesthesia 7. Genetically engineered phenotype that causes pain or distress that will be alleviated. 8. Use of Freund's Complete Adjuvant 9. Ocular and Skin Irritancy testing where pain and distress are relieved 10. Any post procedural outcome resulting in evident pain, discomfort or distress such as that associated with: • decreased appetite/activity level • adverse reactions to touch • open skin lesions • abscesses • lameness • conjunctivitis • corneal edema or • photophobia but are relieved with analgesics 11. Food or water deprivation beyond that necessary for normal presurgical preparation 12. Noxious electrical shock that is not immediately escapable. 13.Paralysis or immobility in a conscious animal	1. Ocular or skin irritancy testing 2. Burns or trauma 3. Radiation sickness 4. Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation until clinical symptoms are evident or death occurs. Experiment induction of disease (i.e., Diabetes, Epilepsy, Parkinson's, etc.) including metabolic and nutritional diseases or disease resulting from exposure to toxicants 5. Mutants with chronic pain or debilitation which is not relieved with analgesics or by appropriate intervention. 6. Food or water deprivation that exceeds ordinary pre-surgical preparation or is stressful to the animal 7. Application of noxious stimuli (i.e. electrical shock) that cannot be avoided or escaped 8. Restraint using paralyzing or immobilizing drugs without anesthesia or prolonged restraint for long periods of time (days to weeks) 9. Exposure to abnormal

			or extreme environmental conditions 10. Psychotic-like behavior suggesting a painful or distressful status whether or not resulting from a procedure 11. Behavior or testing resulting in injury to cage mates or self 12. Studies in which animals are allowed to die without intervention (e.g. LD_{50}, mortality as an end-point), 13. studies that allow endpoints that are painful or stressful (i.e., addictive drug withdrawals without treatment, pain research) 14. Any procedures for which needed analgesics, tranquilizers, sedatives or anesthetics must be withheld for justifiable study purposes. 15. Euthanasia by procedures not approved by the IACUC.