

2004

**APPLICATION FOR APPROVAL TO USE
VERTEBRATE ANIMALS IN RESEARCH and TEACHING
NEW SUBMISSION**

**Institutional Animal Care and Use Committee
University of Pittsburgh, Suite 200, Hieber Bldg.**

Instructions for Filling out this form

1. All teaching and research protocols utilizing living vertebrate animals must be tendered as New Submissions for complete evaluation by the Institutional Animal Care and Use Committee (IACUC). Applications must also be submitted when utilizing dead vertebrate animals or animal parts if the animal was both sacrificed and procured solely and specifically for the research or teaching purpose. All approved protocols must be re-submitted as a new protocol for review every three years.
2. All grants to be funded by the National Institutes of Health (NIH) must be reviewed *prior* to the release of funding to assure that all procedures on animals are included in an approved IACUC protocol. For this purpose, grant PIs must complete the "GRANT APPLICATION REVIEW FORM" and submit the form to the IACUC Office along with the grant application. The "GRANT APPLICATION REVIEW FORM" asks the PI to identify the IACUC protocols that correspond to the grant application animal work. A review of the grant submission and IACUC protocols will be performed to verify that all animal work has been described and approved by the IACUC. The Office of Research must certify that this review has been conducted before NIH will release grant funding. Therefore, all PIs receiving notice of a fundable score from NIH should contact the IACUC Office as soon as possible to initiate a review.
3. Download this Word and Excel based form at www.iacuc.pitt.edu and complete the entire application. Submit the completed application via email to iacuc@pitt.edu. Hardcopy applications will not be accepted. You will receive electronic confirmation of receipt within two days. Your application will be assigned to an appropriate subcommittee for review, and you may be asked to address reviewers' concerns. Following approval from all subcommittee members, you will receive an assurance letter. Should you wish to address the entire IACUC, please contact Dr. Ed Kennah at ekennah@pitt.edu or 383-2014. A minimum turn around time for protocol review is approximately 30 days. There are no expedited reviews since all protocols are required by regulation to go through the same review process.

4. All applications supported by an internal (Departmental) or corporate funding source must be accompanied by certification from the appropriate Dean, Department Head, or Division Chief that the protocol has been reviewed for scientific merit.
5. Postdoctoral fellows, graduate students, and undergraduate students submitting applications as principal investigators must also include sponsorship by an appropriate academic advisor. Please include the sponsor's name and contact information on the certification page of this application.
6. Protocols will not be approved for Principal Investigators who have not completed the required training programs. Contact Marilee Rose, Training Coordinator, at 578-3459 or mrose+@pitt.edu to arrange species-specific training. In addition, all personnel listed as participants in the study must complete the required training. Completion of Modules 1 and 3 is required training for all personnel listed as participants in the study. This can be accessed on the web at <http://www.health.pitt.edu/rpf/>.
7. Work involving rDNA must be approved by the Institutional Biosafety Committee for rDNA (Call 578-3799 for information and complete ~~Form 4~~ the Institutional Biosafety (Recombinant DNA) Application found at www.rcco.pitt.edu/rdna/).
8. If you place any of your animals in Pain and Distress Classification E, an explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anesthetic, analgesic or tranquilizing drugs must be provided on Attachment 1.
9. Work involving radiation must be approved by the Radiation Safety Office (Call 624-2728 for information and complete Attachment 2 of this form).
10. A Registration Workbook for Use of Biological and Chemical Agents must be completed for each IACUC application and approved by the Department of Environmental Health and Safety (see Attachment 3). IACUC protocols will not be reviewed until this Workbook is completed.
11. Investigators wishing to introduce any cell/tumor lines or bodily fluids of human or animal origin into animals in the DLAR, must first receive written permission from the Director of the DLAR or his designee before the first animal is injected. Attachment 4 must be completed.
12. Investigators requesting a dispensation from the nonhuman primate enrichment plan must complete Attachment 5. For information regarding enrichment techniques or any part of the enrichment plan, please contact Sarah Greene at serst28@pitt.edu , (412) 383-7892 or (724) 327-4607.

13. According to University guidelines, any persons receiving commercial funding must complete Module 4 on the Research Practice Fundamentals website - www.health.pitt.edu/rpf/. Investigators also need to complete Attachment 6 – Declaration of Conflict of Interest.
14. All Principal Investigators with approved protocols involving physical, chemical, and/or biological hazards must schedule a pre-study strategy meeting with the Division of Laboratory Animal Resources (DLAR) (648-8950) prior to ordering animals or initiating the project.
15. In an effort to limit the frequency of changes to IACUC submission forms, a single form will be used for each calendar year. Verify that you are using the current application form by checking the date at the top of this page. If changes are required during the year, the changes can be found under the "Additional Addendum" heading of the IACUC web site www.iacuc.pitt.edu/ Before submitting a protocol, check to ensure you are using the current form and that additional addendums (if required) are completed and attached.
16. Please type your response to the questions in the white space to the right of the question. (Do not type your response in the gray shaded areas.)

Consultation with a veterinarian from the DLAR prior to application submission is recommended. IACUC approval does not assure DLAR space availability.

A. Project Administration - Personnel:

1. PI Name (First Name Last Name):	David Bush
2. Co-PI 1 (First Name Last Name):	
3. Co-PI 2 (First Name Last Name):	
4. Co-PI 3 (First Name Last Name):	
5. PI Faculty Title:	Associate Professor
6. PI Department:	Esoteric Medicine
7. PI's Department Chair: (List Dean or Div. Chief if no Chair)	Dr. Mike Smith
8. PI Mailing Address:	100 BST-75
9. PI Office Phone:	412-647-0001
10. PI Lab Phone:	412-647-0002
11. PI Fax:	412-647-0003
12. PI E-mail Address:	davidbush@pitt.edu
13. Emergency Contact (PI - 24 hr phone/pager):	412-123-4567 (PI Cellular Phone)
14. Secondary Contact (CO – PI or Staff - 24 hr phone/pager):	

B. Project Information:

15.	Title:	TNF Receptor mediated pathways in cardiac fibroblasts			
16.	Source of Funding:	NIH			
17.	Total Project Period:	9/30/00-8/31/05			
18.	Grant Title and Number:	NIH R01 HL00001-35, "The Heart is Important"			
19.	Is the grant administered through the University of Pittsburgh?	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO			
20.	If No, what institution is administering the grant?				
21.	Administering Institution's Protocol Approval # and Expiration Date:				
22.	Administering Institution's USDA Registration #:				
23.	Contact Person (name, phone, e-mail):				
24.	Will any aspect of the animal experimentation be performed at another institution (including custom antibody production)?	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO			
If Yes, provide the following: (See www.aphis.usda.gov/ac/ Policy #10 for more information.)					
25.	Institution Name:				
26.	Institution's IACUC Approval # and Expiration Date:				
27.	USDA Registration #:				
28.	AAALAC Accreditation Status:				
29.	Contact Person (name, phone, e-mail):				
<i>License and accreditation info available from the Office of Research at appropriate institution.</i>					
30.	Is this submission a major modification or a renewal of a currently active protocol?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No			
31.	If yes, please list the protocol #:	0106472			
32.	Please list the protocol's title:	Sex-related levels of TNFR and survival in heart failure			

C. Research Staff (List all individuals handling live/dead animals or animal tissues, including PI)

33. NAME	SOCIAL SEC # or PITT ID #	POSITION TITLE	*PROJECT ROLE	E-MAIL	PHONE/PAGER #
David Bush, Ph.D.	001-22-33333	Associate Professor	PI ; handle and manage all aspects of mice; sacrifice mice to isolate tissues and cells; inspect live and dead animals as needed	davidbush@pitt.edu	412-647-0001
George Rice	1003-44-5555	Research Specialist	Technician ; primary duty to euthanize mice to isolate tissues and cells; secondary duty to help manage mouse colony and order mice as needed	georgerice@pitt.edu	412-647-0008
Grand Poobah	007-88-	Undergraduate	technician; primary duty to	grandpoob	412-647-6666

	9999	student	help manage mouse colony (visually inspect mice, manage cage card labelling, order mice); secondary duty to euthanize mice and isolate tissues and cells	ah@pitt.ed	
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***List the duties will this person perform relating to the animal studies.**

D. Animal Usage:

Animals must be obtained from DLAR approved sources/vendors only. Quarantine may be required for certain animals based on their origin before they enter Institutional Animal Housing Space(s). Contact the DLAR (648-8950 or dlar@pitt.edu) for further information. Approximate weight range and age range of experimental subjects at time of arrival should be provided.

34. SPECIES	STRAIN	AGE	WGT.	SEX	Number projected for 3 Years*
mouse	C57BL6 (WT and TNFRIKO, TNFR2KO, ILR1KO)	~4 wks	~ 25 gms	M/F	158

*Only the number of individual animals of each species needs to be denoted here, not the number of individual animals of each strain.

E. Animal Housing And Use Sites:

Housing:

35. SPECIES	HOUSING LOCATION (Building, Room/Floor – if known)	HOUSING DURATION (Days)	MAXIMUM NUMBER ANIMALS HOUSED IN AN APPROVED FACILITY AT ONE TIME
mouse	BST-75 animal room	To~5 weeks	~~35

36. Rodent Caging Requirements: (Complete only for Rodent protocols):

- Microisolator caging is required.**
 Solid bottom caging is requested.
 Standard wire bottom caging is requested (see below.)
 Wire bottom caging is necessary, and I am requesting an exemption from the IACUC policy regarding use of this caging. (Provide justification below.)

*IACUC policy states that animals exceeding 500 grams or maintained for > 9 months cannot be housed in wire-bottom caging unless a specific exemption is granted (See policy at www.iacuc.pitt.edu).

If necessary, please provide scientific justification:

F. Use Sites and Transportation Methods:

37. Are animals transported from the holding room through approved animal facility space to another location/lab? [X] YES [] NO

(Approved animal facility space is defined as DLAR operated facilities as well as other animal housing spaces approved by the IACUC.)

38. If yes, indicate building(s) and room(s) of destination (If using hospital areas, see "Use of Experimental Animals in UPMC Hospital/Patient Areas policy at www.iacuc.pitt.edu/ BST-75 room 20-6666

39. Provide rationale for the need to remove animals from dedicated facilities and justify why such work cannot be performed in dedicated, approved animal facility space? Tissues (hearts) will be harvested for isolation of cells to be cultured in vitro; equipment for sterile isolation and cell preparation cannot be transported to the BST-75 animal room.

40. Will vertebrate animals be housed in these alternate locations for greater than 12 hours? Yes [] No [X]

If yes, these sites must undergo additional evaluation and approval (contact the DLAR director at dlar@pitt.edu for further information).

41. Describe animal transportation route: Via service elevator within BST-75

42. Describe animal containment & transport mechanism: Opaque container with secured lid on lab cart.

(See Animal Transportation SOP at www.iacuc.pitt.edu/.)

43. Indicate laboratory sites (if different than above) where hazardous tissues (e.g., nonhuman primate or tissues from studies involving the use of radiation, residual hazardous chemicals, or BSL-2 or greater containment) are to be taken: Stored tissues or tissue extracts in refrigerators or freezers in BST-75, room 20-6777

Note that if the tissue(s) being transferred will be handled by investigators not listed on this protocol, you are required to inform that person(s) of any inherent dangers associated with the tissues. Your sign off on the certification Section Q verifies you have provided this information.

44. Describe tissue containment & transport mechanism: Carried by hand/cart from lab to freezer room

G. Study Summary:

45. Briefly summarize (in less than three sentences) the aim(s) of the study, and why the study is important to human or animal health, the advancement of knowledge, or the good of society.

Summarize here: TNF α and perhaps IL1 β can induce cardiac remodeling (dilation, fibrosis) and heart failure. Different receptor types mediate these effects on cardiac fibroblasts, important mediators of cardiac remodeling. This study will assess the pathways by which TNF or IL1 may alter cell life, death, and production of protein important to remodeling. Such information could ultimately provide new targets for therapy in heart failure.

H. Animal Welfare Act Required Information:

46. Explain the rationale for animal use, including why non-animal models cannot be used:

Explain here: Congestive heart failure is a complex physiologic adaptation of the body to insufficient cardiac function. The adaptations of the body in turn moderate cardiac function in the failing heart. Non-intact animals cannot mimic the complexities of these interactions, many of which are poorly understood. Even when specific physiologic regulators are to be analyzed in a cultured cardiac fibroblast system, intact animals must serve as a source of cardiac cells as established cardiac fibroblast lines from mice with the desired genetic alterations (ablation of particular cytokine receptors) do not exist.

47. Justify appropriateness of the species selected and why a phylogenetically lower species could not be used?

Justify here: Heart failure in non-mammalian species is almost completely uninvestigated, with very poor documentation of cardiac function and uncertain relevance to human heart failure. Mice are the lowest phylogenetic species in which tools have been developed to create mice with specific genetic alterations, such as the homologous recombination ablation (knockout, or KO) of genes whose products are important to heart function, such as cytokine receptors.

48. Number of experimental/control groups*: 4 mouse genotypes; 2 types of studies

49. Number of animals per group*: 6 mice x 3 repeats x 4 genotypes x 2 studies (apoptosis, biochemistry)

*Number of Experimental/Control Groups X Number of Animals per group should equal the total number of animals delineated in the responses to Question 34.

50. How was sample size determined? (Answer by placing an X in the appropriate bracket(s))

(The number of animals requested should be the minimum required for statistically valid results.)*

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> Numbers and Species of Animals and <ftp://ftp.grants.nih.gov/IACUC/GuideBook.pdf> Fundamental Issues, Criteria)

Pilot study. No report of a similar study exists. The proposed study will utilize a small number of animals (typically less than 10) to obtain preliminary data to justify the performance of a larger study.

Previous experience by this PI (if checked, answer question 51)

Studies cited in the literature (If checked, answer question 51)

51. If previous experience and/or literature review was used to determine sample size, a power analysis should be described if possible, citing type of statistical analysis used. If the experimental approach is not amenable to a power analysis, a thorough justification of the number of animals required must be provided.

Describe and Justify here:

Cardiac cells will be prepared from 4 week old mice (C57BL6 WT, TNFRIKO, TNFRIIKO, or IL1R1KO, purchased from Jackson Laboratory). **Six** mice should provide sufficient cells for 12 culture wells of about 100,000 cells each; these 24 wells constitute 1 experiment of control, IGF treated, TNF (or IL1) treated, and TNF(or IL1) + IGF treated (4 conditions) in six replicate wells, with proteins isolated for western blot analyses. This experimental regimen will be repeated **3** times to confirm reproducibility of results. Mice with **Four** genotypes will be studied (C57BL6 WT, TNFRIKO, TNFRIIKO, or IL1R1KO). Thus 6x3x4=72 mice are needed. Similar experimental design will be used to assess the effects of TNF (or IL1) on IGF induced cell proliferation or apoptosis, equalling another 72 mice. Ten per cent extra are requested to compensate for unexpected cell losses, thus 158 mice (72 x 2 x 1.1) are requested. Since the degree to which TNF may alter IGF-induced responses (such as AKT phosphorylation) are not known, the distribution of mice/repeats may require adjustment. However, within one experiment, estimating an effect difference of 50%, a standard deviation of 20%, 6 replicates per condition, 4 treatments, and an alpha of 0.05%, we have a power of ~90% to detect statistically significant differences b(Russ Lenth's power and sample size page, www.stat.uiowa.edu/~rlenth/Power/index.html).

I. Drug Administration

Anesthesia, Analgesia, Tranquilizing, and Paralytic Drugs (Specify dosage in mg/kg)

52. SPECIES	DRUG	DOSE	ROUTE	ADMIN. FREQUENCY
Mouse	isoflurane	To effect	inhalation	Anesthetic used once preceding euthanasia.

For drugs listed above, please supply the duration of treatment and method of monitoring efficacy:

53. DRUG	Duration of Treatment	Method of Monitoring Efficacy
isoflurane	Once each for 5 minutes	response to foot pinch/pull;

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> -Minimization of Pain & Distress)

54. Are Paralytic Drugs Used: Yes No
If Yes, paralytic drug used:

Describe Health Monitoring Procedures:

- Justification for use of the paralytic agent in this study.
- Provide information and/or data that demonstrate the proposed anesthetic regimen is adequate without the paralytic agent. This can be based on a) literature citations specifically using the same regime, b) documented experience of the PI with this

anesthetic regime without paralytic inclusion or c) the previous documented performance of a “sham” procedure utilizing the regime without paralytic agent use.

- c. Provide a plan to titrate the amount of anesthetic to establish a verifiably adequate plane of anesthesia in each animal prior to inducing paralysis.
- d. Describe the proposed anesthetic monitoring procedures in paralyzed animals (e.g. electroencephalogram, electrocardiogram, blood pressure, etc.) Please note if heart rate or blood pressure is monitored, elevations or depressions of 15-20% from baseline prior to adding paralytics may be indicative of need for alteration of anesthetic levels.

55. Drugs Administered for Therapeutic Purposes (excluding anesthetics, analgesics, and tranquilizers)

Will pharmaceutical agents be introduced to the living and/or dead animals? YES NO
 If Yes,

SPECIES	AGENTS	DOSE	ROUTE	EFFECT

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable:

56. Are any *non-pharmaceutical agents to be introduced into Animal Welfare Act (AWA) regulated species (vertebrate animals excluding rats, mice and birds)? YES NO
*(Please note: As per specific Animal Welfare Act directive, “Non-pharmaceutical-grade chemical compounds should only be used in regulated animals after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. **Cost savings alone are not an adequate justification for using non-pharmaceutical-grade compounds in regulated animals.**”)*

(See policy at <http://www.iacuc.pitt.edu/Policies.asp>)

***Non-pharmaceutical agent** – An agent that was not specifically prepared for injection into animal or human in its current form.

If Yes, provide the scientific rationale for their use, source, proposed sterilization technique, sterile packaging, labeling and expiration information along with proposed methods to determine efficacy.

Describe:

57. Are non-pharmaceutical agents to be introduced into Animal Welfare Act (AWA) non-regulated species (rats, mice or birds) for the purpose of veterinary medical care or to relieve pain and distress (e.g., anesthetics, analgesics, sedatives, antibiotics, or paralytics)? YES NO
(Please note: As per University of Pittsburgh IACUC approved policy, all compounds used in rats, mice or birds for veterinary medical care or to relieve pain and distress (e.g., anesthetics, analgesics, sedatives, antibiotics, or paralytics) must be pharmaceutical grade (when available) unless scientifically justified. Cost savings alone are not considered by the IACUC an adequate justification for using non-pharmaceutical grade drugs for veterinary medical care or to relieve pain and distress in these species.)

(See policy at http://www.iacuc.pitt.edu/Policies.asp)
If Yes, provide the scientific rationale for their use, source, proposed sterilization technique, sterile packaging, labeling and expiration information along with proposed methods to determine efficacy.
Describe:

J. Euthanasia

58. Will animals be euthanized?	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
59. If NO, what is final disposition of animals?	
60. If YES, provide drug and dose or method used.	Cervical dislocation while under isoflurane anesthesia followed by thoracotomy.
See http://www.avma.org/resources/euthanasia.pdf for approved euthanasia methods (Adobe Acrobat software required)	

K. Animal Exposures

Radioactive Agents (Prior to any study involving the administration of radioisotopes to animals, the Radiation Safety Office must review and approve the use.)		
61. Will radioactive substances be administered in live animals? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes, complete Attachment 2 and the table below.		
SPECIES	ISOTOPE	ACTIVITY
62. Will X-Ray equipment, accelerators, CT scanners, irradiators, or external beam radiation be used? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes, complete the table below. (It is <u>not</u> necessary to complete Attachment 2 for a Yes response to this question.)		
EQUIPMENT	LOCATION OF USE	DOSE*

*Dose not applicable to diagnostic imaging studies

Biological Agents (complete Attachment 3)				
63. Will Biological Agents be used in living and/or dead animals? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes,				
SPECIES	BIOLOGICALS	DOSE	ROUTE	EFFECT

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable:

64. Chemical Agents (Do not include drugs used for therapeutic purposes) ?				
Will chemical agents be introduced to the living and/or dead animals? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes,				
SPECIES	CHEMICALS	DOSE	ROUTE	EFFECT

Describe any adverse effects associated with the administration of these agents and a detailed plan for monitoring and alleviating these effects if applicable:

65. Cells/Tumor Lines/Bodily Fluids (complete Attachment 4)				
Will cells, tumor lines, or bodily fluids be introduced to the live and/or dead animals? <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO If Yes,				
SPECIES	AGENTS	DOSE	ROUTE	EFFECT

L. Description of Experimental Design and Animal Procedures:

66. Describe the specific experimental manipulations and treatments of the animals in terms that are intelligible to non-specialists. This description should allow the IACUC reviewer to understand exactly what will be done to all the animals from entry into the study to the endpoint of the study.

It would be helpful to provide a flow chart or table illustrating your experimental design and include animal numbers to be used. The experimental endpoint that is being measured must be indicated for each procedure. (Please do not insert the Methods Section from the grant application. The IACUC is only required to review procedures that are done to animals.)

Describe Experimental Design, Animal Procedures, and Surgical Procedures here:

Preparation of cardiac cells from mice with varying expression of TNF or IL1 receptors Insulin-like-growth factor (IGF) is a potent regulator of cardiac cell growth and survival. TNF appears to blunt the effects of IGF on cardiac cells, perhaps through alteration of AKT-kinase pathways. The pathways by which TNF may alter the effects of IGF, and whether IL1 acts similarly, will be examined in cultured cardiac fibroblasts isolated from mice with various genotypes. Cardiac cells will be prepared from 4 week old mice (C57BL6 WT, TNFRIKO, TNFRIKO, or IL1R1KO, purchased from Jackson Laboratory). Mice will be brought to BST1750, anesthetized in a jar containing isoflurane vapors, killed by cervical dislocation, and hearts removed for cardiac cell isolation by sequential collagenase digestion. **Six** mice should provide sufficient cells for 12 culture wells of about 100,000 cells each; these 12 wells constitute 1 experiment of control, IGF treated, TNF (or IL1) treated, and TNF(or IL1) + IGF treated (4 conditions) in triplicate wells, with proteins isolated for western blot analyses. This experimental regimen will be repeated **3** times to confirm reproducibility of results. Mice with **Four** genotypes will be studied (C57BL6 WT, TNFRIKO, TNFRIKO, or IL1R1KO). Thus 6x3x4=72 mice are needed. Similar experimental design will be used to assess the effects of TNF (or IL1) on IGF induced cell proliferation or apoptosis, equalling another 72 mice. Ten per cent extra are requested to compensate for unexpected cell losses, thus 158 mice (72 x 2 x 1.1) are requested.

67. Describe Experimental Endpoints Here: (At what point is the experiment completed on animals?)

Animals are euthanized so that hearts can be removed for preparation of cardiac fibroblasts.

For each *Non-surgical Procedure* for each species: Specifically address the following:

68. Will blood sampling be conducted? [] Yes [X] No

69. If Yes, provide rationale, method, site, volume, & frequency:

70. Will food scheduling or restriction (other than standard pre-operative fasting) be conducted? [] Yes X] No

71. Provide rationale, method, frequency, & duration:

72. Will water scheduling or restriction (other than standard pre-operative fasting) be conducted? [] Yes [X] No

73. If Yes, provide rationale, method, frequency, & duration:

74. Will restraint methods be utilized? [] Yes [X] No

75. If Yes, provide rationale, method, frequency, & duration*:

***Note:** Provide a detailed description of non-human primate handling methods (other than chemical immobilization) and justify the use of alternative methods and provide assurances of personnel safety.

76. Will stress paradigms be utilized? [] Yes [X] No

77. If Yes, provide rationale, type, frequency, & duration:

(See <http://grants.nih.gov/grants/olaw/GuideBook.pdf> Methodology)

<p>For Each <i>Surgical Procedure</i> for each species, specifically address the following (required): (The Surgical Guidelines are available at www.iacuc.pitt.edu under "Policies.")</p>
<p>78. Will non-survival surgery be performed? Yes [X] No []</p>
<p>79. Will survival surgery be performed? Yes [] No [X]</p>
<p>80. Will multiple survival surgeries be performed on one animal? Yes [] No [X]</p> <p>If Yes, please indicate the rationale for performing multiple procedures. Also, please justify if these surgeries must be performed on separate occasions, versus under one anesthetic event. (Note-If two or more of these survival surgeries involve major procedures, defined as a surgical intervention that penetrates and exposes a body cavity or any procedure that produces substantial or permanent impairment of physical or physiological functions, proceed directly to question 81.) <i>Enter rationale here:</i></p>
<p>81. Will two or more of these survival surgeries involve major procedures (defined as a surgical intervention that penetrates and exposes a body cavity or any procedure that produces substantial or permanent impairment of physical or physiological functions)?</p> <p>Yes [] No []</p> <p>Federal guidelines specify that no animal is to be used in more than one major survival operative procedure except in cases of scientific necessity or to provide adequate veterinary care. If you are requesting to perform multiple major survival surgeries, you must provide substantial scientific justification below. (See http://www.aphis.usda.gov/ac/polmanpdf.html, Policy 14).</p> <p><i>Enter justification here:</i></p>
<p>82. Surgical Site (Building, Room Number): BST-75 room 20-6666</p>
<p>83. Names of Surgeon(s): David Bush and George Rice</p>
<p>84. Has the surgeon(s) performed this procedure on the species requested? Yes [X] No []</p>
<p>85. If no to question 84, please provide training plan:</p>
<p>86. Postoperative Plan (Methods to assess/alleviate pain/distress, Recovery Criteria, Monitoring Criteria -See http://grants.nih.gov/grants/olaw/GuideBook.pdf): none/non-survival</p> <p><i>Enter plans for monitoring animals as well as alleviating pain and distress here:</i> When mice are not responsive to toe pull, they will be euthanized as described for isolation of cardiac cells.</p>

M. Anticipated Effects on Experimental Animals

<p>87. Changes to Health and Well-Being</p>
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Please describe all signs of morbidity (e.g., decreased food and/or water consumption, weakness, infection neoplasia, neurological effects, etc.) expected to occur in the experimental subjects as a result of the procedures performed in this protocol (If mortality is expected please note here and see

[http://www.iacuc.pitt.edu/sop/Pain %20Indicators.htm](http://www.iacuc.pitt.edu/sop/Pain%20Indicators.htm).)

These mice appear normal until euthanized for isolation of cardiac cells.

88. Describe the expected frequency of the complications noted in question 87 and how you will deal with these complications:

Describe here:

Non complications expected

Please complete the following two questions for all procedures.

89. Methods and Frequency of Monitoring Health/Well-Being Changes

Describe in detail the species specific procedures that the investigator will use to monitor the experimental animals' health and well-being, monitor pain and distress, alleviate pain and distress, and the frequency with which these procedures will be performed:

Mice will be housed for 1-2 weeks before isolation of cardiac tissues. Mice will be visually checked once-twice a week by lab personnel before use, and everyday by DLAR staff.

90. Criteria for Premature Removal of Animals from Study (This section applies to acute and chronic studies)

Describe in detail the criteria utilized to determine that an experimental animal must be removed from a study:

Upon veterinary opinion, mice which appear ill will be treated or sacrificed as advised.

N. Pain or Distress Classification

Please note that this section applies to USDA and Public Health Service (NIH) regulated species. This includes all species of animals and is a change from an earlier application.

Pain or Distress Classification

91.	SPECIES (Common Name)	USDA CLASSIFICATION* % of Animals Classified			
		B	C	D	E
		%	100%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%
		%	%	%	%

* **USDA Classifications** (see www.aphis.usda.gov/ac/ -Policy #11 for more information)

Classification B: Animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery, but not yet used for such purposes.

Classification C: Animals upon which teaching, research, experiments, or tests will be conducted involving no pain, no distress, or no use of pain-relieving drugs. Euthanasia must precede any invasive procedure (i.e. tissue harvesting) to be in Classification C.

Classification D: Animals upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used. Acute or terminal surgery is considered a painful procedure, which is alleviated by anesthesia.

Classification E: Animals upon which teaching, experiments, research, surgery (survival or non-survival), or tests will be conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests and/or animals upon which teaching, experiments, research, surgery (survival or non-survival), or tests will be conducted resulting in permanent physiological impairment that may lead to chronic pain or distress.

NOTE REGARDING CLASSIFICATION E: An explanation of the procedures producing pain or distress in these animals and the justification for not using appropriate anesthetic, analgesic or tranquilizing drugs must be provided on **Attachment 1**. This information is required to be reported to the USDA, will be available from the USDA under the Freedom of Information Act, and may be publicly available through the Internet via USDA's website.

O. Consideration of Alternatives To Painful/Distressful Procedures – (Complete only if you are using vertebrate species other than purpose-bred birds, mice of the genus *Mus*, and rats of the genus *Rattus*)

This section applies to all USDA regulated species. Reference Librarian, Alice Kuller is available for consultation in performing literature searches (648-1971).

If any procedures fall into USDA's Classification D or E, causing more than momentary or slight pain or distress to the animals, USDA Policy 12 requires that you must address **all three** of the following issues:

- 1) You have refined potential pain-producing methods as much as possible to minimize distress.
- 2) You have reduced animal use as much as possible without jeopardizing statistical validity.
- 3) You have considered replacing potential pain-producing methods with other techniques (e.g., in vitro techniques, computer simulations, etc.).

As part of the response, you must provide written narratives that will convince the IACUC reviewers you have made a good faith effort to substantively address **each** of these three issues. You must also provide **two sources** of information to show that you have addressed **each** of the three issues. At least one of these sources of information should be a search of a scientific database. For each search executed, you must specify the **database** considered, the **date** of the search, **period** covered by the database searched, and the **keywords** used. If you elect to search two databases to satisfy the consideration of alternatives, please note that both Medline and PUBMED are search engines that consider the same database, the National Library of Medicine, and thus employing both of these search engines is considered to be a single database search. **Note: the investigator should retain all searches, as they are reviewable by federal agencies.**

An alternate method of consideration for alternatives is consulting with an expert (e.g., DLAR veterinarian, another PI with extensive experience with the issue in question) or attending conferences or colloquia that provide relevant and up-to-date information directly related to the issue. When sources other than database searches are included, sufficient documentation, such as the consultant's name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert's knowledge on the availability of alternatives in the specific field of study.

For more information, see www.aphis.usda.gov/ac/ -Policy #12.

Instructions:

For each section below, provide at least 2 database searches, or 1 database search + an expert consultation, then provide a written narrative.

92. Refinement - Provide evidence that you have refined potential pain or distress-producing procedures as much as possible to minimize distress.

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used

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Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation

Written narrative based on this information:

93. **Reduction** - Provide evidence that you have reduced animal use as much as possible without jeopardizing statistical validity.

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation

Written narrative based on this information:

94. **Replacement** - Provide evidence that you have considered replacing potential pain or distress-producing methods with other techniques (e.g., in vitro techniques, computer simulations, etc.).

Date of Search(s)	Database(s) Searched	Inclusive Date(s) of Search	Keywords Used

Source of Expert Information	Qualifications of Expert Source	Extent, Nature, and Content of Expert Consultation	Date of Expert Consultation

Written narrative based on this information:

P. Exemption from Environmental Enrichment For Nonhuman Primates or Exercise Program for Dogs

95. For nonhuman primates, are you seeking an exemption from the institution's plan for environmental enrichment? (see Plan to Promote the Psychological Well-Being of Nonhuman Primates at www.iacuc.pitt.edu/policies.htm) [] YES [] NO If Yes, complete Attachment5
96. For dogs, are you seeking an exemption from the institution's plan for exercise (see www.iacuc.pitt.edu/policies.htm)? [] YES [] NO If Yes, justify below:
<i>Type Justification Here</i>

Q. Principal Investigator Certifications

- I certify that I have determined that the research proposed herein is not unnecessarily duplicative.
- I certify that I will notify the IACUC before initiating any significant changes in this protocol.
- I certify that I will notify the IACUC regarding any unexpected results that impact animals. Any unanticipated pain, distress, morbidity or mortality will be reported to the attending veterinarian.
- I understand that if I cannot be contacted in the event that animals in this project show evidence of distress, illness or pain, emergency care, including euthanasia if necessary, will be administered at the discretion of the veterinary medical staff.
- For all USDA Classification D and E proposals (see Section N): I certify that I have reviewed the pertinent scientific literature and the sources and/or databases as noted in Section O and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
- For any animal body fluids or tissues, derived from the studies described in this application and transferred to persons not listed in Section C, I certify I will notified the recipient of all hazards associated with these materials and instructed them to register with the EHS Department (624-9505)
- I certify that I will share a copy of the approved protocol with all personnel identified in Section C and they will read and understand all elements described for the study.

- I certify that all surgeons involved in this project have read the Surgical Guidelines. (The Surgical Guidelines are available at www.iacuc.pitt.edu under "Policies.")

R. Inclusions*: (Please respond by placing an X in the appropriate bracket):

97. This protocol involves animals in Pain or Distress Classification E: YES NO
If yes, has Attachment 1 been completed? YES NO
98. This protocol involves radiation usage: YES NO
If yes, is this protocol registered with the Radiation Safety Office (Attachment 2)?
 YES NO
99. This protocol involves hazardous chemicals or biologicals: YES NO
If yes, is this protocol registered with the Health and Safety Office (Attachment 3)
 YES NO (**to be submitted**)
100. This protocol introduces cells/tumor lines or bodily fluids into animals: YES NO
If yes, is this protocol registered with the DLAR (Attachment 4)? YES NO
101. This protocol seeks an exemption from the institution's environmental enrichment plan for nonhuman primates: YES NO
If yes, has a dispensation from the University of Pittsburgh's Nonhuman Primate Enrichment Plan (Attachment 5) has been requested? YES NO
102. This protocol involves private or commercial funding: YES NO
If yes, has the Conflict of Interest Form has been completed (Attachment 6)?
 YES NO
103. This protocol involves rDNA: YES NO
If yes, has registration been submitted to the IBC? YES NO
If the rDNA is already registered, has the IBC been notified to add this protocol to your registration? YES NO

***Note – Please delete all Attachments not valid to your protocol before returning this completed form to the IACUC office.**

Principal Investigator Certification: (First Name
Last Name):

David Bush

Date Submitted:

6/11/04

If Applicable, Post-Doctorate and Graduate Student Sponsor (Name, phone #, e-mail

address):

As a sponsor, I agree to take responsibility for all experimental studies performed pertaining to this protocol and agree to comply with all federal regulations applicable to this research YES NO