NOV 2 8 2008

This report is required by law (7 USC 21 43) Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150

Set reverse side for additionalinformation

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. REGISTRATION NO. Certificate Number: 63-R-005
Customer Number: 858

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA include Zip Code)

Vanderbilt University
Asst VC for Research Director Animal Care
(b)(2)High, (b)(7)f
Nashville, TN 37232
Telephone: (b)(2)High, (b)(7)f

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS (Sites)

(b)(2)High, (b)(7)f

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Anima Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, reaearch, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used	E. Number of animals upon which teaching experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in those animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO OF ANIMALS (Cols. C + D + E)
4. Dogs		3	251	5	259
5. Cats			29		29
6. Guinea Pigs			7		7
7. Hamsters			380		380
8. Rabbits		30	151	40	221
9. Non-human Primates			203		203
10. Sheep		3			3
11., Pigs			225	37	262
12. Other Farm Animals					
Goat			74		74
13 Other Animals					
Cotton Rat			47		47
Water Shrew			5		5
Squirrel			2		2

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarianfor this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional Official)
I certify that the above is true, correct, and complete (7 U.S.C Section 2143).

SIGNATURE OF CEO OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF CEO OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
(b)(6), (b)(7)c		11/26/08

API

(AUG 91)

NP

NOV 2 8 2008

This report is required by law (7 USC 21 43) Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150

Set reverse side for additionalinformation

Interagency Report Control No 0580-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. REGISTRATION NO. Certificate Number: 63-R-005
Customer Number: 858

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY (Name and Address, as registered with USDA Include Zip Code)

Vanderbilt University
Asst VC for Research Director Animal Care
(b)(2)High, (b)(7)f
Nashville, TN 37232
Telephone: (b)(2)High, (b)(7)f

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS (Sites)

(b)(2)High, (b)(7)f

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS FORM 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used	E. Number of animals upon which teaching experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in those animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO OF ANIMALS (Cols. C + D + E)
Naked Mole Rat			164		164
Tree Shrew			10		10
Gerbil			147		147
Mole			15		15
Grasshopper Mice			14		14
Ferret			41		41

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarianfor this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional Official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

SIGNATURE OF CEO OR INSTITUTIONAL OFFICIAL	NAME & TITLE OF CEO OR INSTITUTIONAL OFFICIAL *(Type or Print)*	DATE SIGNED
(b)(6), (b)(7)c		11/26/08

APHIS FO

(AUG 91)

Attachment to APHIS FORM 7023
Summary of Exceptions to Regulations and Standards
Vanderbilt University 63-R-0005
Oct 1, 2007 to Sept 30, 2008

1. The following protocols involve exceptions to the requirements for watering nonhuman primates. Fluid access will be controlled for operant conditioning. Experience has demonstrated that short periods (e.g., 24 hours) of markedly reduced or no fluid intake may be required during the initial phases of operant conditioning. The maximum time access to water is limited does not exceed 24 hours. Once an animal has learned a behavior, the daily amount of fluid provided will be increased to the maximum level that will ensure adequate performance of the task. Each exception was approved by the Vanderbilt University (VU) Institutional Animal Care and Use Committee (IACUC) after their determination of scientific justification.

C/06/563: *Organization of Vibrotactile Information in SI Cortex, Optical Imaging of SI in the Awake Primate*
Species Used: Macaque Species (*Macaca mulatta, M. radiata*)
Number Used: 1

C/06/564: *Inter-areal Cooperativity During Perception of Visual Contours, Fast Optical Imaging of Cortical Signals in the Behaving Primate (rewrite of C/05/080 & M/06/359)*
Species Used: Macaque Species (*Macaca mulatta, M. radiata*)
Number Used: 4

M/06/385: *Cognitive Control o f Thalamic Activity*
Species Used: Macaque Species (*Macaca mulatta, M. radiata*)
Number Used: 1

C/06/417: *Neural Control of Voluntary Movement*
Species Used: Macaque Species (*Macaca mulatta, M. radiata*)
Number Used: 5

C/06/416: *Saccade Target Selection – Frontal Cortex (rewrite of C/05/106)*
Species Used: Macaque Species (*Macaca mulatta, M. radiata)*
Number Used: 5

M/06/392: *Visual System Organization and Development (rewrite of M/02/303)*
Species Used: Galago (*Otolemur garnetti)*
Number Used: 1

2. The following protocol involves an exception to the lighting requirements for cats. This study examines the role of visual experience on the development of multisensory processes in the midbrain. From birth, kittens are raised to adulthood in total darkness. Total darkness is required to eliminate all visual experience during development in a reversible manner. Procedures such as enucleation or eyelid-suturing are inadequate because they are not reversible or allow a significant amount of light to penetrate the lids. A portion of the population is returned to full-spectrum lighting at six to twelve months of age. When in darkness, animals are cared for in accordance with all other standards and regulations. Infrared goggles

are used by animal care, veterinary, and research personnel who enter the room daily to perform routine husbandry, health evaluations, enrichment, and research manipulations. All dark-reared animals have remained healthy. Results from this work are important for the improved treatment of children with visual disorders such as blindness, amblyopia, and strabismus. The exception was approved by the VU IACUC after determination of scientific justification.

M/06/375: *Development of Multisensory Cortex: Role of Experience (rewrite of M/05/345)*
Species Used: Cats (*Felis sylvestris catus*)
Number Used : 12

3. The following protocol involves an exception to the exercise requirements for dogs. The objective of the research is to assess the capacity of an implantable stimulator (laryngeal "pacemaker") to reanimate paralyzed muscles of the larynx. The implantable stimulator could obviate the need for a tracheostomy (breathing hole in the throat) in some human patients that would otherwise require one. Following nerve transection to induce laryngeal paralysis, dogs are implanted with a laryngeal muscle stimulation device. To allow normal wound healing and stabilization of the implanted pacemaker, each animal is restricted from exercise for a maximum period of 10 days post-operatively. The exception was approved by the VU IACUC after determination of scientific justification.

M/06/441: *Electrical Stimulation of the Bilaterally Paralyzed Larynx Paced with Respiration (rewrite of M/06/230)*
Species Used: Dogs (*Canis familiaris*)
Number Used: 4

4. The following protocol involves exceptions to the provision of food to dogs. These studies are done to investigate the hormonal and neural control of glucose metabolism, specifically gluconeogenesis and glycogenolysis, the two biologic processes by which glucose is made available. For each protocol listed, a subset of dogs is fasted for 42 hours prior to performing a metabolic study. At 42 hours of fast, glucose production by the liver is approximately 50% derived from *de novo* synthesis and 50% from glycogen breakdown. This is the minimal time in the progression of the switch from glycogenolysis to gluconeogenesis that the experimental techniques and methods allow the investigators to assess significant changes in gluconeogenic rates. The exceptions were approved by the VU IACUC after determination of scientific justification.

M/08/053: *Gluconeogenesis and Glycogenolysis: Role and Regulation (rewrite of M/06/400)*
Species Used: Dogs (*Canis familiaris*)
Number Used: 6

5. The following protocols involve an exception to the provision of food to dogs. This study is done to investigate how the body, especially the liver, responds to glucose oversupply and infection. Animals receive total nutritional support via a central vein catheter for 4 days. During this time, dogs have free access to water, but access to food is limited. Intravenous nutrients (glucose, amino acids, lipid emulsion, electrolytes, and vitamins) are administered in amounts that meet the dogs' daily requirements and estimated energy expenditure. The exception was approved by the VU IACUC after determination of scientific justification.

M/06/369: *Regulation of the Adaptive Response to Increased Nutrient Availability*
Species Used: Dogs (*Canis familiaris*)
Number Used: 11

M/06/368: *Nutrition Infection and Hepatic Carbohydrate Metabolism*
Species Used: Dogs (*Canis familiaris*)
Number Used: 11

6. The following protocols involve an [redacted]
This study creates a [redacted] The goal is to help develop new treatments for [redacted] suffering from [redacted] Some animals will be raised in a hypoxic environment created by delivering 10% oxygen at atmospheric pressure. CO_2 levels are maintained at 3-5 mm Hg by rapid gas flow and the use of soda lime CO_2 absorbent. The animals are kept warm with a heat lamp and the ambient temperature and humidity are monitored and recorded once daily. The exception was approved by the VU IACUC after determination of scientific justification.

M/06/371: [redacted]
Species Used: Swine (*Sus scrofa domestica*)
Number Used: 12

M/06/372: [redacted] *(rewrite of M/06/182)*
Species Used: Swine (*Sus scrofa domestica*)
Number Used: 12

M/06/376: *Hsp90/Client Protein Interactions in the Newborn Lung*
Species Used: Swine (Sus scrofa domestica)
Number Used: 13

7. The following protocols involve an exception to the provision of animal space requirements for rabbits. The purpose of these studies is to see if Tylenol or Dexamethasone administration will lead to protection of the brain from strokes by stopping or limiting the formation of specific chemical compounds that are produced when a blood clot breaks down, thereby, lessening or inhibiting vasospasm following a subarachnoid hemorrhage usually caused by an intracranial aneurysm rupture. To this end, New Zealand white rabbits will undergo a cerebral angiography, followed by injection of autologous blood directly into the subarachnoid space at the cisterna magna. A catheter will be surgically placed into the ipsilateral jugular vein for infusion by a Pegasus pump to be worn in a jacket pocket with the drug in the other pocket for balance. The rabbits will receive constant infusion of Tylenol or Dexamethasone through an IV pump for 3 days. A subset of rabbits (10-20%) is expected to exhibit moderate to severe neurological deficits. To prevent those animals from injuring themselves, they will be housed in a medium-sized airline pet carrier with footed rubber matting over underpads to keep rabbits clean and dry. Pads under the matting will be changed and the cage cleaned whenever wet or soiled. Rolled padding along sides of cage will be provided for comfort and to keep the rabbits from injuring themselves. The exception was approved by the VU IACUC after determination of scientific justification.

M/06/391: *Neuroprotective Effects of Acetaminophen in a Subarachnoid Hemorrhage-induced Cerebral Vasospasm Model in Rabbits (rewrite of M/06/044)*
Species Used: Rabbit (*Oryctolagus cuniculus*)
Number Used: 12

M/07/355: *Continuous Low-Dose Heparin Infusion in the Subarachnoid Hemorrhage-Induced Cerebral Vasospasm Model in Rabbits*
Species Used: Rabbit (*Oryctolagus cuniculus*)
Number Used: 1

8. The Vanderbilt University IACUC had petitioned the USDA/APHIS/AC office to grant an exemption from §2.31(d)(1)(x) AWAR that addresses multiple major operative procedures in a single animal. The exemption was approved by USDA/APHIS for the period from August 14, 2008 to August 13, 2011. The exemption involves four *Macaca mulatta* that had been used in a study requiring multiple major survival surgeries, which were scientifically justified, and approved by the IACUC. This study ended when the Principal Investigator left the university. USDA granted permission to transfer animals RQ2996, RQ

5536, RQ 5540, and RQ 5541 to two different protocols which are similar but not identical in scientific scope to the original protocol and also will involve additional major survival surgeries. As requested, USDA will be notified when surgeries will be performed. Complete health and surgical records will be maintained with sufficient detail and the VU IACUC will be periodically updated on the animal's well-being. In addition, special attention will be paid to ensure adequate anesthesia and analgesia to minimize the potential for pain and/or distress in these animals. The clinical veterinarian(s) are intimately involved in the oversight of the four macaques.

M/07/354: *Multisensory Interactions in the Lateral Geniculate Nucleus*
Species Used: Macaque species
Animals used: 1 *(not used during USDA reporting period)*

C/06/564 *Inter-areal Cooperativity During Perception of Visual Contours, Fast Optical Imaging of Cortical Signals in the Behaving Primate*
Species Used: Macaque species
Number used: 3 *(not used during USDA reporting period)*

Attachment to APHIS FORM 7023

Explanation of Column E Procedures
Vanderbilt University 63-R-0005
Fiscal Year 2007-2008

1.The following protocols involve experiments reported in Column E. Experiments were approved by the Vanderbilt University (VU) Institutional Animal Care and Use Committee (IACUC) after their determination of scientific justification.

M/06/371: [redacted]
(rewrite of M/06/030)
Species Used: Swine (*Sus scrofa domestica*)
Number Used: 12

M/06/372: [redacted] *(rewrite of M/06/182)*
Species Used: Swine (*Sus scrofa domestica*)
Number Used: 12

M/06/376: *Hsp90/Client Protein Interactions in the Newborn Lung*
Species Used: Swine (*Sus scrofa domestica*)
Number Used: 13

Explain the procedure producing pain and/or distress:

This experiment examines the effects of low levels of oxygen (hypoxia) on tiny blood vessels from the lungs of newborn pigs. Piglets are placed in a plastic chamber and a hypoxic atmosphere is provided by delivering 10% O_2 at atmospheric pressure to simulate the environment of newborns born and living at an altitude of 15,000 feet. Control piglets are housed in a similar chamber but with a room air environment. Rapid gas flow and the use of soda lime CO_2 absorbent is used to keep the CO_2 level at approximately 3-5 mm Hg. The temperature and humidity of the chamber are monitored and recorded at least once daily. In this experiment, piglets must remain in the chamber under the control or hypoxic environment for one of two time periods, either 3 days or 10-12 days. Both the control and hypoxic chamber are opened at least 2 times a day to clean the chamber and replenish food. Although animals are not expected to experience significant discomfort, piglets are monitored for difficult breathing and other signs of respiratory distress, and euthanized if such signs are observed.

Provide scientific justification why pain and/or distress could not be relieved. State methods used to determine that pain and/or distress relief would interfere with test results. (For Federally-mandated testing, see next response.):

[redacted] *Any potential discomfort was felt to be scientifically justified since maintaining the piglets in an*

hypoxic environment is the only known method for replicating the condition seen in human infants. To date, no signs of overt distress have been observed and no animals have been euthanized.

What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102):
Agency: *Not applicable* CFR: *Not applicable*

2.The following protocol involves experiments reported in Column E. Experiments were approved by the Vanderbilt University (VU) Institutional Animal Care and Use Committee (IACUC) after their determination of scientific justification.

M/06/441: *Electrical Stimulation of the Bilaterally Paralyzed Larynx Paced with Respiration (rewrite of M/06/230)*
Species Used: Dogs (*Canis familiaris*)
Number Used: 4

Explain the procedure producing pain and/or distress:

The objective of the research is to assess the capacity of an implantable stimulator (laryngeal "pacemaker") to reanimate paralyzed muscles of the larynx. The implantable stimulator could obviate the need for a tracheostomy (breathing hole in the throat) in some human patients that would otherwise require one. Following nerve transection to induce laryngeal paralysis, dogs are implanted with a laryngeal muscle stimulation device. Although unlikely, animals that [redacted] *Although undesirable, such attacks are probably not life threatening, because* [redacted] *will cease if the animal becomes* [redacted] *However, the* [redacted] *for the animal.*

Provide scientific justification why pain and/or distress could not be relieved. State methods used to determine that pain and/or distress relief would interfere with test results. (For Federally-mandated testing, see next response.):

An extensive literature search confirmed that the research proposal was novel and that there were no other alternatives to the above listed procedures. An animal model of unilateral paralysis as used in the past would not allow us to determine whether this new treatment is effective and could restore ventilation in the bilaterally paralyzed human. Additionally, tissue or organ culture studies cannot obtain necessary data to support a subsequent investigation in the human of laryngeal pacing. Computer modeling would not serve as an appropriate replacement for animal modeling. We are unsure whether [redacted]

What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102):
Agency: *Not applicable* CFR: *Not applicable*

3.The following protocol involves experiments reported in Column E. Experiments were approved by the Vanderbilt University (VU) Institutional Animal Care and Use Committee (IACUC) after their determination of scientific justification.

M/06/368: *Nutrition Infection and Hepatic Carbohydrate Metabolism*
Species Used: Dogs (*Canis familiaris*)
Number Used: 1

Explain the procedure producing pain and/or distress:

Glucose oversupply and glucose toxicity associated with it is a major obstacle in the management of diabetic and insulin-resistant patients. This study will focus on the role glucagon and fatty acids play in the liver's adaptation to glucose oversupply and infection. To this end, a non-lethal infection will be induced by placing a fibrin clot containing E. coli into the peritoneal cavity of an anesthetized dog in which catheters for blood sampling had been implanted previously. The dog will awaken and will be studied ~36-48 hours after implantation of the clot.

Provide scientific justification why pain and/or distress could not be relieved. State methods used to determine that pain and/or distress relief would interfere with test results. (For Federally-mandated testing, see next response.):

An extensive literature search confirmed that the research proposal was novel and that there were no other alternatives to the above listed procedures. The metabolic response to infection is markedly altered by the use of opioid analgesia and non-steroidal anti-inflammatory agents. Because alterations in metabolism are the focus of these studies, we are unable to give these agents. While these studies will create a stress, we are giving low doses (non-lethal) of the bacteria so as to minimize the hypotension, pain and discomfort to the animal, while not compromising interpretation of the results. Generally, all animals after receiving fluid resuscitation (1000 ml at a rate of ~9 ml/min; 24 hours after induction of infection) are mobile.

What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102):
Agency: *Not applicable* CFR: *Not applicable*

4.The following protocols involve experiments reported in Column E. Experiments were approved by the Vanderbilt University (VU) Institutional Animal Care and Use Committee (IACUC) after their determination of scientific justification.

M/06/391: *Neuroprotective Effects of Acetaminophen in a Subarachnoid Hemorrhage-induced Cerebral Vasospasm Model in Rabbits (rewrite of M/06/044)*
Species Used: Rabbit (*Oryctolagus cuniculus*)
Number Used: 32

M/07/147: *Dexamethasone Effects on Inflammation in the Subarachnoid Hemorrhage-induced Cerebral Vasospasm Model in Rabbits*

Species Used: Rabbit (*Oryctolagus cuniculus*)
Number Used: 2

Explain the procedure producing pain and/or distress:

The purpose of these studies is to see if Tylenol administration in M/06/391 and Dexamethasone in M/07/147 will lead to protection of the brain from strokes by stopping or limiting the formation of specific chemical compounds that are produced when a blood clot breaks down, thereby, lessening or inhibiting vasospasm following a subarachnoid hemorrhage usually caused by an intracranial aneurysm rupture. To this end, New Zealand white rabbits will undergo a cerebral angiography, followed by injection of autologous blood directly into the subarachnoid space at the cistern magna. A catheter will be surgically placed into the ipsilateral jugular vein for infusion by a Pegasus pump to be worn in a jacket pocket with the drug in the other pocket for balance. The rabbits will receive constant infusion of Tylenol or Dexamethasone (or normal saline for controls) through the IV pump for 3 days.

Provide scientific justification why pain and/or distress could not be relieved. State methods used to determine that pain and/or distress relief would interfere with test results. (For Federally-mandated testing, see next response.):

The purpose of this study is to observe the results of the maximal clinical deterioration and see how the study drug affects the resultant neurological deficit due to the subarachnoid hemorrhage. A small number of rabbits in this model will exhibit these maximal deficits and may not improve substantially during the 3-day time-frame, but they should show some improvement every 24 hours. This group of rabbits is necessary to the study since it models the human disease and terminating the study early would lead to incomplete scientific data. It has been shown in the literature that there is a good correlation between cerebral blood flow and clinical status of the animal. Therefore, we would be reluctant to euthanize an animal with neurological deficits because we need to keep these animals in the study so as not to bias the outcome of the study. It is also clear that we would not want the animal to suffer in any way, so we will apply adequate pain control – Buprenex every 12 hours or more often as deemed necessary by the veterinary staff. If necessary, an anxiolytic like diazepam (Valium) or related benzodiazepine could be used if anxiety is suspected. A scoring system is used to evaluate the animals and discern the humane end-point when animals will be euthanized.

What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102):
Agency: *Not applicable* CFR: *Not applicable*

5.The following protocols involve experiments reported in Column E. Experiments were approved by the Vanderbilt University (VU) Institutional Animal Care and Use Committee (IACUC) after their determination of scientific justification.

M/07/355: *Continuous Low-Dose Heparin Infusion in the Subarachnoid Hemorrhage-Induced Cerebral Vasospasm Model in Rabbits*
Species Used: Rabbit (*Oryctolagus cuniculus*)
Number Used: 6

Explain the procedure producing pain and/or distress:

The purpose of these studies is to see if Heparin administration will lead to protection of the brain by inhibiting the production or effects of endothelin (a blood vessel constrictor) or increasing the availability of nitric oxide (a blood vessel dilator), thereby, lessening or inhibiting vasospasm.

Vasospasm is a dangerous side effect of subarachnoid hemorrhage that irritates and inflames the blood vessels on the surface of the brain causing them to constrict erratically, cutting off the blood flow to the brain. To this end, New Zealand white rabbits will undergo a cerebral angiography, followed by injection of autologous blood directly into the subarachnoid space at the cistern magna. A catheter will be surgically placed into the ipsilateral jugular vein for infusion by a Pegasus pump to be worn in a jacket pocket with the drug in the other pocket for balance. The rabbits will receive constant infusion of Heparin (or normal saline for controls) through the IV pump for 3 days.

Provide scientific justification why pain and/or distress could not be relieved. State methods used to determine that pain and/or distress relief would interfere with test results. (For Federally-mandated testing, see next response.):

The purpose of this study is to observe the results of the maximal clinical deterioration and see how the study drug affects the resultant neurological deficit due to the subarachnoid hemorrhage. A small number of rabbits in this model will exhibit these maximal deficits and may not improve substantially during the 3-day time-frame, but they should show some improvement every 24 hours. This group of rabbits is necessary to the study since it models the human disease and terminating the study early would lead to incomplete scientific data. It has been shown in the literature that there is a good correlation between cerebral blood flow and clinical status of the animal. Therefore, we would be reluctant to euthanize an animal with neurological deficits because we need to keep these animals in the study so as not to bias the outcome of the study. It is also clear that we would not want the animal to suffer in any way, so we will apply adequate pain control – Buprenex every 6-12 hours or more often as deemed necessary by the veterinary staff. If necessary, an anxiolytic like diazepam (Valium) or related benzodiazepine could be used if anxiety is suspected. A scoring system is used to evaluate the animals and discern the humane end-point when animals will be euthanized.

What, if any, federal regulations require this procedure? Cite the agency, the Code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113.102):
Agency: *Not applicable* CFR: *Not applicable*

###