See attached form for additional information.

Interagency Report Control No.:

UNITED STATES DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 32-R-0027

CUSTOMER NUMBER: 803

FORM APPROVED OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Indiana University-Southeast 4201 Grant Line Rd. New Albany, IN 47150

Telephone: (812) -941-2200

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

FACILITY LOCATIONS (Sites) - See Atached Listing

| A.  Animals Covered By The Animal Welfare Regulations | B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes. | C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use or 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 an 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 whithe use of appropriate enesthetic, anatgesic, or tranquility drugs would have adversely affected the procedures, resion interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reask such drugs were not used must be attached to this report.) | TOTAL NUMBER |
|---|---|---|--|---|--------------|
| 4. Dogs   |   |   |  |   |              |
| 5. Cats   |   |   |  |   |              |
| 6. Guinea Pigs  |   |   |  |   |              |
| 7. Hamsters   |   |   |  |   |              |
| 8. Rabbits  |   |   |  |   |              |
| 9. Non-human Primates                                 |   |   |  |   |              |
| 10. Sheep   |   |   |  |   |              |
| 11. Pigs  |   |   |  |   |              |
| 12. Other Farm Animals                                |   |   |  |   |              |
| 13. Other Animals                                     | -   |   |  |   |              |
| Frogs   |   | 144   |  |   | 144          |
| Sprague-<br>Dawley Rats                               |   | 51  | 21   |   | 72           |
| viarsh Rice<br>Rats                                   | 195   | 0   | 238  | 181   | 419          |

- Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual reseteaching, 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 ap 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 into brief explanation of the exceptions, as well as the species and number of animals affected.
- 4) The attending veterinarian for 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)

SIGNATURE OF C.E.O. OR INSTITUTIONS OFFICIAL

(b)(6), (b)(7)c

THANKS TITLE OF OUR OR MOTERITIONAL OFFICE AT

DATE SIGNED

APHIS FORM 7023 ( AUG 91 )

(Replaces VS FORM 18-23 (OCT 88), which is obsolete.)

NOV 9. 4 2008

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## Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

|           | 1. R       | egistration Number:_                             | 32-R-002/                                     |  |   |
|-----------|------------|--|---|--|---|
|           | 2. N       | umber  | 181   | of animals used in this study.   |   |
|           | 3. S       | pecies (common nam                               | ne) Marsh Rico                                | e Rats animals used in the study.  |   |
|           | 4. E       | xplain the procedure p<br>See attached           |   | or distress.   |   |
|           | ue         | remine mar pain and                              | cation why pain and<br>d/or distress relief w | l/or distress could not be relieved. Stat<br>rould interfere with test results. (For F | e methods or means used to ederally mandated testing, see |
| keli ni   | Ite        | em 6 below) See attached                         |   | ( )  | and any managed to sing, see                              |
| ekilur iz |            | See attached                                     |   |  |   |
|           | 6. W<br>(C | hat, if any, federal reç<br>FR) title number and | gulations require thing the specific section  | s procedure? Cite the agency, the cod<br>number (e.g., APHIS, 9 CFR 113.102            | e of Federal Regulations<br>2):                           |
|           | Ag         | ency   |   | CFR  |   |
|           |            |  |   |  |   |

Provide a scientific justification to explain why the use of anesthetics, analgesics, sedatives or tranquilizers during and/or following painful or distressing procedures is contraindicated:

Collection of trunk blood samples in two experiments performed required rapid sacrifice to prevent a potential stress-induced alteration of neurohormone (melatonin) levels. Therefore, no anesthetic or tranquilizing drugs were used prior to sacrifice as is recommended by the AVMA Panel on Euthanasia. One experiment examined the effects of different methods of euthanasia on pineal gland melatonin content and, therefore, drugs could not be used as I did not want to risk altering the data by the use of drugs. Our results showed that the method of euthanasia used did significantly influence pineal melatonin content. Based on this result the second experiment (Effects of Ontogeny on Pineal Melatonin Content in Juvenile Rice Rats) also did not use any drugs as we had definitive evidence that the method of euthanasia could influence pineal melatonin content in rice rats.

In addition, literature searches conducted indicate that rapid euthanasia without the use of anesthesia is a necessary research technique whenever there is a likelihood of anesthesia or stress interfering with the chemistry of the tissues under investigation. As this could potentially happen, I did not want to jeopardize the outcome of the experiments by administering any drugs to these animals prior to decapitation. That would defeat the purpose of the experiments and possibly result in the use of additional animals, an outcome that the IACUC Committee wants to avoid. In support of the use of decapitation only, Nakai et al., 2005 state that it is critical to avoid anaesthetizing experimental animals and that decapitation is the preferred method for euthanasia when conducting neurochemical In addition, Holson, 1992, has shown that euthanasia by decapitation produces prompt, painless unconsciousness in laboratory rodents, while Derr, 1991 has shown that the maximum time that pain and distress could be perceived would be about 2.7 seconds and that decapitation of rats may be considered a humane method of euthanasia. Lastly, Vanderwolf, 1988 concluded the cerebral reaction to decapitation does not resemble the cerebral reaction to painful stimuli and that decapitation would also not be considered inhumane. Recently, the ACLAM Task Force on Rodent Euthanasia issued a report on the effects of decapitation alone on various biological parameters, but no information was included regarding effects on the neurohormone of interest (melatonin from the pineal gland). Since little or nothing is known it was advisable to perform these studies utilizing only decapitation to prevent possible effects on the data being obtained.