

CONFIDENTIAL

To: Christine Parks
RARC
From: [REDACTED]
RE: Protocol G00146
Date: July 1, 2003

[REDACTED] and [REDACTED] cross-compared the protocols (see below) and records obtained from all possible sources (e.g., colony records, laboratory note books, check sheets) and compiled a list of discrepancies between approved protocols and actual records. The apparent and potential violations are summarized below. The period of this investigation is from March 2000 to present. The tabulated information in detail is available upon request.

Protocol approval date: 3/13/00 with final amendment on 4/19/00 Major amendment approval date: 10/15/01 Minor amendment date: 05/20/02 Renewal approval date: 04/14/03

1. Apparent Violations

- Bret left animals during the push-pull-perfusion (PPP) experiment for lunch 4 times. The protocol states that animals will be attended continuously. One animal died in the chair while Bret was at lunch.
- In 24 PPP experiments in 11 animals, 4 different drug challenges were given. The 3/13/00 protocol did not contain this, but in the 10/15/01 protocol the maximum number of drug challenges was added to be three.
- Five drugs (the ATP sensitive potassium channel blockers, tolbutamide and glybenclamide; the purinergic receptor blockers, PPADS and suramin; and the ATP hydrolysor, apyrase) that had not been stated in the 10/15/01 protocol were examined with PPP experiments.
- In 15 PPP experiments ATP doses exceeded the dose range stated in the protocols.
- In 2 animals more than 12 PPP experiments were conducted (1 animal with 13 times; 1 animal with 15 times). The 3/13/00 protocol did not contain this, but in the 10/15/01 protocol the maximum number of PPP was added to be 12.
- One c-section resulting in a 4th major surgery (the 3/13/00 protocol stated that the maximum number of major surgeries was 3. This confusion occurred because the protocol stated that "the animals used for the project 1 (puberty project) with PPP experiment can be used for breeding females in project 2, in which 3 c-sections were allowed").

2. Inadvertent Violations

- Five animals received reheadcap surgeries. This procedure was not described in the 3/13/00 protocol, but it was added in the 10/15/01 protocol.
- In 15 animals blood draws were conducted on project 2, in which no blood draw was described. In projects 1 and 4 blood drawings are described.

- In 12 animals more than 4 ml of bloods were drawn in one week. The protocol stated that taking more than 4 ml/week of bloods requires CBC. (See also related problems in No.3).
- Forty-two animals received higher doses (up to 20 mg/kg) of ketamine. The protocol stated that ketamine dose will be 10-15 mg/kg.
- Antibiotics were given to 18 animals after PPP experiments. In most cases a staff veterinarian approved the treatment, but consent was not in writing. In fact, Carol Emerson suggested antibiotics after PPP experiments on a regular basis.
- Prophylactic antibiotics were given before and after surgeries, such as cranial pedestal implantation as well as cranial pedestal repair and removal, in 59 animals. Staff veterinarians knew of this practice, but we failed to describe it in the protocol.

3. Errors due to Reproductive Services for Breeding in Our Animals

- In 7 animals Reproductive Services took blood samples up to 14 ml in a week under our protocol.
- Reproductive Services conducted ultrasounds in 8 animals under our protocol. Ultrasound examination was not described in the protocol.

4. Record Errors

- Fourteen initials/signatures are missing from check sheets.
- Forty-one hourly check sheets for Chairing/PPP experiments conducted by a former postdoctoral research fellow (Dr. Shinichiro Nakamura) are missing. Since the check sheets had to be displayed on the door of room [REDACTED] when the experiment was conducted, they must have been filled out. However, at this time we cannot locate these records.
- Errors in computer records are found in the following animals:

r94070	6/15/01	Remark by a veterinary assistant says "in chair". However, this animal did not have cranial pedestal at this time and there is no chairing record. So, there is no way that she was in chair.
r96130	7/10/00-7/14/00	Record says she was in chair after cranial pedestal removal. This is not possible.
r96112	8/8/00	The animal should be 96119. Record has been corrected.
r99046	6/12/01	The animal should be 97046. Record has been corrected.
r98003	6/12/01	The animal should be 98033. Record has been corrected.
r98040	7/10/01	The animal should be 98049. Record has been corrected.
r84108	10/3/00	This monkey does not belong to this protocol, because of a male. To take blood Reproductive Services assigned to our protocol.
rhaa56	10/3/00	This monkey does not belong to this protocol, because it is a male. To take blood Reproductive Services assigned to our protocol.
r87100	12/8/00	This monkey does not belong to this protocol, because it is a male. To take blood Reproductive Services assigned

