



DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE  
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:

Office of Laboratory Animal Welfare  
6700B Rockledge Drive, Suite 2500, MSC 6910  
Bethesda, Maryland 20892-6910  
Home Page: <http://grants.nih.gov/grants/olaw/olaw.htm>

FOR EXPRESS MAIL:

Office of Laboratory Animal Welfare  
6700B Rockledge Drive, Suite 2500  
Bethesda, Maryland 20817  
Telephone: (301) 496-7163  
Facsimile: (301) 402-7065

April 17, 2019

Re: Animal Welfare Assurance  
A3433-01 [OLAW Case 2L]

Dr. Prasat Mohapatra  
Vice Chancellor for Research  
University of California, Davis  
1850 Research Park Drive  
Davis, California 95618

Dear Dr. Mohapatra,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your April 1, 2019 letter reporting an adverse event involving nonhuman primates at the University of California- Davis, following up on an initial telephone report on April 24, 2018. According to the information provided, OLAW understands that seven infant macaques died suddenly after they and their mothers had been marked with dye for identification. A switch in dye product had been made because the original formulation faded between semiannual animal round ups. Previous marking of animals with the new product at several facilities had not caused any problems. The infants had face, nose, and mouth exposure to the dye which was present on the mother.

The immediate action taken upon discovery of the compromised infants consisted of providing emergency veterinary care. The treatments were unsuccessful and the animals were euthanized while others had been found dead. Necropsies were conducted and the findings pointed to anaphylaxis as the cause of death in five cases and sepsis in two. To prevent a recurrence, infants aged six months or younger will not be marked with dye, any infants that must be marked will be given the original formulation, heat sources will be used when processing animals in cool weather, anesthetized mothers and babies will be kept apart until recovered to minimize dye transfer, and excess dye will be wiped off. Standard operating procedures have been revised to reflect these changes and no further incidents have occurred.

Based on its assessment of this explanation, OLAW understands that measures have been implemented to correct and prevent recurrence of this problem. OLAW concurs with the actions taken by the institution to comply with the PHS Policy on Humane Care and Use of Laboratory Animals.

Sincerely,

(b) (6)

Axel Wolff, M.S., D.V.M.  
Deputy Director  
Office of Laboratory Animal Welfare

cc: IACUC Chair  
Robert Gibbens, D.V.M., USDA-APHIS-AC

UNIVERSITY OF CALIFORNIA, DAVIS

BERKELEY • DAVIS • IRVINE • LOS ANGELES • MERCED • RIVERSIDE • SAN DIEGO • SAN FRANCISCO



SANTA BARBARA • SANTA CRUZ

OFFICE OF RESEARCH

1850 RESEARCH PARK DRIVE  
DAVIS, CALIFORNIA 95618

**PRASANT MOHAPATRA**  
VICE CHANCELLOR FOR RESEARCH

TELEPHONE: 530.754.7764  
RESEARCH@UCDAVIS.EDU

April 1, 2019

**AXEL WOLFF, M.S., D.V.M.**  
Deputy Director  
Office of Laboratory Animal Welfare  
National Institutes of Health  
RKL1, Suite 360, MSC 7982  
6705 Rockledge Drive  
Bethesda, MD 20892-7982

**RE: Institutional Report - D16-00521 # (A3433-01)**  
**Follow-up to adverse effect report for 2018 Nyanzol-D dye marking**  
**CNPRC Base Grant number P51OD011107**

Dear Dr. Wolf:

In accordance with Assurance D16-00521 # (A3433-01) and PHS Policy IV.F.3., UC Davis is providing a comprehensive summary report of the 2018 infant macaque deaths following routine Nyanzol-D dye marking, as well as the results of infant dye marking practice changes currently in place. This adverse effect from the dye marking was previously reported in 2018 and discussed with you over the phone on multiple occasions with our IACUC Director, Donna Routley. This incident was self-reported to USDA. Below please find the comprehensive summary and practice changes that have been successfully implemented.

**Background**

The CNPRC has been using Nyanzol-D for dye marking animals since 2012. During this time the routine health surveillance round-ups were changed from three times a year to twice a year mirroring standard practice among the other National Primate Centers. Through this process it was determined that the current product used for dye marking at that time, Miss Clairol, was not providing enough coverage between the semi-annual round-ups. An alternative dye marking product was sought out. The Primate Center at Cayo Santiago in Puerto Rico was using Nyanzol-D for dye marking with successful coverage over multiple months. The CNPRC recipe was derived from the recipe used at

Cayo Santiago. Since use of Nyanzol-D began six years ago approximately 24,000 animals have been dye marked using this product.

### **Incidents**

In March and April 2018 seven infants came in to the hospital for emergency care following completion of routine round up. The veterinary staff provided clinical assessment of two of the infants that were brought to the hospital for emergency care. Both infants presenting to the hospital had generalized weakness and respiratory distress. Dye was noted on the lips and tongues of two infants. Endotracheal intubation was attempted. Both infants had severe edema and swelling of the larynx and tongue, making intubation difficult. Emergency drugs were administered, which had minimal effect on the laryngeal swelling. Despite treatment attempts, clinical conditions of the infants did not improve, and humane euthanasia was elected. The other infants were either found dead or euthanized upon arrival at the hospital.

### **Pathology Results**

A total of seven infants were submitted to necropsy for sudden death or respiratory distress within 24 hours of round up. On examination, all 7 had some amount of dye on the fur, skin, and/or around or inside the nose and mouth. 4 monkeys had throat or laryngeal edema, 3 had hyper-inflated lungs, 2 had a mixture of hyperinflation/inflation and heavy/wet lungs, and 1 had diffusely heavy wet lungs. All animals had normal GI contents. Preliminary histology data on the first 3 animals showed 1 animal had an acute bronchopneumonia, unrelated to round up procedures. To arrive at definitive diagnoses, all cases were evaluated by all of the pathologists and findings summarized.

### **Summary of Pathology results**

All pathologists subsequently reviewed the gross findings and histology, which are summarized in the table below.

Anaphylaxis was conclusively diagnosed via histologic evidence of laryngeal/tracheal edema with variably lung pathology. One animal submitted on 3/21 is equivocal due to unavailability of the trachea/larynx for examination. One animal submitted on 4/4 is definitively NOT consistent with anaphylaxis, but rather a severe bacterial pneumonia.

Date	Location	Age	Sex	Gross	Dye	Histo	Anaphylaxis?
3/21/2018	NC10	1 day	F	Red, wet lungs. Hemorrhage over cerebellum. Milk in stomach, rest of GIT normal.	3+	Larynx/trachea not available. Lung-multifocal hemorrhage, neutrophils in alveoli. Brain-minimal hemorrhage.	Sepsis likely, anaphylaxis less likely

Date	Location	Age	Sex	Gross	Dye	Histo	Anaphylaxis?
3/21/2018	NC10	4 days	M	Lungs pink. Milk in stomach, rest of GiT empty	2+	Mild laryngeal and tracheal edema. Vacuolation in vessels of the lungs. Minimal hemorrhage in brain.	Probable
4/4/2018	NC13	16 days	M	Lungs mostly pink, some mottling of two lobes. Milk throughout GIT.	1+	Severe neutrophilic bronchopneumonia with bacteria	No
4/10/2018	NC14	3 days	M	Edema of larynx and subcutaneous tissues of neck. Arborizing red pattern in lungs. Milk throughout GIT.	3+	Laryngeal edema, tracheal edema. Hemorrhage and vasculitis in the lungs.	Yes
4/10/2018	NC14	19 days	M	Laryngeal edema, hyperinflated lungs. Hemorrhage over left temporal lobe. Milk throughout GIT.	1+	Laryngeal edema, tracheal edema. Mild perivascular edema and reactive endothelium in the lung.	Yes
4/10/2018	NC14	7 days	M	Caudal laryngeal edema. Hyperinflated lungs. Milk throughout GIT.	1+	Laryngeal edema, tracheal edema. Perivascular edema in liver and brain.	Yes
4/10/2018	NC 14	13 days	M	Laryngeal/facial, nasal cavity edema. Red arborizing pattern and hyperinflation of lungs. Milk throughout GIT.	3+	Laryngeal and tracheal edema. Minimal perivascular edema. Retinal hemorrhage. Possible neuronal injury in brain.	Yes

### Review of procedures

There were no new procedures introduced into any of these round-ups. The processes were all the same and the dye lot being used had been in use for several months. The infants were not anesthetized, did not receive any vaccinations, had a physical exam, eye ointment was applied to help prevent dye from getting in the eyes, had a blood sample collected, received a tattoo, and then received a dye mark. Post-round-up animal technicians observed all animals until fully recovered from sedation, and performed additional checks through the afternoon.

The Nyanzol-D dye mark is used at the Oregon Primate Center, Tulane Primate Center, and at Cayo Santiago in Puerto Rico. The recipe the CNPRC uses calls for warm water, 3% hydrogen peroxide and a measured amount of dye powder. The dye powder is measured out in advance once a new shipment arrives. The measured amounts are placed in Ziploc bags and placed inside an empty container that previously contained the dye powder.

Historically the CNPRC has only applied a small dye mark on the infants back as an indicator that it has undergone a round-up. In recent years, investigators have been using north colony animals as part of their research studies. It is very common for young infants to venture away from their mothers and "play" and investigators requested individual dye marks on the infants in order to determine individual animals when performing observations. This process of dye marking infants at round-up had been going on for several years or more.

Axel Wolff, M.S., D.V.M

March 28, 2019

Page 4

After these incidents the dye marks of the dams were listed out and one commonality was that most of the dams have arm dye marks which put the wet dye in close proximity to the dam's nipples.

#### Conclusions

The initial findings suggested that the infants had exposure to dye that was present on their dam. Although there hadn't been any official change in procedures, the general consensus was that prematurely placing a young infant on an anesthetized dam with a fresh dye mark could increase the opportunity for the infant to get dye on its face, nose and mouth. Subsequent finding supported this conclusion.

#### Plans Moving Forward

Moving forward the following guidelines to Nyanzol -D dye marking at the CNPRC were agreed upon:

- Infants 6 months and younger will not be dye marked. If there is a special request for dye marking infants < 6 months of age Miss Clairol will be used.
- Infants will be placed on a warming pad when temperatures are cool during round-up, or under the radiant heaters near the staff working the round-up table. All other animals will be placed a distance away from the table for recovery. The plan is that the dams will not look for their infants until they are mostly recovered and the infants who are not anesthetized will not be able to wander into other animals and hopefully minimize the risk of dye transfer onto the infants.
- All animals will have excess dye wiped off after application.

Semi-annual round-ups following the implementation of the steps noted above have since proceeded smoothly without Issues involving infants and dye marking. These steps were incorporated into our standard practice.

If you have any questions, please do not hesitate to contact our IACUC Director at (b) (6) or by email at [dmroutley@ucdavis.edu](mailto:dmroutley@ucdavis.edu).

Sincerely,

(b) (6)

Prasant Mohapatra  
Vice Chancellor for Research

/pk

c: IACUC  
AAALAC

**Morse, Brent (NIH/OD) [E]**

---

**From:** OLAW Division of Compliance Oversight (NIH/OD)  
**Sent:** Wednesday, April 10, 2019 12:17 PM  
**To:** Donna Routley; OLAW Division of Compliance Oversight (NIH/OD)  
**Subject:** RE: Institutional Reports - D16-00521 # (A3433-01)

Thank you for providing these reports Ms. Routley. We will send official responses soon.

Best regards, Brent Morse

Brent C. Morse, DVM, DACLAM  
Director  
Division of Compliance Oversight  
Office of Laboratory Animal Welfare  
National Institutes of Health

Please note that this message and any of its attachments are intended for the named recipient(s) only and may contain confidential, protected or privileged information that should not be distributed to unauthorized individuals. If you have received this message in error, please contact the sender.

---

**From:** Donna Routley [mailto:[dmroutley@ucdavis.edu](mailto:dmroutley@ucdavis.edu)]  
**Sent:** Monday, April 01, 2019 6:28 PM  
**To:** OLAW Division of Compliance Oversight (NIH/OD) <[olawdco@od.nih.gov](mailto:olawdco@od.nih.gov)>  
**Subject:** Institutional Reports - D16-00521 # (A3433-01)

Hello,

Please find the attached five signed reports from UC Davis, assurance D16-00521 # (A3433-01). These had previously been reported to OLAW. For the instances involving USDA covered species, each incident was self-reported to USDA. These reports have been copied to AAALAC.

Please let me know if you have any questions.

Kind regards,  
Donna Routley

Donna Routley, RVT, RLATg, CPIA  
Director, IACUC  
Office of Research  
University of California Davis  
(b) (6)(office)  
[dmroutley@ucdavis.edu](mailto:dmroutley@ucdavis.edu)



## Initial Report of Noncompliance

By: Ann

Date: 4/24/18

Time: 12:00

Name of Person reporting: Donna Bentley

Telephone #: (b)(6)

Fax #: \_\_\_\_\_

Email: \_\_\_\_\_

Name of Institution: U of California - DAVIS

Assurance number: A3433

Did incident involve PHS funded activity? Yes

Funding component: \_\_\_\_\_

Was funding component contacted (if necessary): \_\_\_\_\_

What happened?

Several rhesus babies died after exposure to food dye

Species involved: Rhesus

Personnel involved: \_\_\_\_\_

Dates and times: \_\_\_\_\_

Animal deaths: \_\_\_\_\_

Projected plan and schedule for correction/prevention (if known): \_\_\_\_\_

Necropsy, determine cause

Projected submission to OLAW of final report from Institutional Official: \_\_\_\_\_

OFFICE USE ONLY

Case # \_\_\_\_\_