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
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FOR EXPRESS MAIL:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301) 480-3387

July 25, 2022

Re: Animal Welfare Assurance
#A3046-01 (OLAW Case 1G)

Dr. Kevin W. Burton
Vice President, Research Services
Southern Research
2000 Ninth Avenue South
Birmingham, AL 35205

Dear Dr. Burton,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your July 14, 2022 letter reporting an instance of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at Southern Research, following up on an initial report on December 3, 2021 and an interim report on December 15, 2021. According to the information provided, OLAW understands that specific activities being conducted on a study using rats had not been described in the approved amendment.

The corrective actions consisted of stopping the unapproved activities, submitting a revised amendment which was subsequently approved by the Institutional Animal Care and Use Committee (IACUC), counseling the Study Director, and informing the NIH Contracting Officer Representative about the incident. Post-approval monitoring found a discrepancy in the type of experimental feed provided therefore another amendment was submitted. Additional PAM reviews found no further problems.

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 IACUC to comply with the PHS Policy.

Sincerely,

(b) (6)

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

cc: IACUC Chair

McCoy, Devora (NIH/OD) [E]

From: McCoy, Devora (NIH/OD) [E]
Sent: Monday, July 25, 2022 11:40 AM
To: kburton@southernresearch.org
Cc: OLAW Division of Compliance Oversight (NIH/OD); Mann, Jill
Subject: OLAW Case A3046-1G
Attachments: A3046-1G.pdf

Good morning Dr. Burton,

Attached please find Dr. Wolff's final response to OLAW Case A3046-1G. If you have any questions, feel free to contact us by phone or by e-mail.

Best,
Devora

Devora McCoy, BS, MBA
Program Analyst
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
301-435-2390

A 3046

Southern Research
MOVING SCIENCE

Date: 14 JUL 2022

Ms. Devora McCoy, BS, MBA
Program Analyst
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

Dear Ms. McCoy,

In accordance with Assurance #D16-00025 and PHS -Policy IV.F.3, I am writing to provide the final report for non-compliance that occurred on ACUP #20-12-035B, Neurobehavioral Study of Abacavir/ Dolutegravir/Lamitivudine [TRICOMBOHIV2 (12:1:6)] in Harlan Sprague-Dawley Rats (rattus norvegicus) MOG18001D, Southern Research Study #14140.24.04, Contract No. HHSN27320300010C.

The original ACUP was approved on 28 Dec 2020. Amendment #3 was approved on 05 Nov 2021. Amendment #3 described using a subset of the animals for a pilot study to determine an appropriate dose of lipopolysaccharide (LPS) for a follow-on experiment and indicated that Amendment #3 was intended to cover both the pilot and the follow-on experiment. Amendment #3 included an extensive description of the pilot study, but no description of the follow-on experiment was provided to the IACUC as a part of Amendment #3. The Study Director intended the amendment to include changes to activities performed on the main study animals as well as the pilot study animals, but further description of activities for the main study animals was inadvertently left out of the Amendment.

The pilot study was conducted as approved by the IACUC and the activities for the main study animals began on 30 Nov 2021. Thirty-nine main study animals received a daily oral gavage dose of a previously approved test article as well as one dose of LPS intraperitoneally. The main study animals also had survival blood collected via retro-orbital plexus one time. On 02 Dec 2021 SR personnel recognized that these study activities were not described in Amendment #3 and study activities were immediately suspended.

In response, the Study Director drafted a new amendment, Amendment #5, which describes in detail all of the new study activities that will be performed on the main study animals. A meeting of the IACUC was held on 03 Dec 2021 with a quorum of the IACUC membership in attendance. ACUP 20-12-035B Amendment #5 was approved on Friday, 03 Dec 2021 and study activities were resumed. The Study Director submitted an adverse event report and now understands that she must be complete and thorough in her description of study activities for ACUPs and amendments. The Study Director notified (b) (6) (b) (6) for contract number HHSN273201300010C, of the adverse event on 02 Dec 2021.

The Post Approval Monitoring was conducted by Dr. Jill F. Mann, D.V.M., DACVP and (b) (6) (b) (6) Drs. Mann and (b) (6) performed a side-by-side comparison of the ACUP 20-12-035B and ACUP amendments 1-6 and the Study Protocol 14140.24.04. Only one variance was identified: ACUP Section 5.4 stated that the animals would be fed irradiated NIH-07 feed. Study Protocol Section 9.3 stated that the rats would be fed NTP-2000 wafers or pellets. An ACUP amendment (Amendment #7) was approved, adding the NTP-2000 food in addition to the irradiated NIH-07 feed. Both feeds are acceptable to provide for the nutritional needs of the rats.

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In addition to a comparison of the ACUP and amendments with the Study Protocol and amendments, (b) (6) (b) (6) conducted Post Approval Monitoring of in-vivo activities on 27 June 2022. No departures from the ACUP or the six amendments were observed.

Thank you,

(b) (6)

Dr. Kevin W. Burton, IO
Vice President of Research Services
Southern Research

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Friday, July 15, 2022 7:48 AM
To: (b) (6)
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Final report to OLAW for ACUP 20-12-035B Amendment # 3 non-compliance

Thank you for this report, (b) (6) We will send a response soon.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

From: (b) (6)
Sent: Thursday, July 14, 2022 12:45 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: IACUC <IACUC-BIRMINGHAM@sriemail.sri.org>
Subject: [EXTERNAL] Final report to OLAW for ACUP 20-12-035B Amendment # 3 non-compliance

Hi All,

Please find the attached signed letter from Kevin Burton regarding ACUP 20-12-035B Amendment # 3 non-compliance and let me know if you need anything else.

Thanks,

(b) (6)

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McCoy, Devora (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Friday, June 3, 2022 8:51 AM
To: Mann, Jill
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: draft final report to OLAW for ACUP 20-12-035B Amendment # 3 non-compliance.

Good morning Dr. Mann,

During a recent records check it was observed that we still need the final report for this case. At your earliest convenience, can you please have your IO send the final report along with the results of the post approval monitoring?

Thanks,

Devora McCoy, BS, MBA
Program Analyst
Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health
301-435-2390

From: Mann, Jill <jmann@southernresearch.org>
Sent: Wednesday, December 15, 2021 9:06 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: (b) (6) (b) (6) (b) (6) Mann, Jill <jmann@southernresearch.org>; (b) (6) (b) (6)
Subject: [EXTERNAL] RE: draft final report to OLAW for ACUP 20-12-035B Amendment # 3 non-compliance.

Hello Dr. Wolff,
Our IO, Dr. Corinne Augelli-Szafran, will send the final report with the additional information and the results of the post approval monitoring.
Thanks,
Jill

Jill F. Mann, D.V.M., DACVP
Associate Director, Pathology
Core Services Department
IACUC Chairperson
Southern Research

Wolff, Axel (NIH/OD) [E] A3046

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, December 15, 2021 7:53 AM
To: Mann, Jill
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: draft final report to OLAW for ACUP 20-12-035B Amendment # 3 non-compliance.

Hello Dr. Mann,

I will accept this report as an interim and will add to the file. The final report is to come from the IO on agency letterhead. You can send in the final as advised after this study has run for a few weeks and confirm whether the post approval monitoring has identified any additional problems or whether the issues have now been resolved. Also indicate whether there was communication with the relevant HHS contracting officer. Let me know if you have additional question.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

From: Mann, Jill <jmann@southernresearch.org>
Sent: Monday, December 13, 2021 3:02 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: (b) (6) (b) (6) IACUC <IACUC-BIRMINGHAM@sriemail.sri.org>
Subject: [EXTERNAL] FW: draft final report to OLAW for ACUP 20-12-035B Amendment # 3 non-compliance.

CAUTION: This email originated from outside of the organization. Do not click links or open attachments unless you recognize the sender and are confident the content is safe.

Dr. Wolff,

"In Accordance with Assurance #D16-00025 and PHS policy IV.F.3, I am writing to provide the final report for non-compliance that occurred on ACUP # 20-12-035B, Neurobehavioral Study of Abacavir/Dolutegravir/Lamitivudine [TRICOMBOHIV2 (12:1:6)] in Harlan Sprague-Dawley Rats (rattus norvegicus) MOG18001D, Southern Research study # 14140.24.04, Contract No. HHSN27320300010C. The original ACUP was approved on 28 Dec 2020. Amendment # 3 was approved on 05 Nov 2021. Amendment # 3 described using a subset of the animals for a pilot study to determine an appropriate dose of lipopolysaccharide (LPS) for a follow-on experiment and indicated that Amendment #3 was intended to cover both the pilot and the follow-on experiment. Amendment #3 included an extensive description of the pilot study, but no description of the follow-on experiment was provided to the committee as a part of Amendment #3. The Study Director intended the amendment to include changes to activities performed on the main study animals as well as the pilot study animals but further description of activities for the main study animals was inadvertently left out of Amendment.

The pilot study was conducted as approved by the IACUC and the activities for the main study animals began on 30 Nov 2021. Thirty-nine main study animals received a daily oral gavage dose of a previously approved test article as well as one dose of LPS intraperitoneally. The main study animals also had survival blood collected via retro-orbital plexus one time. On 02 Dec 2021 SR personnel recognized that these study

activities were not described in Amendment # 3 and study activities were immediately suspended. The Study Director drafted a new amendment, Amendment # 5, which describes in detail all of the new study activities that will be performed on the main study animals. A meeting of the IACUC was held on 03 Dec 2021 with a quorum of the IACUC membership in attendance. ACUP 20-12-035B Amendment # 5 was approved on Friday, 03 Dec 2021 and study activities were resumed. The Study Director submitted an adverse event report and now understands that she must be complete and thorough in her description of study activities for ACUPs and amendments. We will be conducting
post-approval monitoring of this study."

Thank you,
Jill F. Mann

Jill F. Mann, D.V.M., DACVP
Associate Director, Pathology
Core Services Department
IACUC Chairperson
Southern Research
2000 Ninth Avenue South
Birmingham, AL 35205
(b) (6)
jmann@southernresearch.org

From: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Sent: Monday, December 6, 2021 6:24 AM
To: Mann, Jill <jmann@southernresearch.org>
Cc: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: RE: Southern Research Assurance # D16-00025

Thank you Dr. Mann. We will use the attached description to start a new case file and look forward to receiving the final report from the IO after the IACUC has completed its investigation.
Axel Wolff

From: Mann, Jill <jmann@southernresearch.org>
Sent: Friday, December 3, 2021 4:35 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: (b) (6) IACUC <IACUC-BIRMINGHAM@sriemail.sri.org>
Subject: RE: Southern Research Assurance # D16-00025

Dr. Wolff,
Thank you for your quick response. Our IACUC has determined that there has been a significant change on the study covered under ACUP 20-12-035B described below, without prior IACUC approval. This email serves as a preliminary report. We will send a more extensive report with specific details early next week.

Thank you for your help,
Jill Mann

Jill F. Mann, D.V.M., DACVP
Associate Director, Pathology
Core Services Department
IACUC Chairperson
Southern Research
2000 Ninth Avenue South
Birmingham, AL 35205
(b) (6)
jmann@southernresearch.org

From: Mann, Jill
Sent: Thursday, December 2, 2021 2:49 PM
To: 'OLAW Division of Compliance Oversight (NIH/OD)' <olawdco@od.nih.gov>
Cc: (b) (6)
Subject: Southern Research Assurance # D16-00025
Importance: High

Dr. Wolff,
In Accordance with Assurance #D16-00025 and PHS policy IV.F.3,
I am writing to gain advice on a question that has been raised concerning one of our current studies and if we are out of compliance. Long story short, the study director has an approved amendment on an existing ACUP which describes the addition of dosing with LPS to main study animals as well as using a subset of the study animals for a pilot study. An extensive description was given of the pilot study, but not of the procedures to be performed on the main study animals. The study director intended the amendment to include changes to activities performed on the main study animals. The pilot study was conducted and the activities for the main study animals are currently underway.

To give more details:
This study is ACUP # 20-12-035B, Neurobehavioral Study of Abacavir/Dolutegravir/Lamitivudine [TRICOMBOHIV2 (12:1:6)] in Harlan Sprague-Dawley Rats (*rattus norvegicus*) MOG18001D, Southern Research study #14140.24.04, Contract No. HHSN27320300010C. The original ACUP was approved on 28 Dec 2020. The question concerns activities being conducted based on Amendment # 3, which was approved on 05 Nov 2021. To provide background information of our question I will summarize the main sections of Amendment # 3 discusses the addition of intraperitoneal dosing with lipopolysaccharide (LPS) in addition to the test article Abacavir/Dolutegravir/Lamivudine [TRICOMBOHIV2 (12:1:6)] at a dose of 300/25/150 mg/kg/day. Amendment section 2.2

describes the addition of a dose of LPS intraperitoneally to animals on study along with the test article Abacavir/Dolutegravir/Lamivudine [TRICOMBOHIV2 (12:1:6)] at a dose of 300/25/150 mg/kg/day for approximately 3 weeks (these aspects of the study protocol were inadvertently left out of the original ACUP). Blood will be collected from animals just prior to euthanasia via the retro-orbital plexus while under CO_2/O_2 anesthesia. The amendment also describes the addition of a pilot study that will be conducted under this core ACUP to confirm the correct dose of LPS to be used on study 14140.24.04. An additional item mentioned in section 2.2 is the use of animals from Group 2 of the main study to be used for the pilot study. Group 2 animals were pre-determined as "back-up" animals in the original ACUP and had only body weights and clinical observations collected previously. Ten Group 2 Animals will receive a dose of lipopolysaccharide (LPS) intraperitoneally at 2 mg/kg and have detailed clinical observations performed and body weights collected.

Amendment section 2.3 describes in detail that of 10 animals would be used to conduct a pilot study to confirm the correct dose of LPS (including dose, decapitation as the method of terminal euthanasia approximately 24 hours after dosing for the 10 pilot study animals, after dosing monitoring, potential adverse side effects of LPS, and the fact that animal in the pilot study would be euthanized immediately with euthasol if adverse side effects were observed. Additional requested changes in Amendment section 2.3 included adding CO2/O2 as a method of anesthesia and the use of back up animal for gavage practice with saline to so that technicians would have experience gavaging large adult rats.

Amendment section 2.4 provided an explanation for the changes requested in amendment 3. "Inadvertently, two key aspects of the study protocol were left out of the original ACUP. The purpose of the 14140.24.04 study is to utilize an acute inflammatory response to exacerbate any underlying neurological defects present from dosing with TRICOMBOHIV2 (12:1:6). This method has been used in the literature to accelerate an adverse neurological phenotype that would be localized in the prefrontal cortex and/or hippocampus. Additional study work is being added to determine the appropriate dose of LPS needed for the 14140.24.04 study. Method of euthanasia via decapitation is being added. The objective of the study is to determine the effects of acute neuroinflammation in the prefrontal cortex and/or hippocampus. Because these areas of the brain are likely to be affected with the administration of chemical anesthesia, or prolonged CO2 exposure, this method of euthanasia would likely confound the endpoint readout of the results. The alternative of exsanguination by decapitation provides a rapid and humane means of euthanasia while maintaining the integrity of the neural tissue for biochemical analysis. The addition of the oral gavage via saline to 20 animals is to provide the technical staff with the needed experience in dosing large animals, prior to administration of the test article. We do not have any animals available in the training colony that are

comparable in weight and size, therefore we propose using 20 of the previously designated back-up animals."

Does this amendment sufficiently describe the activities to be performed on the main study animals so the LPS dosing of the main study animals can continue?

Thank you,
Jill F. Mann

Jill F. Mann, D.V.M., DACVP
Associate Director, Pathology
Toxicology and Pathology Services Department
IACUC Chairperson
Southern Research
2000 Ninth Avenue South
Birmingham, AL 35205
(b) (6)
jmann@southernresearch.org

Wolff, Axel (NIH/OD) [E] A30466-1G

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Monday, December 6, 2021 7:24 AM
To: Mann, Jill
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Southern Research Assurance # D16-00025

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Axel Wolff

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Thank you,
Jill F. Mann

Jill F. Mann, D.V.M., DACVP
Associate Director, Pathology
Toxicology and Pathology Services Department
IACUC Chairperson
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Birmingham, AL 35205
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jmann@southernresearch.org