DEPARTMENT OF HEALTH & HUMAN SERVICES

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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) 480-3387

DATE: January 27, 2022

TO: Michael M. Gottesman, M.D.
Deputy Director for Intramural Research, NIH

FROM: Director
Division of Compliance Oversight, OLAW

SUBJECT: Animal Welfare Investigation (NIDA #12-22) – Animal Welfare Assurance A4149-01 [Case 16F]

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your January 12, 2022 memo regarding two related incidents of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at the National Institute on Drug Abuse (NIDA). According to the information provided, OLAW understands that for:

Incident #1

On 12/3/2021 the facility veterinarian determined that an investigator used Equithesin (a mixture of pentobarbital, chloral hydrate and magnesium sulfate) for a surgical procedure on a rat instead of ketamine/xylazine which was approved in the protocol.

The investigator agreed to return Equithesin to the pharmacy and suspend all animal activities indefinitely pending the outcome of an ACUC meeting to discuss the case. The veterinarian agreed to work with the investigator to optimize anesthesia protocols using ketamine/xylazine and consider other alternatives. The rat which had undergone surgery and recovered was euthanized. The investigator was suspended from performing all animal work until January 24, 2022. After that date the investigator is allowed to perform work with additional veterinary oversight. Both the Scientific Director and the Branch Chief responsible for supervising this individual have also been made aware of this offense and the ACUC decision.

Incident #2

In July 2021, rats had been observed with bloating. At that time, laboratory personnel indicated that pentobarbital was being used as a surgical anesthetic, consistent with what was stated in the protocol. On December 3, 2021, additional animals with gastric distress were observed. Lab personnel were again queried about the surgical anesthetic used. It was revealed that animals had received Equithesin during surgeries performed in September and October. The researchers stated that they were not aware of the differences between Equithesin and pentobarbital.

On December 13, 2021, it was agreed that a senior scientist, not involved on the protocol, would collect, and return all Equithesin to the pharmacy and that no further work would be performed on the protocol pending the outcome of an ACUC meeting to discuss the case. The investigator was suspended from performing all animal work until January 24, 2022. After that date the investigator is allowed to perform work with additional veterinary oversight. Again, both the Scientific Director and Branch Chief have been made aware of this violation and the resulting ACUC decision.

As of December 21, 2021, all Equithesin has been confirmed as having been returned to the pharmacy. The pharmacy has been instructed to no longer make this compound available to investigators.

The actions taken to resolve the issues and prevent recurrence were appropriate and accepted by OLAW. We appreciate being informed of this matter and find no cause for further action by this office.

Sincerely,

Brent C. Morse -S Digitally signed by Brent C. Morse -S
Date: 2022.01.27 13:09:09 -05'00'

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

cc: Dr. Stephen Denny
Dr. Richard Wyatt
Alexander F. Hoffman, Ph.D., Chair, NIDA ACUC

A4149-16F

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
Bethesda, Maryland 20892
www.nih.gov

January 12, 2022

TO: Brent C. Morse, D.V.M.
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare

FROM: Deputy Director for Intramural Research, NIH

SUBJECT: Animal Welfare Investigations - Assurance D16-00602 (NIDA 01-22)

This correspondence conveys the results of an animal incident investigation by the NIH National Institute on Drug Abuse (NIDA) ACUC in accordance with Assurance D16-00602 and PHS Policy IV.F.3. The animal incident involved the administration of an anesthetic drug to rats that was not listed on the ACUC-approved animal study proposal and not approved by the ACUC for use in animals. The use of the drug was suspended by the principal investigator.

The event was first reported to the NIH Office of Animal Care and Use by the ACUC Chair on December 6, 2021.

Please contact me or Dr. Stephen Denny, Director, Office of Animal Care and Use, if additional information or clarifications are required.

Michael M. Gottesman -S
Digitally signed by Michael M. Gottesman -S
Date: 2022.01.12 09:28:24 -05'00'

Michael M. Gottesman, M.D.

Attachment

cc: Dr. Wyatt
Dr. Hoffman
Dr. Denny

National Institute on Drug Abuse
Intramural Research Program

National Institutes of Health
National Institute on Drug Abuse
251 Bayview Boulevard
Suite 200
Baltimore, MD 21224

TO: Dr. Michael Gottesman, Deputy Director for Intramural Research
FROM: Alexander F. Hoffman, Chair, NIDA Animal Care and Use Committee
DATE: 1/3/2021
SUBJECT: Reportable incident on protocol non-compliance

The National Institute on Drug Abuse Intramural Research Program, in accordance with Assurance A4149-01 and PHS Policy IV.F.3., reports two separate, but closely related incidents of protocol non-compliance involving the unapproved use of Equithesin in rats. A summary of the incidents, major findings, and actions taken to mitigate against recurrence are described below.

Summary of incidents and immediate actions taken

Incident #1

On 12/3/2021, the facility veterinarian was asked by care staff to evaluate a rat that had difficulty in recovering from surgery on a protocol entitled "Electrochemical studies of drug-induced changes in brain glucose and oxygen." Upon questioning the investigator and trainee present during the surgery, the veterinarian learned that Equithesin (a mixture of pentobarbital, chloral hydrate and magnesium sulfate) was used for the surgery. The investigator stated that this agent was needed to maintain continuity with previous studies, and was most suited for prolonged surgeries. However, neither of the investigator's most recent protocols (approved in 2019 and 2020) had approved the use of Equithesin, and it was stated that ketamine/xylazine was used for all surgical procedures. The incident was reported to both the ACUC Chair and Animal Program Director that afternoon. The Chair notified the Office of Animal Care and Use (OACU) on 12/6/2021.

On December 6, 2021, the Chair, Animal Program Director, and facility veterinarian met with both the investigator and the Branch Chief. During that meeting, the investigator readily admitted that he had been exclusively utilizing Equithesin for surgeries. The investigator agreed to return Equithesin to the pharmacy and suspend all animal activities indefinitely pending the outcome of an ACUC meeting to discuss the case. The veterinarian agreed to work with the investigator to optimize anesthesia protocols using ketamine/xylazine and consider other alteratives. The rat which had undergone surgery and recovered was euthanized.

Incident #2

On 12/10/2021, the facility veterinarian reported to the ACUC Chair and Animal Program Director a violation involving the protocol entitled "Roles of cannabinoid and hormonal systems in drug addiction." The veterinarian noted that in July, 2021, animals from this protocol had been observed

with bloating. At that time, laboratory personnel indicated that pentobarbital was being used as a surgical anesthetic, consistent with what was stated in the protocol. The veterinarian asked the lab personnel to discuss with the investigator and consider alternatives to pentobarbital. No additional issues were reported in the interim. However, on December 3, 2021, additional animals with gastric distress were observed. The veterinarian, in consultation with the investigator, performed a necropsy on 13 animals, and sent a specimen to for pathological examination to Bethesda. During followup on these results, lab personnel were again queried about the surgical anesthetic used. In this instance, it was revealed that animals had received Equithesin during surgeries performed in September and October. The researchers stated that they were not aware of the differences between Equithesin and pentobarbital.

This incident was communicated by the Animal Program Director to the Office of Animal Care and Use on 12/13/2021.

On December 13, 2021, the Chair and Animal Program Director met with both the investigator and a senior scientist from the same laboratory who was listed on the protocol (but not involved in the procedures performed on these animals). At the meeting, it was agreed that the senior scientist would collect and return all Equithesin to the pharmacy, and that no further work would be performed on the protocol pending the outcome of an ACUC meeting to discuss the case.

Additional details

Prior to 2020, only a few investigators at NIDA IRP utilized Equithesin in any studies. The ACUC and the Animal Program Director worked for many years to eliminate the use of this agent based on the well known propensity of chloral hydrate to cause adynamic ileus in rodents[1,2]. Nevertheless, a few investigators who had a long history of using this compound claimed that they did not observe major reported health issues in their animals, perhaps owing to lower concentrations of chloral hydrate used in the formulation of Equithesin.[3] These investigators could receive ACUC approval for Equithesin based on (1) scientific justification (for example, known interference of alternative anesthetics with the experimental endpoint) and (2) an assurance of regular monitoring for signs of adynamic ileus following its use. However, following some reports that reviewers of scientific manuscripts raised animal welfare objections to Equithesin use, in December 2020 the ACUC Chair advised all PIs who had previously used this compound to transition to alternatives if they had not already done so. This decision was made to avoid putting the ACUC in a position of potentially approving a compound that could jeopardize publication of work, resulting in a waste of animals and scientific resources. As of December 2021, only a single investigator had an ACUC-approved protocol with a scientifically justified use of Equithesin (to complete studies already in progress and because ketamine interferes with the experimental endpoint), and that investigator is now actively shifting to use isoflurane as an alternative. Thus, until these incidents were reported, the ACUC had no reason to suspect that Equithesin was being improperly dispensed by the pharmacy to investigators who did not have approval to use it.

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[1] Fleischman, R. W., McCracken, D., Forbes, W., 1977. Adynamic ileus in the rat induced by chloral hydrate. Lab Anim Sci 27, 238-243.
[2] Silverman, J., Muir, W. W., 3rd, 1993. A review of laboratory animal anesthesia with chloral hydrate and chloraloseibid. 43, 210-216.
[3] Vachon, P., Faubert, S., Blais, D., Comtois, A., Bienvenu, J. G., 2000. A pathophysiological study of abdominal organs following intraperitoneal injections of chloral hydrate in rats: comparison between two anaesthesia protocols. Laboratory animals 34, 84-90.

Following the discovery of the second incident, the Chair communicated directly with the NIDA IRP Scientific Director, and relayed the concern that additional labs could have access to Equithesin without ACUC knowledge. As a result of this meeting, the chief NIDA pharmacist was instructed to provide a two year record of all Equithesin supplied to NIDA IRP laboratories. A review of the records revealed no additional evidence of unapproved Equithesin use in any other lab. In addition, PIs who had received Equithesin previously were instructed to (1) confirm with their laboratory staff and (2) review their pharmacy records to ensure that no Equithesin was present in their lab. As of 12/21/2021, all Equithesin has been confirmed as having been returned to the pharmacy. The pharmacy has been instructed to no longer make this compound available to investigators.

Deliberation of the ACUC and Outcome

On December 16, 2021, at the regularly convened meeting of the ACUC, the committee met to discuss the violations. The committee unanimously voted to approve the following:

For incident #1, the investigator was suspended from performing all animal work until January 24, 2022. After that date, the investigator will be allowed to perform work with additional veterinary oversight. Specifically, for a period of 6 months, the investigator will be required to inform the facility veterinarian of when surgical procedures will be performed. Initial surgeries will be performed in the presence of the facility veterinarian and retraining of both the investigator and laboratory personnel in surgical practices and procedures will be conducted by the facility veterinarian. The veterinarian will continue to work with the lab to optimize anesthetic protocols for the procedures described in the ASP. As additional surgeries are performed, the veterinarian must either be present during or as soon as possible after surgeries (as available) to check the health status of the animals and the procedures of the lab. The veterinarian will report back monthly to the ACUC as to the investigator's compliance and overall conduct of procedures in the lab. If, following that 6 month period, the ACUC and veterinarian feel that the investigator has made significant improvements, then the investigator may proceed with reduced supervision.

For incident #2, the investigator was suspended from performing all animal work until January 24, 2022. After that date, the investigator will be allowed to perform work with additional veterinary oversight. Specifically, for a period of 6 months, the investigator will be required to inform the facility veterinarian of when surgical procedures will be performed. Initial surgeries will be performed in the presence of the facility veterinarian and retraining of both the investigator and laboratory personnel in surgical practices and procedures will be conducted by the facility veterinarian. As additional surgeries are performed, the veterinarian must either be present during or as soon as possible after surgeries (as available) to check the health status of the animals and the procedures of the lab. In addition, the senior scientist in the lab will also be available to provide oversight of the procedures and coordinate with the veterinarian. The veterinarian will report back monthly to the ACUC as to the investigator's compliance and overall conduct of procedures in the lab. If, following that 6 month period, the ACUC and veterinarian feel that the investigator has made significant improvements, then the investigator may proceed with reduced supervision.

Concluding statement

In a highly unusual occurrence for our program, two reportable animal welfare incidents involving the use of an unapproved anesthetic agent occurred within a single week. The ACUC is extraordinarily grateful to our facility veterinarian for the prompt reporting of these violations. While the laboratories involved were different, both investigators clearly engaged in procedures

not approved in their protocols, in violation of standards set forth in the *Guide for The Care and Use of Laboratory Animals*, PHS Policy, and NIH Guidelines. In the first instance, the investigator willfully submitted a protocol to gain approval by the ACUC, but then continued using Equithesin anyway. The deceptive nature of this violation represents a clear and deliberate act of defiance against the ACUC. By suspending the investigator's work for several weeks, and by placing stricter oversight on this laboratory for several months, the committee has made clear that this behavior will not be condoned. Both the Scientific Director and the Branch Chief responsible for supervising this individual have also been made aware of this offense and the ACUC decision.

In the second instance, the investigator claimed that he was unaware of the difference between pentobarbital and Equithesin. This claim is a dubious one, as the first report of bloating in these animals should have immediately raised a concern by the investigator and led to heightened sensitivity to the health of the animals. In addition, there is no reason for an investigator or trainee to not be familiar with the protocol in its entirety, and to fully understand which compounds are being used at all times. All individuals are required to acknowledge receipt and understanding of the content of the protocols in which they perform procedures. In this case, animals clearly were harmed by the actions of the investigator. Here again, the committee sends a strong message that such failure to provide adequate training and supervision—whether intentional or not- is simply unacceptable. Again, both the Scientific Director and Branch Chief have been made aware of this violation and the resulting ACUC decision.

In addition to the sanctions taken against the individual labs, the Chair and Animal Program Director, working with the Scientific Director, have taken steps to ensure the complete removal of Equithesin from any use at NIDA IRP. While a recent review indicates that chloral hydrate is still used in an estimated 6% of rat studies,[4] it is clear that this agent represents an unacceptable risk to animal health when many other safer alternative anesthetics are widely available.[5] We have long argued that convenience or an investigator's historic "comfort" with the use of this compound does not provide a sufficient justification for its continued use. Absent a compelling *scientific* rationale for its use, we do not see a need for our pharmacy to make it available to investigators.

The NIDA IRP ACUC remains committed to maintaining the highest animal welfare standards, and appreciates the support of OLAW in this regard. Should you have any additional questions or concerns regarding this report, please do not hesitate to contact me.

Sincerely,

(b) (6)

Alex Hoffman, Ph.D.
Chair
NIDA IRP Animal Care and Use Committee

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[4] Herrmann, K., Flecknell, P., 2019. Retrospective review of anesthetic and analgesic regimens used in animal research proposals. ALTEX 36, 65-80.
[5] Diane J. Gaertner, T. M. H., F. Claire Hankenson, and Margaret A. Batchelder, 2008. Anesthesia and Analgesia for Laboratory Rodents. ANESTHESIA AND ANALGESIA IN LABORATORY ANIMALS, pp. 239-297.

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Friday, January 14, 2022 7:34 AM
To: Denny, Stephen (NIH/OD) [E]
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: D16-00602 NIH Animal Incident Report (NIDA 01-22))

Thank you for this report, Dr. Denny. We will send a response soon.
Axel Wolff

From: Denny, Stephen (NIH/OD) [E] <stephen.denny@nih.gov>
Sent: Wednesday, January 12, 2022 5:17 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: D16-00602 NIH Animal Incident Report (NIDA 01-22))

Dear OLAW/DCO,

The attached documents from the NIH Institutional Official and the NIH National Institute on Drug Abuse (NIDA) ACUC address an animal incident involving the use of an unapproved anesthetic in rats. No rats appeared to have been harmed by unapproved anesthetic used. The event was first reported to the NIH Office of Animal Care and Use by the NIDA ACUC Chair Veterinarian on December 6, 2021..

If you have any questions please contact me via email or at the phone number listed below. Thank you, Steve

STEPHEN DENNY, DVM, MS, DACLAM, DACVPM | Director, Office of Animal Care and Use | NIH Bethesda Campus, Building 31/Room B1C37 | Phone: (301) 435-2188 | ***NIH . . . Turning Discovery Into Health*** |