



University of Pittsburgh

Reactivation of *Macacine herpesvirus 1* In Non-Human Primates: A Case Study in Risk Assessment and Institutional Response

University of Pittsburgh
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and Division of Laboratory Animal Research

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Adverse Event Report

- Attending Veterinarian observes several animals treated with high doses of chemotherapeutic agent have developed oral and genital ulcers indicative of active Herpes B virus infection



http://ocw.tufts.edu/Content/60/lecturenotes/896102/869276_medium.jpg

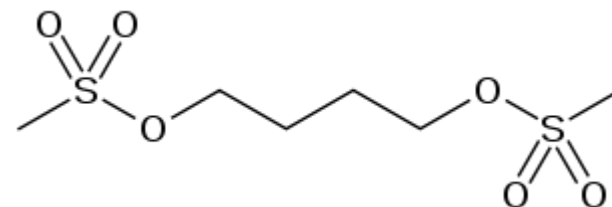


http://www.ccac.ca/images/ETC/module4/zoonosis_herpes_BRhesus.jpg



Research Study Description

- Examination of male fertility in cancer treatment model
- Compare sperm count and efficiency of spermatogenesis pre- and post-treatment with chemotherapeutic agent
 - High dose busulfan
 - Routinely used for treatment of Chronic Myelogenous Leukemia



Research Study Description

- Research protocol required collection of samples from animals during period of immunosuppression
- Herpes B virus detected by PCR in samples collected from one animal with active lesions





Immediate Risk Mitigation

- EH&S and Attending Veterinarian work with PI to suspend work on protocol: No additional busulfan treatments performed
- Current cohort of immunosuppressed animals required care
 - EH&S risk assessment of facility work practices, emergency response and injury treatment protocols, personal protective equipment



Immediate Risk Mitigation

- EH&S recommendations:
 - Immediate discontinuation of sample collection
 - Mandated chemical restraint for direct handling of animals
 - Enhanced decontamination methods
 - PPE enhancements



Other Risk Mitigation

- New cohort of B virus seropositive animals in quarantine reassigned to other studies
 - Not exposed to high-dose busulfan treatment



Retrospective Review of Veterinary Records

B Virus Serology Status	Number of Animals in Study	Evidence of B Virus Reactivation Post-Busulfan Treatment
Seropositive	16	14
Seronegative	14	0

- University program has many years of experience with other NHP model systems involving immunocompromised animals
 - SIV
 - Infectious disease models
 - Transplant models
- No evidence of reactivation of B virus in these models



Post-Incident Review: How did this happen?

- Investigator new to research with non-human primates
 - Generally recognized that immunocompromised animals are at increased risk for reactivation of B virus but new investigator was not well-versed in the species
 - Literature review found isolated cases of reactivation of B virus in specific models of immunosuppression
 - Chemical immunosuppressive agents
- Sourcing NHPs from Herpes B seropositive colonies
 - Historically, the majority of NHPs sourced for studies at the University were not *required* to be sourced from B virus SPF colonies or to be seronegative



Post-Incident Review: University Biohazards Committee

- Provide advice regarding University Biosafety programs
 - Review incident reports involving biohazardous materials or biological agents and recommend corrective actions
 - Coordinated review and revision of guidelines



Post-Incident Review: Concerns Identified

- High risk to personnel
- Animal welfare
- Adverse effects on research and data collection
- University's current mechanisms for IACUC protocol review and animal ordering did not identify or educate investigators of this potential hazard



Animal Research Protocol Review In Place Prior to Incident

- University's IACUC sends all protocols out for designated review
 - EH&S designated reviewer for all
 - Generate a risk assessment for every protocol

Section 1: Hazard Assessment Overview		Protocol Date	9/27/12	Assessment Completion Date	10/1/12
Project Title					
PI Name	Office		Phone		
PI E-Mail	Lab Location/s				
IACUC #	IBC #	Location Animals Transported To			
Animal Species Used	Non-Human Primate				
Hazard Summary	Rabies virus, Surgical and Research Hypocytiles, Isoflurane, formalin, Fast Blue, Diamidino yellow				
Use of Agents in Lab	<p>Rabies virus, (BST-3 4023-4025, 4053C, 4081)</p> <ul style="list-style-type: none">• Agent should be handled in a BSL-2 facility, using BSL-2 practices and containment.• Lab should be signed as BSL-2, being agent in use, agent should be handled in a certified biosafety cabinet.• Animals exposed to agent must be housed in ABSL-2 housing rooms; housing room doors must be labeled with the name of the agent.• It is the responsibility of the PI to label cage cards with the name of the agent, biohazard stickers, and dosing information.• Any centrifugation of agent must include the use of rotor lids or individual rotor cups with safety lids.• Liquid contaminated waste must be disinfected before disposal, solid contaminated waste such as used plastic ware should be disinfected and then disposed as biological waste.• Agent should be transported in a leak-proof container, including absorbent material and an appropriate biohazard label.• Per the OSHA Bloodborne Pathogen standard, EH&S requires the use of approved safety engineered sharps devices (SESIPs) when working with this BSL-2 agent.• Use of agent in protocol can present an exposure hazard via needlestick injury. Appropriate practices and engineering controls should be followed to minimize exposure (sharps containers, safe needle devices, no recapping of needles, etc). <p>Surgical and Research Hypocytiles (BST-3 4th Floor Animal or Surgical Area)</p> <ul style="list-style-type: none">• Care must be exercised when manipulating (weighing, solubilizing) powder or crystalline forms of these agents to decrease the risk of inhalation exposure. <p>Use of Agents in Animals</p> <ul style="list-style-type: none">• Routine laboratory handling using appropriate chemical hygiene practices should not pose significant exposure risk. However, accidental ingestion or injection may produce significant adverse effects.• Use of agent in protocol can present an exposure hazard via needlestick injury. Appropriate practices and engineering controls should be followed to minimize exposure (sharps containers, safe needle devices, no recapping of needles, etc). <p>Fast Blue, Diamidino yellow (BST-3 4025)</p> <ul style="list-style-type: none">• Based upon initial animal studies for o-dianisidine and o-dianisidine-based dyes, this agent may pose a cancer risk following human exposure.• Specific engineering controls should be utilized to scavenge or remove agent from the air surrounding any operations (if scavenging is not possible, usage should occur in a chemical fume hood).• Routine laboratory handling using appropriate chemical hygiene practices should not pose significant exposure risk. However, accidental ingestion or injection may produce significant adverse effects.• It is the responsibility of the PI to label cage cards with the name of the agent, a chemical hazard sticker and dosing information.• Entry doors to facilities housing animals exposed to agent must be labeled with the name and also state "Suspect Carcinogen".• Gloves should be worn at all times; double gloving (nitrile over latex) is recommended. Other personal protective equipment (PPE) including lab coat and eye protection should be worn when working with this agent.• Use of agent in protocol can present an exposure hazard via needlestick injury. Appropriate practices and engineering controls should be followed to minimize exposure (sharps containers, safe needle devices, no recapping of needles, etc). <p>Isoflurane (BST-3 4th Floor Animal or Surgical Area)</p> <ul style="list-style-type: none">• Specific engineering controls should be utilized to scavenge or remove agent from the air surrounding any operations (if scavenging is not possible, usage should occur in a chemical fume hood). <p>Formalin/Paraformaldehyde/Paraldehyde (BST-3 4019, 4024-4026, 4081)</p> <ul style="list-style-type: none">• Specific engineering controls should be utilized to scavenge or remove agent from the air surrounding any operations (if scavenging is not possible, usage should occur in a chemical fume hood).• Gloves should be worn for all manipulations of agent (butyl or nitrile gloves are recommended).				




BHC Recommendations:

1. EH&S to identify studies that involve immunosuppression of NHPs
2. EH&S to develop administrative controls/work practice enhancements for these protocols
 - Disinfectant recommendations
 - Review of NHP-associated injury response procedures
 - Identification of high risk tasks and specialized work practice controls and/or PPE.
3. EH&S to educate animal care and research staff
4. EH&S to trigger mandatory meeting with all research personnel to review specific hazards prior to start of project



BHC Recommendations: Division of Laboratory Animal Resources

5. Investigators should be alerted to the potential risk of reactivation of latent Herpes B virus infection in immunocompromised Macaques

 UNIVERSITY OF PITTSBURGH
Division of Laboratory Animal Resources
Non-Human Primate Ordering Purchase Requisition
PROCEDURE 07-27-06 Rev 7-19-11 Rev 10/20/11

FORMS MUST BE PROCESSED THROUGH DEPARTMENTAL PURCHASING APPROVAL CHANNELS

E-mail this order form as an attachment to: dlersch@dlar.pitt.edu
Orders will only be accepted via e-mail

DEADLINE INFORMATION:

- Orders should be submitted as far in advance as possible to avoid unnecessary delays, as NHP sourcing, health evaluation, and coordination of transportation following federal guidelines is a complicated process. Also quarantine space is limited.
- Missing information or lack of proper departmental approvals may delay placement of the order.

Principal Investigator (the name that appears on the IACUC protocol):
Secondary Investigator (secondary name you may want on cage card):
IACUC Protocol Number:

Vendor(s) preference: _____ Genus and Species: _____
Weight range: _____ Age Range: _____
Requested delivery date: _____

Quantity: Male: Female: Either

Surgically Naïve Yes No

Important occupational health consideration: if you plan to immunocompromise any of these animals during your study and macaque species are to be used, the risk of reemergence from latency of Herpes B virus is a concern and the recommendation is to source or verify during quarantine serologic negativity of the Macaques spp. used for such studies.

NOTE: Animals are not routinely screened for the following pathogens unless requested. If you need negative status for successful completion of your studies please indicate below:

Viral and Pathogen Free Status Requested: SRV SIV STLVI Herpes B Virus
 Other Herpes Measles CMV EBV MALARIA OTHER:
None

Would you like to speak with a DLAR Veterinarian regarding choice of animal type, species, genotype, geographic source/subspecies, specific viral status, or other specific research requirements using NHP models?
 Yes No

OTHER SPECIAL INSTRUCTIONS (If you have any other information or requests related to placing this order please insert the information here):



BHC Recommendations

6. IACUC, EH&S, and DLAR collaborate to monitor adverse events in NHP populations in studies involving immunosuppression
 - IACUC/EH&S act promptly to suspend research and reassess risk to personnel if evidence of active Herpes B infection
 - Require that only Herpes B seronegative animals be used in certain types of studies



Final Results

- PI voluntarily discontinued work with known Herpes B virus seropositive animals in this model
- Identified opportunities for improvement
 - Cooperation between research oversight groups
 - Education of research community
 - Improved communication between parties involved



Final Results

- New institutional awareness of potential implications of this type of study
- Improved EH&S risk assessment and training programs for the University's non-human primate research community



University of Pittsburgh

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