AN INTERNATIONAL ORGANIZATION DEDICATED TO PROTECTING THE RIGHTS OF ALL ANIMALS

PETA FOUNDATION

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS FOUNDATION

Washington
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2154 W. Sunset Blvd.
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323-644-PETA

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757-622-PETA

September 29, 2022

Via mail

CDR U.S. Army Medical Research and Development Command
Attn: Freedom of Information/Privacy Acts Office
810 Schreider St.
Fort Detrick, MD 21702-5000

RE: Appeal of Department of the Army's Decision to Withhold Information in Response to FOIA Request FA-22-0021

Dear Sir or Madam:

People for the Ethical Treatment of Animals (PETA) submits this appeal pursuant to 5 U.S.C. § 552(a)(6) and 32 C.F.R. § 286.11 of the Department of the Army's (Department's) decision to withhold information responsive to PETA's Freedom of Information Act (FOIA) Request FA-22-0021. *See* Ex. 1.

On March 18, 2022, PETA submitted the following FOIA request:

a. For the period of January 15, 2020, to the date of processing this request. All protocols and/or modification to protocols approved by the U.S. Army Medical Research and Development Command (USAMRDC) Animal Care and Use Review Office (ACURO) that involve the use of a weapon—as defined by USAMRDC Policy 84 titled "Animal Research and Medical Training Involving Wounding"—to inflict wounding in dogs, cats, nonhuman primates, and/or marine mammals for the purposes of conducting medical research, development, testing, or evaluation.
b. All photos and videos of animals used in these aforementioned protocols.

PETA received the final response to this request from the Department on July 15, 2022.[1] The final response stated the

[1] Although the Department of the Army's response was dated May 2, 2022, the response was not transmitted to PETA via e-mail until July 15, 2022. *See* Ex. 2. Because appeals of adverse FOIA determinations are due within ninety days of the Department of the Army's response, *see* 32 C.F.R. § 286.11(a), PETA's appeal is timely.

Department identified one record, a protocol, responsive to PETA's request but denied access to the record pursuant to FOIA Exemption 1, 5 U.S.C. § 552(b)(1). Exemption 1 excludes from disclosure matters that are "specifically authorized under criteria established by Executive order to be kept secret in the interest of national defense or foreign policy and . . . are in fact properly classified pursuant to such Executive order." *Id.* § 552(b)(1). The Department stated the record at issue is classified pursuant to Executive Order 13526.

PETA appeals the Department's denial of its FOIA request because the Department has failed to fulfill its obligation to segregate and disclose non-exempt, non-classified information. PETA does not seek information that would be deemed classified pursuant to Executive Order 13526, such as the characteristics of the weapons used pursuant to the protocol identified in the request. Rather, PETA seeks all segregable, non-classified information, *including but not limited to* the number and species of animals used, husbandry considerations, the pain/distress assessment, whether any anesthesia or analgesics were administered to these animals prior to or subsequent to when they were wounded pursuant to this protocol, and the study endpoints. Again, this list is not exhaustive of the categories of information that should be easily segregable without jeopardizing information that is legitimately in need of classified status.

Information can only be classified pursuant to Executive Order 13526 if the information's "unauthorized disclosure could reasonably be expected to cause identifiable or describable damage to the national security," and the information pertains to one or more of subject areas enumerated. Executive Order 13526 § 1.4. Information concerning the weapons used pursuant to the protocol identified in PETA's FOIA request could arguably fall under several of these subject areas, including "(a) military plans, weapons systems, or operations;" "(e) scientific, technological, or economic matters relating to the national security;" or "(g) vulnerabilities or capabilities of systems, installations, infrastructures, projects, plans, or protection services relating to the national security." Nevertheless, it is difficult to imagine how the specific information PETA seeks, such as the numbers and species of animals used, endpoints for the animals, and any medication administered to alleviate pain and suffering, could fit into any of the listed categories and could "reasonably be expected to cause identifiable or describable damage to the national security."

Invoking Exemption 1 to withhold responsive records does not relieve the Department from its legal obligation to segregate and disclose non-classified information. FOIA requires that "[a]ny reasonably segregable portion of a record shall be provided to any person requesting such record after deletion of the portions which are exempt . . . ." 5 U.S.C. § 552(b). Records withheld pursuant to Exemption 1 are still subject to FOIA's segregability requirement. *See Citizens for Responsibility and Ethics in Wash. v. U.S. Dep't of State*, Civ. Action No. 19-1344, 2022 WL 1801054, at *15 (D.D.C. June 2, 2022); *Khatchadourian v. Def. Intelligence Agency*, ---F. Supp. 3d---, 2022 WL 971206, at *5 (D.D.C. 2022). "[B]ecause '[t]he focus of the FOIA is information, not documents, [ ] an agency cannot justify withholding an entire document simply by showing that it contains some exempt material." *Citizens for Responsibility and Ethics in Wash.*, 2022 WL 1801054, at *15 (quoting *Mead Data Ctr., Inc. v. U.S. Dep't of Air Force*, 566 F.2d 242, 260 (D.C. Cir. 1977)). When portions of records contain classified information, "non-classified portions of a document still must be disclosed unless they are inextricably intertwined with exempt portions." *Khatchadourian*, 2022 WL 971206, at *5 (quoting *Mead Data Cent., Inc.*, 566 F.2d at 260). Furthermore, the Department bears the burden to demonstrate with "reasonable specificity the bases for its segregability determinations." *Khatchadourian*, 2022 WL 971206, at *5

(quoting *Armstrong v. Exec. Off. of the President*, 97 F.3d 575, 578 (D.C. Cir. 1996)) (internal quotation marks omitted). Blanket invocations of Exemption 1 to withhold entire documents and/or cursory explanations of the Department's efforts to segregate non-exempt information do not fulfill the Department's legal duties. See *Citizens for Responsibility and Ethics in Wash.*, 2022 WL 1801054, at *15; *Khatchadourianv. Def. Intelligence Agency*, 453 F. Supp. 3d 54, 83 (D.D.C. 2020) (ordering defendant agency to supplement the record regarding its segregability analysis pursuant to Exemption 1 because the agency did not explain with reasonable specificity how it analyzed and segregated non-classified information).

There is no evidence in the Department's final response to PETA's FOIA request that the Department even attempted to perform the requisite segregability analysis. The specific information PETA seeks, such as the species and numbers of animals used pursuant to the identified protocol, the pain/distress assessment, endpoints for the animals, and the administration or lack thereof of anesthesia and analgesics, should be easily segregable from any classified information justifiably withheld under Exemption 1. This is a fair assumption in light of the typically universal format of protocol forms, which separate the various categories of information pertaining only to the animals themselves from the specifics of the experiment or training undertaken. *See* Ex. 3. To fulfill its legal obligations pursuant to FOIA, the Department must reexamine the responsive record and provide PETA with any information that is reasonably segregable from that correctly withheld under Exemption 1.

In accordance with 5 U.S.C. § 552 (a)(6)(A)(ii) and 32 C.F.R. § 286.11(c), PETA looks forward to receiving your decision in writing within twenty working days. Thank you for your attention to this matter.

Sincerely,

Caitlin Zittkowski
Counsel
PETA Foundation
2154 W. Sunset Blvd.
Los Angeles, CA 90026
216-408-3721
caitlinz@petaf.org

EXHIBIT 1

DEPARTMENT OF THE ARMY
HEADQUARTERS, U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
810 SCHREIDER STREET
FORT DETRICK, MARYLAND 21702-5000

May 2, 2022

Freedom of Information Act Office
FOIA Case: FA-22-0021

Kaylie Flaugher
People for the Ethical Treatment of Animals
kaylief@peta.org

Dear Ms. Flaugher:

This letter is in response to your Freedom of Information Act (FOIA) request sent on March 18, 2022. Your request had been assigned the following tracking number: FA-22-0021.

a. For the period of January 15, 2020, to the date of processing this request. All protocols and/or modification to protocols approved by the U.S. Army Medical Research and Development Command (USAMRDC) Animal Care and Use Review Office (ACURO) that involve the use of a weapon—as defined by USAMRDC Policy 84 titled “Animal Research and Medical Training Involving Wounding”—to inflict wounds in dogs, cats, nonhuman primates, and/or marine mammals for the purpose of conducting medical research, development, testing, or evaluation.

b. All photos and videos of animals used in these aforementioned protocols.

After a search for documents responsive to your request using methods which can reasonably be expected to produce the information requested resulted in one record found. The U.S. Army Medical Research and Development Command (USAMRDC) determined the record(s) is/are denied because it is currently and properly classified in accordance with Executive Order (EO) 13526. We are withholding the record in full pursuant Title 5 U.S.C. § 552(b)(1) (“Exemption 1”). Exemption 1 protects from disclosure information that has been deemed classified "under criteria established by an Executive order to be kept secret in the interest of national defense or foreign policy" and is "in fact properly classified pursuant to such Executive order." For more information regarding EO 13526, please visit https://www.archives.gov/isoo/policy-documents/cnsi-eo.html.

Because your request has been denied you are advised of your right to appeal this determination to the Secretary of the Army. If you decide to appeal at this time, your appeal must be submitted within 90 days of the date of this notification. In your appeal, you must state the basis for your disagreement with the denial and the justification for the release of information associated with your request for this command. Your appeal should be addressed to: CDR U.S. Army Medical Research

and Development Command, Attention: Freedom of Information/Privacy Acts Office, 810 Schreider Street, Fort Detrick, MD 21702-5000, for forwarding, as appropriate, to the Office of the Secretary of the Army. Please enclose a copy of this response along with your Appeal. To ensure proper processing of any appeal, the letter and the envelope should both bear the notation, "Freedom of Information Act Appeal."

Should you have any questions regarding this response, please email the USAMRDC FOIA office at usarmy.detrick.medcom-usamrmc.mbx.foia@mail.mil.

Sincerely,

HARRIS.AUDRICIA.MCKINNEY.1085970506

Digitally signed by HARRIS.AUDRICIA.MCKINNEY.1085970506
Date: 2022.07.14 12:49:52 -05'00'

Audricia M. Harris
Colonel, U.S. Army
Communication Director

EXHIBIT 2

Caitlin Zittkowski

From: USARMY Ft Detrick MEDCOM USAMRMC Mailbox FOIA <usarmy.detrick.medcom-usamrmc.mbx.foia@mail.mil>
Sent: Friday, July 15, 2022 9:26 AM
To: Kaylie Flaugher
Cc: USARMY Ft Detrick MEDCOM USAMRMC Mailbox FOIA
Subject: FA-22-0021 / Response Letter
Attachments: FA-22-0021 (PETA_Flaugher) Final Response.pdf

Ms. Flaugher,

Please see the attached response to your FOIA request (FA-22-0021). This is the final response regarding this FOIA request.

V/R

JOHN J. STUART
MAJ, GS
Freedom of Information Act (FOIA) Officer U.S. Army Medical Research and Development Command (USAMRDC) Fort Detrick, MD
Email: usarmy.detrick.medcom-usamrmc.mbx.foia@mail.mil

EXHIBIT 3

DoD Animal Use Protocol Format

Requirements

All DoD animal use protocols must use the format shown in this appendix. This protocol format includes requirements of the Animal Welfare Act Regulations, the Guide, and other applicable Federal regulations and DoD directives.

Protocol cover sheet

Before the protocol is submitted for Institutional Animal Care and Use Committee (IACUC) review, at least three signatures are required on the protocol cover sheet. They must include those of the Principal Investigator (P.I.); either the department or division chief or the scientific review committee chairperson, and the individual performing the statistical review (when necessary). Site IACUCs may require more signatures before submission.

I. Name of Facility

II. Proposal Number

III. Title

IV. Principal Investigator(s)/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

V. Scientific Division Review/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

VI. Statistical Review/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name); Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

VII. Attending Veterinarian/Division/Phone/E-mail

a. Printed Name (First Name, MI, Last Name}; Title; Division

b. Signature; Date (YYYYMMDD); Phone, Fax

DoD Animal Use Protocol Cover Sheet

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a. Scientific/division review. This signature verifies that the animal use proposal received appropriate scientific peer review and is consistent with good scientific practice.

b. Attending veterinarian. The Animal Welfare Act Regulations require that an attending veterinarian must be consulted in the planning of procedures/manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics. It is good practice for a consultant veterinarian to review any animal-use protocol before IACUC submission.

c. Statistical review. A person knowledgeable in biostatistics is required to review all proposals to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

DoD animal use protocol outline

a. The outline format shown below is designed to be used with several word-processing programs on a personal computer as a "fill-in-the-blank" type of document. It is available electronically through the appropriate DOD component oversight office listed in MSR 6025.02. Each paragraph and subparagraph in the format must have a response. Title headings do not require a response. Portions of the protocol format that are not applicable will be marked "N/A." There are no space limitations for the responses. Pertinent standing operating procedures or similar documents that are readily available to the IACUC may be referenced to assist in the description of specific procedures. Each IACUC might require additional information in the protocol submission process.

PROTOCOL TITLE
PRINCIPAL INVESTIGATOR(S)
CO-INVESTIGA TOR(S)
I. NON-TECHNICAL SYNOPSIS
II. BACKGROUND
II.1. Background
II.2. Literature Search for Duplication
II.1.1. Literature Source(s) Searched
II.1.2. Date of Search
II.1.3. Period of Search
II.1.4. Key Words of Search
II.1.5. Results of Search
III. OBJECTIVE/HYPOTHESIS
IV. MILITARY RELEVANCE
V. MATERIALS AND METHODS
V.1. Experimental Design and General Procedures
V.1.1. Experiment 1
V.1.2. Experiment 2
V.2. Data Analysis
V.3. Laboratory Animals Required and Justification
V.3.1. Non-animal Alternatives Considered
V.3.2. Animal Model and Species Justification
V.3.3. Laboratory Animals
V.3.3.1. Genus and Species
V.3.3.2. Strain/Stock
V.3.3.3. SourceNendor
V.3.3.4. Age
V.3.3.5. Weight
V.3.3.6. Sex
V.3.3.7. Special Considerations
V.3.4. Number of Animals Required (By Species)
V.3.5. Refinement, Reduction, Replacement
V.3.5.1. Refinement
V.3.5.2. Reduction
V.3.5.3. Replacement
V.4. Technical Methods
V.4.1. Pain/Distress Assessment
V.4.1.1. APHIS Form 7023 Information (See attending veterinarian for assistance)
V.4.1.1.1. Number of animals
V.4.1.1.1.1. Column C: _(Animal#)
V.4.1.1.1.2. Column D: _(Animal#)
V.4.1.1.1.3. Column E: _(Animal#)
V.4.1.2. Pain Relief/Prevention
V.4.1.2.1. Anesthesia/Analgesia, tranquilization
V.4.1.2.2. Pre- and Post-procedural Provisions
V.4.1.2.3. Paralytics
V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures
V.4.1.3.1. Sources Searched
V.4.1.3.2. Date of Search
V.4.1.3.3. Period of Search
V.4.1.3.4. Key Words of Search
V.4.1.3.5. Results of Search
V.4.1.4. Unalleviated Painful/Distressful Procedure Justification
V.4.2. Prolonged Restraint
V.4.3. Surgery

V.4.3.1. Pre-surgical Provisions
V.4.3.2. Procedure
V.4.3.3. Post-surgical Provisions
V.4.3.4. Location
V.4.3.5. Surgeon
V.4.3.6. Multiple Major Survival Operative Procedures
V.4.3.6.1. Procedures
V.4.3.6.2. Scientific Justification
V.4.4. Animal Manipulations
V.4.4.1. Injections
V.4.4.2. Biosamples
V.4.4.3. Adjuvants
V.4.4.4. Monoclonal Antibody (MAbs) Production
V.4.4.5. Animal Identification
V.4.4.6. Behavioral Studies
V.4.4.7. Other Procedures
V.4.4.8. Tissue Sharing
V.4.5. Study Endpoint
V.4.6. Euthanasia
V.5. Veterinary Care
V.5.1. Husbandry Considerations
V.5.1.1. Study Room
V.5.1.2. Special Husbandry Provisions
V.5.1.3. Exceptions
V.5.2. Veterinary Medical Care
V.5.2.1. Routine Veterinary Medical Care
V.5.2.2. Emergency Veterinary Medical Care
V.5.3. Environmental Enrichment
V.5.3.1. Enrichment Strategy
V.5.3.2. Enrichment Restriction
VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING
VII. BIOHAZARD/SAFETY:
VIII. ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the P.I. unless directed by the IACUC.

IX. ASSURANCES: The law specifically requires several written assurances from the Principal Investigator. Please read and sign the assurances as indicated.

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations/observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R," namely "Responsibility, which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedures: (A signature for this assurance is required by the Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL or WILL NOT (circle one or both, if applicable) be relieved with the use of anesthetics, analgesics, and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

(PRINT) First Name, MI, Last Name of Principal Investigator

Signature Date {YYYYMMMDD)

DOD Animal Use Protocol Format

a. Some information may be added to the format to meet local IACUC needs. However, all labeled paragraphs and subparagraphs will remain in the same relative order. The added information will be similar or complementary to the information requested. Other types of requirements specific to a given Service, command, or locale (such as budgeting information, local coordinating requirements, or specific scientific review requirements, and so forth) can be added by placing them in front or behind the standard format.

Protocol format

The format shown below is the same protocol format previously outlined. Explanations have been added to aid in completing the protocol proposal.

PROTOCOL TITLE: Title must include species of animal(s) used in research.

PRINCIPAL INVESTIGATOR(S)
CO-INVESTIGATOR(S)

I. NON-TECHNICAL SYNOPSIS: Provide a brief, narrative description of the proposal that is easily understood by a high school graduate. Include animal use in your description.

II. BACKGROUND

II.1. Background: Include a brief statement of the requirement or need for the information being sought Lengthy explanations are not required. Typically, the literature or the experience that led to the proposal will be briefly reviewed, references cited, and a description of the general approach will be provided.

II.2. Literature Search for Duplication: This search must be performed to prevent unnecessary duplication of previous experiments. A search using multiple relevant research databases should be performed; requirements for database search criteria are at the discretion of the IACUC.

II.1.1. Literature Source(s) Searched

II.1.2. Date of Search

II.1.3. Period of Search

II.1.4. Key Words of Search

II.1.5. Results of Search: Provide a narrative description of the results of the literature search.

Ill. OBJECTIVE/HYPOTHESIS: State the objective of this protocol or the hypothesis to be accepted or rejected.

IV. MILITARY RELEVANCE: Provide a brief and succinct military justification for the research with regard to military needs and mission requirements. If applicable, state the Science and Technology Objective (STO) that this work supports.

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures: This section includes an explanation of experimental design. Technical methodology need not be described in this section, rather, it should be described under paragraph V.4, Technical Methods. Provide a complete description of the proposed use of animals to include a summary table of the experimental groups. Succinctly outline the formal scientific plan and direction of experimentation. If several experiments or sequential studies are to be included in the protocol, describe the experimental design of each separate experiment in sub-parts to this section. The length and detail required in this section depends largely on the complexity of the study. A clearly understandable description of the numbers of animals and their distribution into experimental groups is essential. The number requested must equal the minimum number required to complete the study yet be sufficient to yield meaningful results. The minimum number includes animals necessary for control or technique development, and so forth. Inclusion of a summary table or flow chart showing the distribution of animals by experimental group is highly recommended. The total number of animals required for the study is listed in section V.3.4.

V.1.1. Experiment 1

V.1.2. Experiment 2

V.2. Data Analysis: List the statistical test(s) planned or describe the strategy intended to evaluate the data. Describe the statistical methodology used to determine group size and total number of animals. A power-based assessment of the sample size is the preferable method of determining the minimum number that is likely to yield significant results with given alpha and beta errors, estimated effect size and expected variability. Be certain to include animals necessary for controls or technique development, and so forth.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered: State all non-animal alternatives (for example, computer modeling, in vitro cell culture work, etc.) that were considered. Explain why animals are needed.

V.3.2. Animal Model and Species Justification: Provide a scientific justification for the choice of animal model(s). What physiological and morphological characteristics does this animal possess that make it the best possible model? If less sentient (invertebrate versus vertebrate) animal models were considered but not chosen, explain **why.**

V.3.3. Laboratory Animals

V.3.3.1. Genus and Species

V.3.3.2. Strain/Stock: If inbred or specialized animals are required, use proper terminology. (See the attending veterinarian for assistance.)

V.3.3.3. Source Vendor: Provide a preferred source for the animals. Animals will be legally obtained from suppliers licensed by the U.S. Department of Agriculture (USDA) in accordance with Code of Federal Regulations, Title 9, Animals and Animal Products, Chapter 1, Subchapter A, Animal Welfare, Parts 1, 2, and 3. (See the attending veterinarian for assistance.)

V.3.3.4. Age

V.3.3.5. Weight

V.3.3.6. Sex

V.3.3.7. Special Considerations: List specialized requirements for animals here (for example, simian immunodeficiency virus or herpes antibody free, *Pasteurella* free, and so forth).

V.3.4. Number of Animals Required (By Species): The number of animals stated here must correspond exactly to that described in section V.1. If, during the completion of the protocol, additional animals are needed owing to technical or unavoidable circumstances, or to exploit a serendipitous finding, follow IACUC procedures for requesting approval of additional animals.

V.3.5. Refinement, Reduction, Replacement (3 Rs): Investigators are required to consider the 3 Rs when preparing an animal use research protocol. In the paragraphs below, describe all provisions in this protocol that refine, reduce, or replace the use of animals. Discuss what provisions were considered and why they were not chosen. If N/A is used, explain why.

V.3.5.1. Refinement: Procedures or measures taken to eliminate or minimize pain or distress in the animal(s) or enhance animal well-being. Examples of refinement include but are not limited to the use of analgesia to decrease pain or distress, the use of remote telemetry, which decreases the distress of restraint, or the use of adjusted early experimental endpoints. In addition to listing refinements, list refinement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.2. Reduction: Procedures or measures taken to reduce the number of animals used. Examples of reduction include but are not limited to the use of shared or historical control groups, preliminary screening in non-animal systems, and innovative statistical packages. In addition to listing reductions that will be used, list reduction alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.3.5.3. Replacement: Procedures or measures that eliminate the use of animals. Examples of replacements include but are not limited to the use of non-animal models or less sentient animal species. In addition to listing replacements that will be used, also list replacement alternatives that would allow you to meet your scientific objectives and were considered but not adopted. Explain why they were not adopted.

V.4. Technical Methods: This information must be presented in sufficient detail, documented or referenced, so that the IACUC can adequately review the procedure, obtain a clear understanding of what is to be done and how the animals will be handled, and make a reasonable determination as to whether this proposed use of laboratory animals is in compliance with DOD regulations, guidelines, and Federal law.

V.4.1. Pain/Distress Assessment: The law defines a painful procedure as one that would "reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure was applied; that is, pain in excess of that caused by injections or other minor procedures." If a procedure may involve pain or distress, even if relieved by anesthetics or analgesics, the P.I. must consult with the attending veterinarian.

V.4.1.1. APHIS Form 7023 Information: (See your attending veterinarian for assistance.) The protocol must contain an estimate of the number of animals that will be counted in columns C, D, and E of the APHIS Form 7023, Annual Report of Research Facility. Columns C, D and E represent specific pain categories. (See below paragraphs, V.4.1.1.1.1.-V.4.1.1.1.1.3.) The animal should be listed in the column corresponding to the most painful or distressful procedure experienced by the animal. It is possible for one protocol to have animals listed in several columns. For instance, control animals may be placed in Column C while experimental animals may be placed in Column D, depending upon the nature of the protocol. Reflect use of more than one species of animals in a duplicate table. The total numbers reflected in these three columns will add up to the number of animals requested for the entire protocol in paragraph V.3.4.

V.4.1.1.1. Number of Animals

V.4.1.1.1.1. Column C: (animal #)
Examples of research procedures/manipulations that would require an animal to be placed in Column C are studies involving not more than slight or momentary pain and/or distress in a human being to which that procedure is applied.

V.4.1.1.1.2. Column D: (animal #)
Examples of procedures/manipulations that would require an animal to be placed in Column D are procedures where anesthesia or analgesia will be administered to avoid or effectively relieve pain or distress. General anesthesia given for surgical procedures, or the use of analgesia or anti-inflammatory agents are examples of this category.

V.4.1.1.1.3. Column E (animal #)
Examples of procedures/manipulations that would require an animal to be placed in Column E are procedures in which alleviation of pain or distress are contraindicated for a scientifically justifiable reason such as the experimental results are likely to be confounded if drugs relieving pain or distress were administered. Detailed justification for putting animals into this category is required below in paragraph V.4.1.4.

V.4.1.2. Pain Relief/Prevention

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Describe the methods or strategies planned to effectively relieve or prevent pain or distress if the study will cause more than slight or momentary pain or distress. If pain/distress relief/prevention is planned, specify agents to be used and when these agents will be given (preemptive or post-procedural). Provide agent, dosage, and frequency and duration of administration.

V.4.1.2.2. Pre- and Post-procedural Provisions: Describe the provisions for both pre- and post-procedural care, including provisions for post-procedural observations, frequency, and duration of observations. (Information concerning pre- and post-surgical care should be listed in paragraphs V.4.3.1 and V.4.3.3). If analgesics are used for pain/distress relief, provide the frequency and duration of administration, observational criteria utilized to determine if animals are experiencing pain or distress, and the location for the post-procedural care.

V.4.1.2.3. Paralytics: The use of paralytic agents without anesthesia is prohibited. Describe the monitoring method that will be used to ensure adequate depth of anesthesia while the animal is under the influence of the paralytic agent and adequate vital signs.

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures: Respond N/A if the animals will experience not more than momentary or slight pain or distress and are placed in column C of APHIS Form 7023. (See paragraph V.4.1.1.)

V.4.1.3.1. Source(s) Searched: Examples are AGRICOLA, MEDLINE, BIOSIS, Altweb, etc.

V.4.1.3.2. Date of Search

V.4.1.3.3. Period of Search

V.4.1.3.4. Key Words of Search: Examples are pain, surgery, alternatives, LD 50, analgesia, anesthesia, death as an endpoint, distress, species of animal(s) to be used, name of painful or distressful experimental procedure, etc.

V.4.1.3.5. Results of Search: Provide a narrative summary of the results of the literature search for alternatives. The Animal Welfare Act specifically states that the P.I. **MUST** provide a narrative description of the methods and sources, e.g., the Altweb (Johns Hopkins Center for Alternatives to Animal Testing), MEDLINE, Life Sciences Ab tracts, AGRICOLA, and BIOSIS) that he/she used to determine that alternatives to the painful procedure were not available. Discuss alternatives (those that would meet your scientific objectives) considered but not chosen. The alternatives literature search **MUST** be performed even when animals are placed in Column D (the pain or distress is alleviated through the use of analgesics or anesthetics) and Column E.

V.4.1.4. Unalleviated Painful/Distressful Procedure Justification: Procedures that cause more than slight or momentary pain or distress that is not alleviated through the effective use of anesthetics or analgesics must be justified on a scientific basis in writing by the P.I. This paragraph must be completed if there are ANY animals in this protocol that will experience unalleviated pain or distress.

V.4.2. Prolonged Restraint: Describe (period of restraint, method, and timing of animal observations, habituation/training of animal to restraint device) and justify In detail any prolonged restraint greater than 12 hours for nonhuman primates or in accordance with IACUC policy for other species. Examples of restraint methods are primate chairs, restraint boards, metabolism cages, and so forth. This section is not intended for short-term actions such as rabbit restraint for bleeding, and so forth.

V.4.3. Surgery: Major survival operative procedures on non-rodent species will be conducted only in dedicated facilities intended for that purpose, and operated and maintained under aseptic conditions. Non-survival operative procedures do not require a dedicated facility, but they should be performed using surgical gloves, mask, and clean instruments. Additionally, the surgical site should be clipped and cleaned prior to surgery. Major survival rodent surgery does not require a dedicated facility, but it **must** be performed using aseptic technique; that is, aseptic patient preparation, surgical gloves, mask, and sterile instruments. A major operative procedure is defined as a procedure that penetrates and exposes a body cavity, or causes substantial or permanent impairment of physical or physiological function.

V.4.3.1. Pre-surgical Provisions: Describe the provisions for pre-surgical care, including provisions for pre-surgical observations and frequency of pre-surgical observations. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the pre-surgical care.

V.4.3.2. Procedure: Describe in detail any surgical procedures planned.

V.4.3.3. Post-surgical Provisions: Describe the provisions for post-surgical care, including provisions for post-surgical observations, frequency of post-surgical observations and criteria for early euthanasia owing to surgical complications or pain that cannot be relieved. If analgesics are utilized for pain or distress relief, provide the time schedule for administration, observational criteria utilized to determine if animals are experiencing pain/distress, and the location for the post-surgical care.

V.4.3.4. Location: Give the location/room number for the proposed surgical procedure.

V.4.3.5. Surgeon:

V.4.3.6. Multiple Major Survival Operative Procedures: The principal investigator must scientifically justify multiple major survival operative procedures performed on the same animal.

V.4.3.6.1. Procedures:

V.4.3.6.2. Scientific Justification:

V.4.4. Animal Manipulations: Describe any injections, sampling procedures, or other manipulations of the animals necessary for the study. A reference or SOP may be furnished to the IACUC to document a particular procedure in lieu of a detailed description.

V.4.4.1. Injections: Information must include route of injection, dosage, frequency, duration, volume injected, needle size, anatomic injection site and osmolarity, pH, pyrogenicity, sterility of non-pharmaceutical substances.

V.4.4.2. Biosamples: Examples include cerebrospinal fluid taps, blood sampling, and biopsies. List volumes taken, sampling site, frequency of sampling, needle size, and method of sampling. Procedures performed or biosamples obtained during a necropsy need not be described here.

V.4.2.3. Adjuvants: List any adjuvants used and the plan for their use. Provide a scientific justification for the use of Complete Freund's Adjuvant (CFA) and discuss why other less reactive adjuvants cannot be used. Provide dosages, volumes, route, number of injection sites, and injection locations. Specify frequency and method of injection site monitoring and include a response plan (for example, alternative endpoint and veterinary medical treatment) in the event of an adverse reaction.

V.4.2.4. Monoclonal Antibody (MAbs) Production: Provide a scientific justification for in vivo MAbs production. What in vivo methods of MAbs were considered but not used? For in vivo MAbs production, specify the priming agent, animal monitoring frequency, number and frequency of abdominal taps, and fluid replacement therapy. Include a response plan (for example, alternative endpoint(s) and veterinary medical treatment) in the event of an adverse reaction.

V.4.2.5. Animal Identification: Describe the method of animal identification used in this study. Examples include: microchips, tattoos, eartags, and cage cards.

V.4.2.6. Behavioral Studies: Fully describe the use of aversive stimuli, food or water restriction, and so forth, that would affect the study animals. Include methods of monitoring physiologic or behavioral indexes, including criteria (for example, weight loss or state of hydration) for temporary or permanent removal of the animal from the study. Provide an appropriate scientific justification for this type of behavior modification. An IACUC policy may be included where applicable.

V.4.2.7. Other Procedures: Describe all procedures which have not been explained in other sections of this proposal that will be performed while conducting this research. Examples include electrocardiograms, radiology, and aerosol exposure.

V.4.2.8. Tissue Sharing: List what tissues will be shared, with whom, and for what purpose.

V.4.5. Study Endpoint: State the projected study endpoint for the animals (for example, recovery and return to issue pool, euthanasia, or death without early euthanasia). Indicate whether recovery, euthanasia, or death is expected; and the specific plan for determining when the animal experimentation phase will be stopped. The P.I. must ensure that unnecessary pain or distress is prevented by carefully considering "When is the experimental question answered?" so that the animals can be expeditiously removed from the study. Define specific criteria that will be used to determine study endpoint (for example, weight loss, loss of locomotion and significant lowering of body temperature, decreased food or water consumption, and decreased activity). Specifically address and scientifically justify any proposal in which critically ill or moribund animals are allowed to die as a result of the experimental procedures without the benefits of veterinary medical treatment or early euthanasia. Explain the plan for the disposition of surviving animals or animals removed from the study prior to its completion.

V.4.6. Euthanasia: If applicable, discuss the euthanasia method. The Animal Welfare Act defines euthanasia as "humane destruction of an animal by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death." The current American Veterinary Medical Association (AVMA) guidelines for euthanasia must be followed. Exceptions to the AVMA guidelines will be considered by the IACUC on a case-by-case basis. If requested, the attending veterinarian will assist in selecting the best method for euthanasia.

V.5. Veterinary Care: If requested, the attending veterinarian of the facility will assist P.I.s with preparing this section.

V.5.1. Husbandry Considerations: Federal regulations require that animal housing and living conditions must be appropriate to their species and contribute to their health and comfort. Briefly describe animal husbandry to include routine animal observations, caging methods, feed and water provisions, environmental parameters, sanitation schedules, and light cycles.

V.5.1.1. Study Room: Where will the experimental procedure be conducted? Will the animal be housed in this room for more than 12 hours?

V.5.1.2. Special Husbandry Provisions: Examples include micro-isolators, metabolic cages, food and water restriction.

V.5.1.3. Exceptions: Describe any deviations/exceptions to The Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act regulations, or IACUC policy that have an impact on animal housing space, watering, feeding, and sanitation. Deviations/exceptions must be justified by the P.I. and approved by the IACUC.

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care: Describe the routine veterinary medical care. State if the animals will be observed daily or more frequently. Indicate what will happen if the animal becomes ill or debilitated during the study and requires evaluation. List the criteria used for health evaluation while the animals are on study (for example, weight loss, ruffled fur, dehydration, decreased activity, and hunched body position). Include a response plan (for example, alternative early endpoint(s) and veterinary medical treatment) in the event of debilitating illness or an adverse reaction.

V.5.2.2. Emergency Veterinary Medical Care: Describe emergency veterinary medical care.

V.5.3. Environment Enrichment

V.5.3.1. Enrichment Strategy: Discuss enrichment provided to animal species listed in this protocol.

V.5.3.2. Enrichment Restriction: Provide written justification for restricting enrichment programs or activity programs of dogs, cats, or nonhuman primates. Single housing of social species such as rodents, pigs, nonhuman primates, and dogs without sensory contact with conspecifics must also be justified and approved by the IACUC.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING: List the names, qualifications and training by procedure of all personnel working with animals assigned to this protocol. Personnel performing observations, procedures, and/or manipulations described in the protocol must be identified and appropriately trained and qualified to perform these procedures. Contact the attending veterinarian for assistance with this requirement (please see endnotes below).

VII.BIOHAZARD/SAFETY: Provide a list of any potential biohazards associated with the chosen animal model and this research proposal (for example, viral agents, toxins, radioisotopes, oncogenic viruses, and chemical carcinogens). Describe safety precautions and programs designed to protect personnel from biohazards associated with this research and any surveillance procedures in place to monitor potential exposures.

VIII.ENCLOSURES: Enclosures such as IACUC policies on adjuvants, monoclonal antibody production, tissue sharing, food and/or water restriction, prolonged restraint, pathology addenda, and pain assessment criteria may be included at the discretion of the P.I. unless directed by the IACUC.

Personnel qualifications.

a. Study Personnel Qualifications in a training table must be included in section VI of the protocol description. The table format is preferred by the IACUC for ease of reviewing the protocol. The table will contain at minimum the following four column headings:

(1) Name of the person(s) performing protocol activities.

(2) Name of the activity (for example, the procedure, observation, or manipulation to be performed, such as the venous catheterization of a dog). Itemize each activity being performed in the protocol. List per species if there are multiple species in the protocol. If more than one individual is performing the activity, list each individual separately.

(3) Qualifications and experience of the person performing the activity (for example, assistant laboratory animal technician (ALAT), three years of experience).

(4) Training of the person performing the activity (for example, Canine Procedures Workshop, 2018).