

Staff Summary for June 19-20, 2024

23. Restricted Species Permit Application (consent)**Today's Item**Information Action

Review application approved by the Department for a permit to possess transgenic Hawaiian bobtail squid (*Euprymna scolopes*).

Summary of Previous/Future Actions (N/A)**Background**

State regulation (Section 671.1, Title 14, California Code of Regulations) prohibits possession or use of restricted species without a permit issued by the Department; this includes transgenic aquatic animals, for which additional procedures apply. Subsection (a)(8)(H) of Section 671.1 mandates Commission review before the Department issues a permit for importing, possessing, transporting, rearing or conducting research on transgenic aquatic animals. The Commission can deny issuance of the permit if it determines the applicant is unable to meet regulatory requirements.

The California Institute of Technology (Caltech) submitted to the Department a restricted species permit application proposing to use transgenic Hawaiian bobtail squid (a marine invertebrate) for biomedical research; the proposal includes an emergency preparedness plan to meet regulatory requirements. The Department has fulfilled its notification requirement to the Commission (Exhibit 1).

Unlike research involving transgenic finfish, which requires notification to both the Commission and the California State Legislature's Joint Committee on Fisheries and Aquaculture (Fish and Game Code, Section 15007, subdivision (e)), notification for invertebrates is not mandatory.

Following a thorough review, the Department determined that the applicant has met the regulatory requirements for permit approval and supports issuing a restricted species permit to Caltech for the possession of transgenic squid. Refer to Exhibit 1 for the application and Department memo. Unless the Commission determines that the applicant is unable to meet regulatory requirements and takes action to deny issuance of the permit, the Department will issue the permit.

Significant Public Comments (N/A)**Recommendation**

Commission staff: Receive the restricted species permit application and accept any public comment, under a motion to adopt the consent calendar. No further action is recommended.

Department: No action recommended.

Exhibits

1. [Department memo and permit application, received May 20, 2024](#)

Staff Summary for June 19-20, 2024

Motion

Moved by _____ and seconded by _____ that the Commission adopts the staff recommendation for Agenda Item 23 on the consent calendar.

Memorandum

Date: April 8, 2024

To: Melissa Miller-Henson
Executive Director
Fish and Game Commission

From: Charlton H. Bonham
Director

Subject: **Agenda Item for the June 19-20, 2024 Meeting: Approval of Restricted Species Permit Application to Possess Transgenic Squid**

The California Institute of Technology (Caltech) has applied for a Restricted Species Permit to possess transgenic Hawaiian bobtail squid (*Euprymna scolopes*). According to California Code of Regulations (CCR), Title 14, Section 671.1(a)(8)(H), all approved applications to possess a transgenic aquatic animal shall be reviewed by the California Fish and Game Commission (Commission) at a regularly scheduled meeting. The Commission may deny the issuance of a permit if it determines that the applicant is unable to meet the regulatory requirements for the importation, transportation, possession, and confinement of transgenic aquatic animals.

The transgenic squid will be used for biomedical research. Caltech has agreed to comply with containment and security conditions as specified in CCR, Title 14. California Department of Fish and Wildlife (Department) Marine Region staff have coordinated with the Fish Health Lab in reviewing the permit. The Department recommends issuing Caltech a Restricted Species Permit to possess transgenic squid.

If you have any questions or need additional information on this matter, please contact Dr. Craig Shuman, Marine Regional Manager at R7RegionalMgr@wildlife.ca.gov.

Attachment

cc: Chad Dibble, Deputy Director
Wildlife and Fisheries Division

Craig Shuman D.Env., Regional Manager
Marine Region

Kirsten Ramey, Program Manager
Marine Region
State Managed Finfish and Nearshore Ecosystem Program

Adam Frimodig, Senior Environmental Scientist
Marine Region
[Nearshore and Bay Management Project](#)

Sara Briley, Environmental Scientist
Marine Region
[Nearshore and Bay Management Project](#)

Colleen Burge, PhD, Shellfish Pathologist and Research Scientist Supervisor
Fisheries Branch



State of California – Department of Fish and Wildlife
2023 RESTRICTED SPECIES PERMIT AMENDMENT REQUEST
 DFW 1313b (REV. 10/18/22) Page 1 of 2

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 BY LRB

IMPORTANT! YOU MAY NOT OBTAIN ANIMALS PRIOR TO AMENDMENT APPROVAL

Fees include a nonrefundable three percent (3%) application fee, not to exceed \$7.50 per item. (Section 700.4, Title 14, California Code of Regulations CCR).

FEE: \$73.13 (Nonrefundable application fee must accompany this amendment request.)

SEE INSTRUCTIONS ON PAGE 2. TYPE OR PRINT CLEARLY.

FIRST NAME Karen	M.I.	LAST NAME Lencioni	PERMIT NUMBER
BUSINESS NAME California Institute of Technology			DAY TELEPHONE

LOCATION OF ANIMAL(S) HOUSING: Note: Animals being held at multiple locations require inspection certification by the Department that each of those facilities meet the minimum applicable housing requirements as set forth in subsection 671.1(a)(8)(A-F), Sections 671.3-671.4 and/or 671.7(b), Title 14, of the CCR.

ADDRESS 1200 E. California Blvd	CITY Pasadena	STATE CA	ZIP CODE 91125	COUNTY Los Angeles
ADDRESS	CITY	STATE	ZIP CODE	COUNTY

List all restricted animals **TO BE ACQUIRED** in the following order: mammals, birds, fish, or reptiles. Group animals by order, family, and species. Use the following letters to denote sex: M=Male, F=Female, and U=Neutered or Unknown. Mark an "X" in the **TO BE ACQUIRED** column for animals to be acquired within the next year. **Unique Identifiers:** Use the following letters to denote unique identifying methods (See Section 671.1(c)(3)(J), Title 14, of the CCR): M=Microchip, T=Tattoo, and A=Alternative Method. Aquaculture and fish permittees: Identify the actual number in the ID number field and identify either W=Weight, V=Volume or C=Count in the method field. Remember to complete the Importation Only Section below for animals being imported into California. For California Residents Only: **All native species obtained from a licensed California Wildlife Rehabilitation Facility require a Native Species Exhibiting Permit.** Contact the License and Revenue Branch at (916) 928-5846 or SPU@wildlife.ca.gov if you need additional information.

COMMON NAME	SCIENTIFIC NAME	ID NUMBER	METHOD	SEX	AGE
Hawaiian bobtail squid	Euprymna scolopes	N/A	count	N/A	0-5d

WILL ANIMALS BE IMPORTED INTO CALIFORNIA? YES, COMPLETE IMPORTATION SECTION NO, EXPLAIN:
 Eggs will be collected from broodstock tanks, injected with CRISPR Cas9 and guide RNA, then incubated in Petri dish in a temperature controlled table top incubator until hatching. After hatching, individual transgenic squid will be held in 20ml glass scintillation vials for colonization with *V. fischeri*, and will be transferred to another prepared scintillation vial for euthanasia after imaging and/or experimentation.

IMPORT ONLY: COMPLETE THIS SECTION IF YOU ARE IMPORTING ANIMALS INTO CALIFORNIA

LIST SPECIES TO BE IMPORTED	NUMBER OF ANIMALS	ORIGIN (State or Country)
PERSON/BUSINESS SHIPPING ANIMALS		DAY TELEPHONE
ADDRESS	CITY	STATE ZIP CODE
NAME OF CARRIER	POINT OF ENTRY INTO CALIFORNIA	

I certify under penalty of perjury under the laws and regulations of the State of California that all information on this application is true and correct and I am not violating any city or county laws. I agree to comply with the provisions of Section 671, Title 14, of the CCR. I understand it is unlawful to use or possess a permit which was obtained by fraud or deceit (Fish and Game Code Section 1052b). I understand that in the event that this information is found to be untrue or incorrect, the permit will be considered invalid and must be surrendered where purchased and I will be subject to criminal prosecution. I further understand that failure to comply with the terms and conditions of a permit may result in revocation of current permit and/or denial of future permits. Violation of this section is a misdemeanor, punishable by fine of not more than \$1,000.00, imprisonment in the county jail for not more than six months, or both the fine and the imprisonment. In addition, I may be subject to civil penalties as stated in Fish and Game Code Section 2125.

APPLICANT'S SIGNATURE (Must be in ink) *Kathleen* DATE 8/9/2023

FOR DEPARTMENT OF FISH AND WILDLIFE USE ONLY

REVIEWED BY/DATE <i>W 9/7/23</i>	TRANSACTION#	ISSUED BY/DATE
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Pdbycc



INSTRUCTIONS FOR COMPLETING THE RESTRICTED SPECIES PERMIT AMENDMENT REQUEST

Use this form to: 1) add species you are not currently authorized to possess; 2) increase the number of animals where there are condition limitations; or 3) add/change facility locations.

Please allow 45 business days for processing your request. Amendments for transgenic species must go before the Fish and Game Commission, so you must allow an additional 30 business days. Incomplete requests will be returned and could delay the issuance of your amendment. Contact the Department of Fish and Wildlife (Department), License and Revenue Branch at (916) 928-5846 or SPU@wildlife.ca.gov if you need additional information regarding Restricted Species Permits.

To complete this application, you must:

1. It is mandatory to complete all items unless exempted.
2. Sign and date the amendment request in ink (an **original** signature is required).
3. Provide a list of animals to be acquired.
4. Provide a statement of purpose describing in detail the planned use for each animal. Applicants shall include relevant materials, as appropriate, including any lists of prospective clients with their contact information or contracts with clients or websites, scripts, brochures or flyers promoting or describing the planned use of the animals. If the animals will be used in an educational program, the applicant shall provide an explanation why live restricted species are necessary and samples of the educational material and message that will be distributed (not required for animal care, AZA, breeding, research and single event breeding permittees).
5. Provide a resume that provides dates and details documenting you or your full-time employee's qualifying experience caring for restricted animals at a facility engaged in a similar or directly related activity to the permit requested and for the animal(s) to be acquired. This experience shall have been acquired within the previous five years and include a total of at least one year full-time, hands-on experience caring for a species in the same family or closely related taxonomic family as the species requested (required for breeding, exhibiting, nuisance bird abatement, shelter and single event breeding permittees only).
6. Provide a letter of recommendation, written within the previous five years on **letterhead stationery, with an original signature**, from the facility where you or your full-time employee gained the experience. Document the quality and extent of the knowledge and experience, as related to the species and permit requested (required for breeding, exhibiting, nuisance bird abatement, shelter and single event breeding permittees only).
7. Provide an updated copy of your Emergency Action Plan that includes the new species.
8. Provide an updated Breeding Plan that includes the new species (required for breeding and single event breeding permittees only).
9. Provide photograph(s) of the enclosure(s) for animal(s) to be acquired that includes all required elements of the minimum standards as specified in Section 671.3.
10. Provide any other supporting documentation required by regulations.
11. Mail the completed application and supporting documentation with a cashier's check, money order, personal or business check*, or credit card** authorization form with the appropriate fee to the Department of Fish and Wildlife, License and Revenue Branch, PO Box 944209, Sacramento, CA 94244-2090 or apply in person. **DO NOT SEND CASH.**

IMPORTANT INFORMATION FROM THE DEPARTMENT OF PUBLIC HEALTH

The Department of Public Health (CDPH) has regulatory authority over the importation of specified carnivores (including skunks and raccoons), nonhuman primates and bats, due to potential health hazards.

Section 2606.8, Title 17, of the CCR, prohibits the importation of skunks because the hazard to the public from exposure to rabies is extremely high. The CDPH is concerned that certain wild animals could carry rabies and introduce new strains of rabies into the state of California. Therefore, the Department routinely denies requests for the importation and possession of skunks and raccoons. Exceptions may rarely be made for zoological or research institutions demonstrating an extraordinary need. The importation of other specified carnivores, bats or nonhuman primates may be allowed under a CDPH permit in certain circumstances. **For more information on CDPH permits, please contact them at (916) 552-9740.**

NOTICE

Disclosure Statement—Under Section 671.1, Title 14, of the CCR, the Department of Fish and Wildlife is authorized to collect information from applicants to maintain a record of licensure. All information requested on this application is mandatory unless otherwise indicated. All information except the street address and telephone number of the applicant may be provided to the public, if requested. All information related to a business may be released, including the residence address if it is the same as the business address. Other personal information submitted on this application may be released for law enforcement purposes, pursuant to court order, or for official natural resources management purposes.

A licensee may obtain a copy of his/her license records maintained by the Department by submitting a written request to the Custodian of Records, at the Department of Fish and Wildlife, License and Revenue Branch, PO Box 944209, Sacramento, CA 94244-2090. All requests must include the requester's name, address, and telephone number.

PAYMENT POLICY

***Personal or business checks** will be accepted by the Department if name and address are imprinted on the check. Checks returned to the Department due to insufficient funds will render your permit invalid. The Department may also deny the issuance or renewal of any permit if a person has failed to reimburse the Department for the amount due. Any activity performed without a valid permit is a violation of the Fish and Game Code and therefore subject to enforcement action.

****Credit Cards**—Licenses, permits, tags, stamps, or registrations may be purchased with a Visa or MasterCard.

Caltech

Karen Lencioni, D.V.M.
Director, Office of Laboratory Animal Resources
1200 E. California Blvd.
MC 114-96
Pasadena, CA 91125
(626) 395-8664

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8/20/23
BY LAB

August 8, 2023

Ms. Lucy Lopez
California Department of Fish and Wildlife
License and Revenue Branch
1740 N. Market Boulevard
Sacramento, CA 95834

Dear Ms. Lopez:

Please find the 2023 Restricted Species Permit Amendment Request along with payment enclosed. Caltech is a non-profit Higher Education and Research institution and has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare/National Institutes of Health. The Assurance number is [REDACTED]. In addition, Caltech is fully accredited with the AAALAC International.

An emergency preparedness plan for the species covered by this permit is attached. Pictures of the animal enclosures are attached as well.

Please do not hesitate to contact me should you have any questions or concerns with this renewal application.

Sincerely,

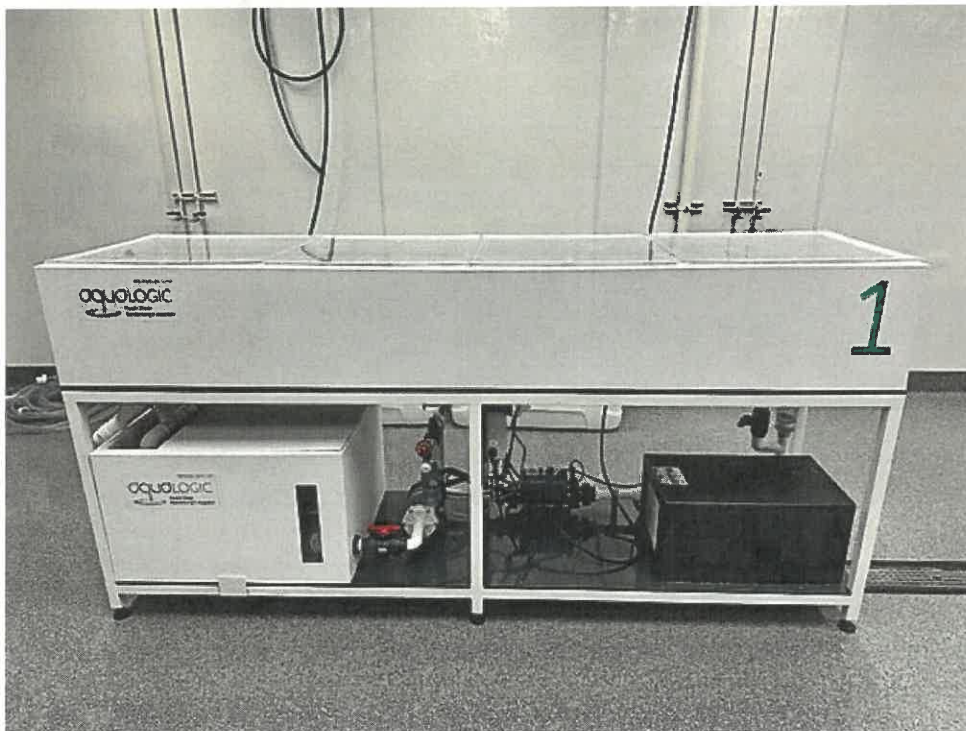


Karen Lencioni, D.V.M.
Attending Veterinarian
Director, Office of Laboratory Animal Resources

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Proposed representative housing for Transgenic Squids.

Eggs will be collected from broodstock tanks, injected with CRISPR Cas9 and guide RNA, then incubated in Petri dishes in a temperature controlled table top incubator until hatching. Water exchanges will occur daily. After hatching, individual transgenic squid will be held in 20ml glass scintillation vials for colonization with *V. fischeri*, and will be transferred to another prepared scintillation vial for euthanasia after imaging and/or experimentation.



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SCOPE OF WORK (TRANSGENIC SQUIDS):

This species is being amended to our permit for the following scope of work:

This work is part of an IACUC approved research project to further understanding of host-microbe interactions. Our approach relies on the use of a powerful model, the binary symbiosis between the Gram-negative bacterium *Vibrio fischeri* and its host, the Hawaiian bobtail squid, *Euprymna scolopes*. This binary system, with its unparalleled resolution, has been studied for over 30 years, providing insights into evolutionary conserved mechanisms by which hosts, including humans, initiate and maintain, throughout their lifetimes, interactions with bacteria. Eggs will be collected from broodstock tanks, injected with CRISPR Cas9 and guide RNA, then incubated in Petri dishes in a temperature controlled table top incubator until hatching. Water exchanges will occur daily. After hatching, individual transgenic squid will be held in 20ml glass scintillation vials for colonization with *V. fischeri* and will be transferred to another prepared scintillation vial for euthanasia after imaging and/or experimentation to evaluate colonization parameters.

California Institute of Technology

Restricted Species Permit # [REDACTED] - Emergency Action Plan

August 2023

1. Background Information:

- a. The list of restricted species maintained at the California Institute of Technology is limited to the following species: Octodon degus, Xenopus frogs, lamprey, tiger salamander (aquatic larval stage), tree shrews, African spiny mice, Lesser Egyptian jerboa, Axolotl, genetically altered zebra fish and genetically altered squid.
- b. The restricted species maintained do not pose a hazard of a physical nature to humans due to their very small size.

2. List of recapture equipment available, including but limited to darting equipment, nets, traps and chemical immobilization:

- a. All aquatic animal housing areas (for Xenopus frogs, lamprey, tiger salamanders, Axolotls, zebra fish and squid) will be equipped with nets and buckets/tanks suitable for recapture of animals should the need arise.
- b. Octodon degus, tree shrews, African spiny mice, and Lesser Egyptian jerboa will be recaptured by hand, net or humane live traps and each animal facility will maintain this equipment should the need arise.

3. Description of humane lethal dispatch methods for various animals and a list of qualified personnel who are trained to carry out the methods:

- a. With exception of the transgenic squid, aquatic species will be immersed in Tricaine or benzocaine, depending on the species, in the event that euthanasia is required. For squid, 4% ethanol is used as a euthanasia solution. Each aquatic facility will maintain adequate supply of Tricaine (tricaine methanesulfonate), benzocaine (for Xenopus laevis), and/or ethanol (for squid) in order to humanely euthanize all aquatic species in the lab should the need arise during an emergency.
- b. Octodon degus, Tree shrews, African spiny mice, and Lesser Egyptian jerboa will be euthanized by overdose of ketamine/xylazine or a pentobarbital-based euthanasia solution. African spiny mice may also be euthanized by CO2 inhalation.
- c. Research personnel listed on an Institutional Animal Care and Use Committee (IACUC) animal research protocol whose training has been reviewed by the IACUC, as well as licensed veterinary or trained animal care personnel on site, are authorized to euthanize animals in the event of an emergency.

4. List of medical supplies/first aid kits and where they are located:

- a. A first aid kit is located in close proximity to all restricted species housing areas.

5. Description of mobile transport cages and equipment on hand:

- a. Routine housing tanks and/or buckets will be used to transport aquatic species should the need arise during an emergency.
- b. Standard microisolator rodent housing cages will be used to transport tree shrews should the need arise during an emergency.

6. List of emergency telephone numbers that includes the local departmental regional office, 911 and animal control agencies:

- a. In the event of an emergency, promptly notify the California Institute of Technology Security Office (ext. 5000). This office is manned 24/7 365 days each year and has access to an extensive telephone tree which is activated in the event of an emergency situation. The responding Security Officer will notify Caltech emergency personnel in accordance with internally approved procedures (a detailed written plan of response to emergency events is maintained within the California Institute of Technology Security Office). Also, contact a member of the Caltech Veterinary Staff (phone contact information for the Veterinary Staff is posted near the telephone area in all aquatic species housing areas). The Veterinary Staff will contact regulatory agencies as deemed necessary. Regulatory agencies include:

Regulatory Agency	Contact Information
CA Fish and Wildlife South Coast Region	858-467-4201
PHS/NIH Office of Laboratory Animal Welfare	301-496-7163
Pasadena Humane Society Animal Control	626-792-7151

7. Written plan of action for emergencies:

- a. See attached page.

Restricted Species Emergency Response Plan

1. GENERAL INFORMATION

- A. Emergencies, by their very nature, are unplanned events that require an immediate response. This emergency response plan contains a general guideline for response to emergencies that may affect the restricted species maintained at Caltech.
- B. All employees are responsible for becoming familiar with the procedures for emergency response listed in the Caltech Emergency Response Guide.
- C. On a continuous basis, a minimum of a 72-hour supply of food and water for the animal population will be maintained. In the event that minimum food and water requirements and established environmental controls and husbandry standards cannot be maintained, and the health or welfare of the animals is threatened, animals may be humanely euthanized under the direction of the veterinary staff. Euthanized animals will be bagged and placed in sealed plastic containers if freezers are not functional.
- D. In the event that a disaster results in actual harm or the death of an animal, a report will be submitted to the appropriate regulatory agencies.

2. NATURAL DISASTER

In the event of an emergency resulting from a natural disaster, such as a fire or earthquake, animals will be sheltered in place unless otherwise determined by Caltech management. The following plan of action is to be followed by Caltech employees.

A. Personnel Safety

1) Non-Business Hours

- a. Contact your area supervisor by phone to report on your ability to report to work during regularly scheduled work hours.
- b. Do not enter buildings until building entry is deemed safe by emergency personnel.
- c. Call the information phone line for emergencies.

2) Regular Business Hours

- a. Evacuate the building as directed by emergency personnel. Report immediately to assigned assembly points.
- b. Do not reenter building until emergency personnel have deemed the building safe for reentry. When reentering the building, proceed with caution and immediately report any safety issues or unusual findings to the area supervisor.

B. Immediate Emergency Response

- 1) Assess condition of restricted species in assigned work areas. Notify the area supervisor or the veterinary staff of any immediate animal health or welfare concerns.
- 2) Monitor room temperatures hourly in assigned work areas until stabilized. Report any deviations from normal to area supervisor or veterinary staff.

3. LOSS OF ESSENTIAL SERVICES

A. LOSS OF ELECTRICAL POWER

1) Aquatic species life support systems are provided with emergency power. Ventilated caging that may house African spiny mice are also provided with emergency power. All other species on the Caltech Restricted Species permit should not be immediately impacted by a loss of electrical power. Animal housing facilities are provided with emergency lighting for safe egress.

B. LOSS OF HEATING, VENTILATION, AIR CONDITIONING SYSTEM

- 1) Immediately notify area supervisor of problem.
- 2) Contact Service Center to report problem.
- 3) If necessary, use heaters to raise room temperatures.
- 4) If area temperatures are altered (high/low) such that physical harm to the animals may result, move the animals to an alternate location where the temperature can be controlled. Consult with the Veterinary Staff to determine the location of the best alternate housing area.

C. LOSS OF WATER SERVICE

- 1) Aquatic systems are provided with emergency power and are largely recirculating. Turn off water discharge from system in event of loss of water service to conserve water.
- 2) Tree shrews, African spiny mice, jerboas and degus can be provided water from emergency water storage on campus.

D. FLOOD

- 1) Locate source of water and attempt to stop flow by turning off faucet or water valve.
- 2) Secure animals so that their health and welfare is not at risk.
- 3) Secure equipment so that damage is minimized.
- 4) Contact the Service Center to report problem if plumbing assistance is required.

E. LOSS OF SECURITY SYSTEM

- 1) Report security system failure to area supervisor or Security Office.

4. BREACH OF SECURITY

- A. Report any breach of security immediately to the Security Office and to the Caltech Attending Veterinarian.