

# **Guidelines for Care and Use of Nonhuman Primates**

**Version 3**

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**Primate Research Institute, Kyoto University**

## Definitions of terms

According to the definitions proposed by the Science Council of Japan, the following terms used in the present guidelines are defined below.

- 1) Animal experiment: Utilization of animals for education, research, manufacture of biological products or other scientific purposes.
- 2) Facilities: Facilities and equipment used to perform animal experiments.
- 3) Laboratory animals: Animals of mammalian, avian or reptilian species used in animal experiments. In the present guidelines, this term in most cases refers to monkeys and thus the term “monkeys” is often used as a synonym for “laboratory animals”.
- 4) Institutions: Organizations (university, institute, independent administrative body, company, etc.) where animal experiments are performed.
- 5) Director of the institution: A person with overall responsibility in the institution for the proper and safe conduct of animal experiments (dean, director of an institution, principal of a school, chairperson of the board of directors, president, head of an institute, etc.).
- 6) Animal experiment protocol: A protocol drafted in advance regarding the conduct of an animal experiment.
- 7) Animal experiment researcher: A person(s) performing the animal experiment.
- 8) Principal investigator: A researcher who supervises all duties related to the animal experiment protocol.
- 9) Manager: A person(s) who undertakes the management of laboratory animals and facilities under supervision of the director of the institution (head of the animal experimentation facilities, department head, etc.)
- 10) Laboratory animal manager: A laboratory animal manager assists the manager and is in charge of management of the laboratory animals.
- 11) Animal caretaker: A person(s) in charge of the care and management of laboratory animals under supervision of the laboratory animal manager or animal experiment researcher.
- 12) Manager etc.: Refer to the director of the institution, manager, laboratory animal manager, animal experiment researcher and animal caretaker.

13) Regulations: In-house regulations of research institutions specified for the proper conduct of animal experiments and the proper care and management of laboratory animals based on applicable laws/regulations and guidelines.

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## **Chapter I: Basic policies**

Research and education activities using animals in life science, medicine and veterinary medicine are conducted for the promotion of human health/well-being or the protection or improvement of the welfare of wild and captive animals. Animal experiments/studies are indispensable for promoting these research/education activities and advances in science. These animal experiments should be managed and conducted voluntarily under the responsibility of each research institution. Through such ethical management, Japanese life science and medical and veterinary research activities have been developed in a free and creative manner and have contributed to impressive developments across the world. For the proper conduct of ethical management, the care, management and use of laboratory animals for research and education purposes should be performed properly based on scientific and specialized knowledge and a full understanding of the characteristics of each animal species. In addition, the animal experiment protocol should be prepared based not only on scientific reasonability but also on sufficient humanitarian/ethical considerations for laboratory animals.

Research activities must be conducted in compliance with the internationally accepted 3R principles of animal experimentation, which were included in the Act on Welfare and Management of Animals amended in 2005 (effective since June 1, 2006). The “3Rs” refer to replacement (use of alternative methods; i.e. non-animal research methods or substituting animals), reduction (use of fewer animals while maintaining scientific reliability) and refinement (refining experimenter’s technique and knowledge to minimize pain and distress experienced by laboratory animals). Moreover, consideration must be given to the four Rs, which additionally includes the responsibility of researchers. Nonhuman primates (NHPs) are more closely related to humans than other animal species and have highly social lives and advanced mental function. Therefore, research activities using NHPs must be conducted based on sufficient consideration of the significance of using NHPs and it must be ensured that the research objectives can only be achieved with NHPs. This document describes basic knowledge and regulations required for conducting such research activities.

This document was prepared based on the provisions of the Guidelines for Proper Conduct of Animal Experiments (June 1, 2006; Science Council of Japan), Basic Policies for the Conduct of Animal Experiments in Research Institutions under the Jurisdiction of the Ministry of Health, Labor and Welfare (effective on June 1, 2006; Ministry of Health, Labor and Welfare (MHLW)), Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Notice No. 71 of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) dated June 1, 2006) and Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notice No. 88 of the Ministry of the Environment dated April 28, 2006).

This document does not simply describe minimal requirement, but also actively provides abstract guidelines that would help in identifying objectives to be achieved and resolving current problems through ceaseless efforts. These guidelines primarily concern the NHPs that are housed and used in research activities in the Primate Research Institute of Kyoto University (hereinafter, referred to as “PRI”). For other laboratory animals, special instructions only are presented in this document.

This chapter describes the basic policy for the care and use of laboratory animals at PRI.

### **1. Responsibility of the director of the institution**

The president of Kyoto University (hereinafter, referred to as “the President”) bears the final responsibility for all animal experiments and related activities conducted in Kyoto University. The President organizes PRI as an institution required for the proper care and management of laboratory NHPs and proper and safe conduct of animal experiments and related activities, and appoints the director of PRI (hereinafter,

referred to as “the Director”) as the manager of the institution. The President delegates all responsibilities for animal experiments conducted in PRI to the Director and the Director assumes the responsibilities on behalf of the President.

The Director performs all administrative duties concerning the care, management, breeding and supply of NHPs kept in PRI. The President may also appoint, based on the Director’s recommendation, a person with knowledge and expertise related to laboratory animals as the head of the Center for Human Evolution Modeling Research (hereinafter, referred to as “the Head of CHEMR) as the laboratory animal manager and allow the director of CHEMR to perform the duties of the Director of PRI on his/her behalf. The Director of PRI must also establish, based on relevant policies, regulations including the authority and responsibilities of the Director of the institution, procedures for the conduct of animal experiments, proper care and management of laboratory animals and methods of maintenance and management of facilities.

## **2. Establishment of Monkey Committee**

For the effective execution of the basic policy for the care and use of NHPs (i.e. the guidelines described in this document), the “Animal Welfare and Animal Care Committee ( Monkey Committee)” shall be established as the organization to prepare animal care/management policies, assess the validity of the animal experiment protocol and supervise the conduct of the protocol.

The Monkey Committee is composed of:

- one or more council member(s) from each department and
- one or more council member(s) from CHEMR who are qualified veterinarians.

The Committee shall also be attended by the Head (or designee) of CHEMR and one of its technical staff. One of the administrative staff members of the Research Aid Division shall also attend the Committee to prepare meeting minutes and retain related documents, such as the animal experiment protocol. In case of problems that cannot be managed by these members only, the attendance of additional temporary members may be requested upon consultation with council members. For the ethical review of an animal experiment protocol, one or more outside monkey expert(s) and one or more non-research personnel shall be included in the Committee.

The Director of PRI consults with the Monkey Committee regarding whether the submitted animal experiment protocol complies with the guidelines specified in this document and related regulations, and approves the protocol if judged appropriate based on the review results of the Monkey Committee or issues necessary instructions/advice if the protocol is judged inadequate. The Director also gives necessary instructions/advice based on the results of the Monkey Committee inspection of the status of animal care and experiments. Laboratory animals other than NHPs should be handled in the same manner. The Monkey Committee shall be convened periodically or when deemed necessary by the Director of PRI or the Chair of the Monkey Committee, to discuss the items listed below. The Monkey Committee also conducts periodic inspections and implements necessary measures, such as recommendations for improvement, when deemed necessary. The procedures for conducting animal experiments are described in Chapter IV.

### **Inspection and assessment of animal care facilities and care conditions**

Faculty staff members who are qualified veterinarians periodically conduct the inspection and assessment of animal care facilities and care conditions. Inspection/assessment results shall be reported by the person in charge of inspection/assessment to the Chair of the Monkey Committee. The Chair of the Monkey Committee shall convene the Committee to review the inspection/assessment results and report to the Director of PRI when deemed necessary. The Director provides the animal care staff with instructions/advice when deemed necessary.

### **Review of validity of the animal experiment protocol for educational and research purposes and advice thereon**

Following the guidelines specified in this document, the Monkey Committee conducts ethical review of the animal experiment protocol

submitted by a researcher and report the review results to the Director of PRI. The Director approves the protocol if judged appropriate based on the review results or gives necessary instructions/advice if the protocol is judged inadequate.

**Allocation of NHPs to researchers and arrangements for the care and use of NHPs in PRI**

With cooperation of the faculty members of CHEMR, the Monkey Committee determines the number of NHPs to be allocated to each researcher and where to keep them, taking into consideration the species and number of NHPs requested for each experiment.

**Assessment of current status of animal experiments and issuance of recommendations for improvement or orders to discontinue the experiment through the Director of PRI when deemed necessary**

Faculty staff members who are qualified veterinarians periodically conduct the inspection and assessment of laboratories and the status of the experiment. Inspection/assessment results shall be reported by the person in charge of the inspection/assessment to the Chair of the Monkey Committee. The Chair of the Monkey Committee shall convene the Committee to review the inspection/assessment results and report to the Director of PRI when deemed necessary. The Director issues instructions, advice and recommendations to the animal experiment researcher and may order the discontinuation of the experiment if no improvement is seen.

**Assessment of animal experiment reports**

The Monkey Committee reviews the conduct of the experiment based on the Animal Experiment Completion Report submitted at the end of the study and reports the evaluation results to the Director of PRI. Based on the evaluation, the Director may issue instructions/advice to the animal experiment researcher when deemed necessary.

**Planning and adjustment of the breeding and introduction of NHPs and proposal to CHEMR**

In view of future research and educational activities to be performed at PRI, the Monkey Committee selects the species of NHPs to be bred/reared and prepares and reviews an introduction schedule.

**Safety measures for the handling of NHPs and management of the working environment**

The Monkey Committee shall implement safety measures for the handling of NHPs and maintenance of the working environment in cooperation with Inuyama Health Committee.

**Other activities to be performed as required by Kyoto University, PRI or relevant laws and regulations**

### **3. Roles of the Center for Human Evolution Modeling Research**

The administrative duties related to the care, management, breeding and supply of NHPs should basically be performed by faculty members at CHEMR (i.e. animal caretakers) in an integrated manner under the supervision of the Director of CHEMR. CHEMR shall seek to provide a wide range of services, such as clinical veterinary, disease control, animal welfare and research support services. CHEMR shall also cooperate with the Monkey Committee to ensure that laboratory NHPs are kept in a healthy state and properly used for experiments.

The specific duties of CHEMR include the following.

- 1) All types of duties relating to the care, breeding, management and supply of NHPs.
- 2) Maintenance of established model NHPs.
- 3) Maintenance of facilities and equipment.
- 4) Training program provision for animal experiment researchers and animal caretakers under instruction from the Monkey Committee and familiarizing them with relevant laws and guidelines.
- 5) Planning and arrangement of the breeding/introduction of NHPs under instruction from the Monkey Committee.

6) Hazard prevention and health management measures, including maintenance of environment and prevention of disease, at PRI in cooperation with the Monkey Committee, Inuyama Health Committee and other related committees.

7) Improving personnel's awareness of safety management in cooperation with the Monkey Committee, Inuyama Health Committee and other related committees.

8) Other duties required for the smooth management of CHEMR.

#### **4. Principles of the use of homebred NHPs**

Basically, only systematically bred NHPs shall be used for experiments. Wild NHPs, even if they are derived from harmful wildlife control, shall not be accepted in principle to prevent unnecessary wildlife control. When the introduction of wild NHPs is required for particular research purposes, the use of wild NHPs shall be described in the animal experiment protocol and reviewed by the Monkey Committee in accordance with the guidelines established by the Field Research Committee ("Guidelines for Studying Wild Primates or Using Wild Primates in Research"). The replacement of animals or introduction of foreign species should be carefully considered in view of the abundance and breeding status of the relevant species, not to mention in compliance with regulations concerning the import/export of animals, such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and the Law for the Conservation of Endangered Species of Wild Fauna and Flora (Ministry of the Environment, effective in April 1993). The purchase of species established as laboratory animals (i.e. those with an established breeding/supply system) and use of them for education/research purposes shall also be reviewed by the Monkey Committee. The Monkey Committee contacts the Director of CHEMR to determine whether the relevant species can be introduced into the Center.

#### **5. Restrictions on the use of NHPs species not established as laboratory animals for research purposes**

The protocol for a research project using NHPs species not established as laboratory animals (i.e. those with no established breeding/supply system and which cannot be continuously supplied without introducing wild animals) shall be reviewed by the Monkey Committee in accordance with the principles described below. The Committee reports the review results to the Director of PRI. The Director approves or does not approve the protocol based on the Committee's review results.

The principles are as follows.

- i. Research projects involving experimental sacrifice of animals, including euthanasia, or those that cause animals to have irreversible loss of physical functions required for normal living activities, including social activities, are acceptable only if deemed absolutely necessary.
- ii. There must be no alternative methods and must be a reasonable, compelling reason for using the relevant species.

#### **6. Care and management of animals from the standpoints of veterinary medicine and animal welfare**

The care and management of NHPs must be performed based on veterinary knowledge and experience. These management procedures include assessment of whether animal welfare is adequately taken into account such that not only the physical but also behavioral and psychological conditions of the animals can be maintained in a healthy state. Animal welfare refers to the state of animals being able to cope with the surrounding environment. For evaluating the status of animal welfare, it is essential to have appropriate physiological, psychological and behavioral markers, in addition to evaluating the presence or absence of illness, injury or pain. The details of the care/management procedures are separately described in Chapters III and IV. Users are required not only to comply with the Act on Welfare and Management of

Animals and other relevant laws, but also always to conduct themselves with animal welfare in mind.

## **7. Principles of humane handling and authority of the Monkey Committee**

The conduct of experiments using pathogens or toxic chemicals, causing stress and pain to animals, requiring repeated surgical procedures, involving long-term restraint of animals in a monkey chair or other equipment, involving feeding and/or water restriction or using very young animals shall be reviewed with extra care by the Monkey Committee and other relevant committees during assessment of the validity of the animal experiment protocol. Depending on the degree of pain and health status of NHPs, the Monkey Committee may provide the principal investigator and animal experimental researchers with instructions and/or advice, such as recommendations for changes to the experimental schedule and experimental procedures. The details of experimental procedures, including blood sampling and biopsy, are described in Chapters IV and VI. When it has been determined that NHPs are being handled inappropriately, the Monkey Committee shall immediately request the principal investigator to improve the method for handling NHPs. The Committee may report to the Director of PRI when deemed necessary, and if no improvement is seen, the Director may recommend the discontinuation of the experiment or impose such penalties as prohibiting the use of NHPs.

## **8. Training programs and licensing system for PRI personnel involved in the care and use of NHPs**

The care, management and use of NHPs should be performed, in principle, only by PRI personnel. All PRI personnel involved in the care and use of NHPs (i.e. animal experiment researchers and animal caretakers, including permanent or part-time staff, graduate students, research fellows/trainees/students and collaborating research fellows) are required to complete the animal experiment training programs provided by Kyoto University. Because the conduct of experiments using NHPs requires different knowledge and skills depending on the degree of contact with animals and invasiveness of experimental procedures, those who are conducting these experiments are required to acquire in advance the necessary knowledge and skills. Those who wish to use NHPs in an experiment shall thus attend lectures and practical training programs provided by the Monkey Committee and CHEMR to be trained in the proper handling of NHPs and obtain appropriate licenses required according to the degree of contact with animals and invasiveness of experimental procedures. A written pledge and copies of the results of chest X-ray examination and measles antibody testing shall also be submitted. Each license has a specific expiration date and must be renewed through attendance of lectures/practical training programs when expired. Details are given in Chapter VI.

## **9. Health and safety management for PRI personnel involved in the care and use of NHPs and responsibilities of relevant committees**

Many of the diseases affecting NHPs are also observed in humans (for more details about infectious diseases, see Appendix “Infectious Diseases in NHPs” and Chapter II of the Safety and Health Manual issued by PRI). The health and safety management for researchers who perform experiments using NHPs and animal caretakers is important in terms of protection against disease transmission from NHPs. The care and use of NHPs is associated with the risks of bites, scratching and disease transmission from animals (or transmitting disease TO animals) as well as health hazards and contamination caused by washing/disinfection agents. The Monkey Committee and CHEMR shall implement appropriate measures against these risks in cooperation with Inuyama Health Committee. In the event of accidents associated with these risk factors, the person involved in the accident or someone who has witnessed the accident shall report to the Monkey Committee, CHEMR and General Affairs Division without delay (see the Safety and Health Manual). The Monkey Committee and CHEMR shall implement necessary

measures to cope with the accident. Sterilization and disinfection procedures are described in Chapter II.

Those who are expected to be involved in research activities using biological (e.g. pathogens), chemical (e.g. toxic chemicals) or physical (e.g. electromagnetic wave, radioactive materials) hazardous materials or equipment must be trained in the handling of hazardous materials and equipment and, if applicable, obtain necessary qualifications. The overall risk associated with a particular animal experiment protocol involving NHPs is reviewed by the Monkey Committee while risks associated with individual experimental procedures are reviewed by the Disease Control Committee, Biosafety Committee, Chemical Substance Control Committee, Radiation Safety Committee or Inuyama Health Committee, depending on their details. Waste materials arising from animal experiments shall be disposed of in accordance with the guidelines established by Inuyama Health Committee.

## **10. Principles of information disclosure**

The documents pertaining to the care, management and use of NHPs for research and education purposes, as listed below, shall be disclosed as required based on the principle of information disclosure, within a range that does not affect personal privacy or research benefits.

### **Conventions and guidelines**

Guidelines for the care, management and use of NHPs

### **Monkey Committee related**

Monkey Committee meeting minutes

Animal experiment protocol

Laboratory animal use plan

Report of completion of experiment using primates

Euthanasia report

Monkey Committee review results

### **CHEMR related**

CHEMR meeting minutes

Animal care records (e.g. records of the species, number and age of animals used, rearing conditions, dietary components and feeding schedule)

Documents related to the use of animals in experiments (e.g. records of the number of animals used and the details of procedures performed)

Application for delivery of primates to PRI

### **Shared use and collaborative research related**

Application for Shared Use/Collaborative Research

Shared Use/Collaborative Research Report

### **In-house inspection/assessment related**

In-house inspection/assessment results

## **11. Research activities conducted by PRI personnel outside PRI**

Researchers whose primary affiliation is with PRI and who wish to conduct a research project using NHPs at another research institution shall also submit an animal experiment protocol to the Monkey Committee for review and obtain approval from the Director of PRI before

conducting the research project. If the protocol has passed ethical review at another research institution, a copy of the written approval must be submitted to the Monkey Committee. The conduct of the research project must be in accordance with the guidelines specified by PRI.

## Chapter II: Facility design and equipment

A laboratory animal facility that is adequately examined during its planning, designing and building and is appropriately maintained is an essential element of the proper management and use of laboratory animals, and facilitates proper facility management in terms of efficiency, economy, safety and animal welfare. The design and scale of a laboratory animal facility is determined according to research area, the species and number of animals to be housed, positional relationship with non-animal areas and geographical condition.

This chapter describes facility designs and architectural characteristics to be considered when designing and managing animal care facilities for laboratory Nonhuman primates (NHPs) and factors to be considered when repairing existing facilities.

### 1. Specified animals and invasive alien species

According to Article 26 of the Act on Welfare and Management of Animals, specified animals are defined as animals that may harm human life, health or property. A Ministry of the Environment ordinance specifies that these animals should be reared and kept based on approval by the governor of the relevant prefecture for each species. NHPs species falling under this category include: all species of the genus *Alouatta*, *Ateles*, *Brachyteles* and *Lagothrix* of the family Cebidae; all species of the genus *Macaca* (excluding Taiwanese monkeys, cynomolgus monkeys and rhesus monkeys), *Cercocebus*, *Papio*, *Mandrillus*, *Theropithecus*, *Ceropithecus*, *Erythrocebus*, *Colobus*, *Procolobus*, *Pygathrix*, *Rhinopithecus*, *Nasalis* and *Presbytis* of the family Cercopithecidae; all species of the family Hylobatidae; and all species of the genus *Pongo*, *Pan* and *Gorilla* of the family Hominidae. Therefore, almost all species of NHPs, excluding small NHPs such as marmoset, fall under the category of specified animals. Taiwanese monkeys, cynomolgus monkeys and rhesus monkeys are excluded from this category because these species are subject to regulation under different laws (described later).

The application form to be submitted to the prefectural governor for approval for the rearing of these animals shall specify the species and number of specified animals to be housed, purpose of rearing or keeping them, the location, structure and scale of the specified animal housing facility and the methods for rearing or keeping them, and shall be accompanied by the documents specified by a Ministry of the Environment ordinance (PRI is under the jurisdiction of the Owari Office of Aichi Animal Protection and Management Center). The approval expires in 3 years in Aichi Prefecture, as prescribed by the Regulations for the Humane Treatment and Management of Animals (Regulation No. 21 dated March 27, 2001).

The standards for the structure and scale of the specified animal housing facility and methods for rearing or housing specified animals are described in Article 17 of the Ordinance for Enforcement of the Act on Welfare and Management of Animals. Key criteria include that the facility has a structure and strength sufficient for preventing the escape of specified animals and preventing someone not authorized to handle specified animals from easy access to the relevant specified animals, and that the methods for rearing and housing specified animals are not considered inappropriate for preventing harm to human life, health or property. More details of the criteria are described in the Details of Standards for the Structure and Scale of Specified Animal Housing Facilities and Details of Methods for Rearing and Housing Specified Animals. Specified animal housing facilities for NHPs are required to be cage-type, wall-type or mobile housing facility with sufficient robustness, anti-escape features (e.g. small grid space, mesh size and water feeding/draining holes and a retaining wall with sufficient height) and double entrance doors. Requirements regarding the rearing and housing of animals include the following: 1) appropriate individual identification methods must be adopted (e.g. microchips, tattoo); 2) appropriate measures shall be taken to prevent third parties from easy access to specified animals, whether an appropriate sign stating that the relevant specified animals may harm human life, health or property and

shall not be accessed by third parties, is presented in the specified animal housing facility or around it; and 3) any increase or decrease in the number of specified animals shall be reported. See Appendix “Infectious Diseases in NHPs” for more details.

Taiwanese monkeys, cynomolgus monkeys and rhesus monkeys fall under the category of “invasive alien species” (alien species that harm or may harm local ecosystems, human life/health and agriculture, forestry and fisheries industries), as defined by the Invasive Alien Species Law (Ministry of the Environment Law No. 78, 2004). In addition to these 3 species, all species of the genus *Macaca*, excluding Japanese monkeys, are also defined as “uncategorized alien species” and import of these species must be accompanied by the certificate of species name, which proves that they are non-invasive alien species. Currently, squirrel monkeys do not fall under the category of invasive alien species and thus are free from these regulations; however caution shall still be exercised when keeping these NHPs, as they may affect ecosystems by competing with other species etc., and animal caretakers shall be cautious to avoid escape of these NHPs.

The import and rearing of invasive alien species must be approved by the relevant ministers (for the 3 monkey species, approval from the Minister of the Environment alone is sufficient) through submission of the Application for Approval for Rearing of Invasive Alien Species (Form 1-A for new submission or change of approved matters; Form 1-B for renewal of approval) (PRI is under the jurisdiction of the Ministry of the Environment Chubu Office). The standards for the structure and scale of housing facilities for invasive alien species are basically the same as those for specified animals, although approval must be obtained from the Minister of the Environment for invasive alien species, whereas it can be obtained from the prefectural governor for specified animals. In addition, the approval for the rearing of invasive alien species expires in 5 years. Ledgers must be disclosed to government officials from the Ministry of the Environment upon request.

The import of NHPs for research purposes is permitted from only certain areas of world in order to prevent the entrance of Ebola hemorrhagic fever and Marburg disease into Japan, under the Law Concerning the Prevention of Infections and Medical Care for Patients with Infections (hereinafter, referred to as “the Infectious Disease Law”), government ordinances and ministerial ordinances issued by the Ministry of Health, Labour and Welfare (MHLW) and Ministry of Agriculture, Forestry and Fisheries (MAFF). When importing NHPs from those areas, approval by the ministers of MHLW and MAFF are still required as well as a certificate issued by the relevant government agency of the exporting country. Also mandatory are 30-day pre-export quarantine carried out in the exporting country and 30-day quarantine carried out after arriving in Japan (by the MAFF Animal Quarantine Service or designated testing sites). See Appendix “Infectious Diseases in NHPs” for more details.

## 2. Relation of NHPs housing/experiment facilities to other areas

For PRI personnel to stay healthy and comfortable while conducting proper care and management of laboratory animals, it is necessary to separate offices, conference rooms, laboratories (i.e. personnel areas) and uncontaminated laboratories (i.e. non-animal experiment areas) from monkey housing areas (i.e. animal housing facilities) and animal experiment facilities in which live NHPs and possibly contaminated materials are handled (e.g. animal experiment rooms). For this purpose, it is preferable to have animal housing facilities and animal experiment rooms in a different building from personnel areas. It is unacceptable to keep NHPs in a place other than animal housing facilities for more than 48 hours.

To newly establish or abolish animal housing facilities or animal experiment rooms, the Head of CHEMR (for animal housing facilities) or manager of each animal experiment room (for animal experiment rooms) must submit an application beforehand, as prescribed by the Regulations on the Conduct of Animal Experiments in Kyoto University. After receiving the application, the Monkey Committee proceeds with the review processes, including field inspection, and reports the review results to the Director of PRI. The Director approves the establishment

or abolishment of animal housing facilities or animal experimental rooms based on the Monkey Committee's review results.

### 3. Components of NHPs housing/experiment facilities and locations of each room

Laboratory animal facilities basically consist of animal housing rooms, in which laboratory animals are constantly housed, and animal experiment rooms, in which animal experiments are carried out. These components may vary depending on the purpose/scale of facilities and other factors. It is preferable to prepare multiple facilities with same functions so that the handling of contaminated materials that are highly likely to affect the sanitary and health conditions of animal experiment researchers and animal caretakers can be performed separately from the handling of uncontaminated materials that are less likely to have such risks. If it is not feasible, those who have handled contaminated materials are required to eliminate these risks by disinfecting the room or taking other appropriate measures. Facilities should be designed to clearly distinguish between contaminated and uncontaminated areas, with sufficient consideration given to the movement (i.e. traffic lines) of humans, animals and materials within and between these areas. Especially in those facilities in which animals and/or samples infected with zoonotic pathogens, such as herpes B virus, are handled, sufficient care must be taken to avoid infection of workers and non-infected animals as well as contamination of the facilities, and every effort must be made to separate infected animals/samples from non-infected animals/samples in both animal housing facilities and laboratories.

Laboratory animal facilities (i.e. animal housing facilities and animal experiment rooms) intended for NHPs are required to satisfy the following functional requirements.

- (1) Sufficient considerations are given to the care, sanitary and behavioral managements of laboratory NHPs.
  - (2) Designed to allow for the separation of different species as needed or separation of animals as required by individual research projects.
  - (3) Designed to allow for the quarantine and isolation of NHPs.
  - (4) Laboratories are located adjacent or close to monkey housing rooms.
  - (5) Equipped with surgery rooms, intensive care rooms and working rooms for diagnosing, treating and coping with diseases.
  - (6) Equipped with separate autopsy rooms for NHPs infected with zoonotic pathogens, such as B virus, and for those not infected with such pathogens for such purposes as infection prevention. Extra care must be taken in the handling of infected animals and samples derived from these animals.
  - (7) Where biological, physical or chemical hazardous materials are to be used, must be equipped with containment facilities and equipment.
- In relation to this, facilities must be designed with special consideration to safety based on such guidelines as the Safety and Health Manual and Chapter VI of "Guidelines – Building of Laboratory Animal Facilities and Required Equipment Ver. 3 (Edited by the Architectural Institute of Japan)".
- (8) Equipped with space for washing and sterilization of equipment.
  - (9) Equipped with storage space for receipt and storage of feed and equipment.
  - (10) Equipped with workshops for repair of cages and equipment.
  - (11) Equipped with waste storage facilities for the temporary storage of waste to be incinerated or exported out of the institution.
  - (12) Equipped with changing rooms for people entering and exiting the personnel area, etc.

### 4. Building structure and equipment

As mentioned in the beginning of this chapter, Taiwanese monkeys, cynomolgus monkeys and rhesus monkeys are designated as invasive

alien species and their rearing is regulated by the Invasive Alien Species Law. In addition, apes and all species of the families Cercopithecidae and Cebidae not falling under the categories of invasive alien species are designated as specified animals and their rearing is regulated by the Act on Welfare and Management of Animals. Animal housing facilities intended for these species are required to satisfy requirements specified by the relevant laws. Especially, the doubling of safety precautions, such as double-door entry vestibules, is essential.

Building structure and equipment should be carefully designed also from the viewpoint of infection prevention. For general principles, see “Guidelines – Building of Laboratory Animal Facilities and Required Equipment Ver. 3 (Edited by the Architectural Institute of Japan)” and other guidelines.

#### 1) Building materials

Building materials shall be selected that allow for the management of monkey housing facilities in an efficient and sanitary manner. The interior surface should be covered with water-, fire- and chemical-proof materials with seamless design. For painting and finish coating, high-pressure sprays or impact-resistant paints shall be used. In addition, non-toxic paint shall be used for surfaces that can come into direct contact with NHPs.

#### 2) Corridors

Corridors shall be wide enough ( $\geq 180$  cm) for easy movement of animal experiment researchers and animal caretakers and transport of equipment. The floor-wall junction shall be finished so as to allow for easy cleaning. Water supply/drainage pipes and electric wires should preferably be operated through an operation panel or other control equipment located outside the animal housing facilities. Fire alarms, fire hydrants and telephones shall be placed so that they are not damaged during transport of large equipment.

#### 3) Doors to animal housing facilities

Doors to animal housing facilities shall be designed to open inward for personnel's safety. There shall also be an anteroom in each animal housing rooms. To prevent escape of animals, there shall be at least two doors on the traffic line extending from a monkey room to outside the building. Each door should preferably be made of metal and equipped with an observation window for safety confirmation. The observation window may be equipped with a shutter if there is concern that lighting or activities carried out in the corridor may cause stress to animals. For easy transfer of cages and equipment in and out of facilities, doors should preferably have a width of  $\geq 90$  cm for single swing doors or  $\geq 120$  cm for double swing doors and a height of  $\geq 200$  cm. Doors shall provide complete sealing function to prevent insect pests from entering or remaining in the facilities. Doors shall be lockable. Where special security control is required, multiple locks shall be installed on a door.

#### 4) Exterior windows

From the standpoints of animal welfare and environmental enrichment, monkey housing facilities should preferably be equipped with exterior windows. However, exterior windows shall be designed with sufficient consideration given to their effects on room temperature/humidity (e.g. installation of double windows to prevent dew condensation), lighting periods (e.g. installation of shading curtains) and security (e.g. installation of a fence or wire net for prevention of animal escaping). Installation of exterior windows is not mandatory if strict control of room temperature and lighting period is required.

#### 5) Floor

Floors shall be smooth but not slippery, not absorbing water and liquid, and resistant to erosion by acid, solvent, detergent or disinfectant, abrasion and the weight of cages and equipment. The floors shall be composed of a solid timber or floor materials with minimal seams.

Suitable floor materials include epoxies, hard concrete with smooth surface, very hard rubber and glass fibers (FRP). The waterproof membrane shall be inspected on a regular basis and repeatedly applied as needed. If sills are installed at the entrance to a room, they should be designed to allow for convenient passage of equipment.

#### 6) Drainage system

The drainage system is an essential component of monkey housing facilities. The drainage system shall be carefully designed to prevent high humidity in housing rooms, allow the floor to dry within a short period of time and enable immediate drainage. Floors shall be sloped at a minimum pitch of 2.1 cm/m. The diameter of drain pipes should preferably be  $\geq 15$  cm. Drain holes shall be equipped with a large disposer for waste treatment or a trap bucket with holes. Drain pipes leading to the main pipe shall be designed as short as possible or steeply sloped. When drain holes are not in use, traps shall be placed to prevent the backflow of sewage gas or the drain holes shall be sealed with lids. Appropriate measures shall also be taken to prevent escape of NHPs through drain holes.

#### 7) Walls

Walls shall be free of breaches and seamlessly connected with doors, ceilings, floors and corners. Wall surfaces shall be constructed of materials durable to intensive cleaning with detergents or disinfectants and to impact of high water pressure. Appropriate measures shall also be taken to avoid damage to walls caused by transport of mobile equipment.

#### 8) Ceilings

Ceilings shall be humidity-resistant and seamless. Ceilings may be constructed of smooth or pored materials or metallic grids, depending on the species of NHPs used and facility environment. Ceilings are always required to prevent animal escape. Suspended ceilings shall generally be avoided, except for standardized products that are seamless and impervious to water and air. Exposed piping or equipment installed directly on the ceiling shall also be avoided.

#### 9) Air conditioning

Ideally, temperature shall be maintained between 18–29°C and individually adjusted to  $\pm 2^\circ\text{C}$ . Humidity shall be maintained between 40–70%, depending on the species of NHPs used and room temperature, throughout the year (see Chapter III). For this purpose, a temperature/humidity controller will be installed in each room, in principle. Consideration shall also be given to pressure adjustment; for example, quarantine rooms, animal isolation rooms, contaminated equipment storage rooms and infection experiment rooms shall be maintained under negative pressure while rooms accommodating clean equipment shall be maintained under positive pressure. In addition, measures in case of power outage and mechanical failure should preferably be in place, such as duplication of equipment.

#### 10) Ventilation

See Chapter III for the reason that ventilation is required. Animal housing rooms and other areas shall be ventilated separately.

When selecting ventilation/air conditioning systems, sufficient considerations shall be given to their functions, such as durable periods and durable ventilation frequencies. In view of easiness of duplication, function, maintainability and fault resistance, either a central duct system or separate temperature setting system shall be selected. Installation of an air conditioner exposed in an animal room shall be avoided. Those systems that do not provide sufficient ventilation frequencies, such as fans, shall also be avoided. For ventilation/air conditioning systems, it is preferable to perform at least biannual system inspections and performance checks and at least quarterly replacement of filters.

#### 11) Electric power and lighting

In regard to the electrical system, sufficient electric power is required for appropriate lighting and conduct of work. Consideration shall also be given to safety; in particular, water-proof outlets shall be installed in places where water is used for cleaning. Lighting must be

distributed evenly to all parts of a room for easy conduct of management activities, with an amount of light suited for animals (see Chapter III). Lighting apparatuses shall be installed on the ceiling with their surface sealed or covered to prevent insect pests from accumulating inside them. Fluorescent lights are economical and convenient as they can be installed in various ways. To maintain regular lighting cycle, timers shall be installed and regularly inspected. Emergency power sources shall be available to ensure ventilation and lighting in case of power outage.

#### 12) Noise control

Noise control is another important issue to be considered when designing laboratory animal facilities (see Chapter II). Concrete absorbs sound more efficiently than metals or plasters. Sound containment is effectively achieved by removing all windows or using double-paned windows. The installation of sound insulating equipment on the ceiling of an animal room or their use to construct a suspended ceiling shall be avoided for hygienic and pest control reasons. Meanwhile, embedding insulating materials in the ceiling is an effective strategy for noise control. Placing doors in corridors also helps reduce noise transmission from corridors.

#### 13) Equipment washing/disinfection facilities

There should preferably be facilities for washing, disinfection and sterilization of cages and equipment. When designing these facilities, the following must be into consideration.

- a. Located close to monkey housing facilities and equipment storage areas.
- b. Equipped with piping for hot/cold water supply and vapor vent, floor drainage and ventilation and electric power systems.
- c. Noise control.
- d. Equipped with a door wide enough for easy transport of equipment.

#### 14) Facilities for sterile surgical procedures

Facilities required for sterile surgical procedures include operation rooms, operation support areas, operation preparation areas and areas/rooms for intensive care and supportive treatment. The interior surfaces of these areas should be constructed from materials that are resistant to high humidity and easily washable. An operation support area should be available for the storage, washing and sterilization of equipment (see Chapter II). A separate operation preparation area, in which laboratory animals are prepared for operation, shall be located close to the operation room. This area shall be equipped with a sink on which surgical procedures are performed. A changing area shall also be available in which animal experiment researchers and animal caretakers change into surgical gowns. Where explosive anesthetics, such as ether, are to be used, the floor shall be conductive for electricity, exhaust ports shall be explosion proof and electric outlets shall be placed at least 150 cm away from the floor. A system to scavenge used anesthetic gas shall also be available. Additional measures to prevent infection shall be considered, such as keeping operation rooms under positive pressure.

### 5. Outdoor housing facilities

Some of the outdoor housing facilities, including group cages for group housing, sunrooms and loose housing yards, shall be designed based on different policies than those for indoor housing facilities. Therefore, the design of these facilities shall appropriately incorporate different requests from the standpoints of veterinary management, behavioral management, care management and research while complying with relevant laws and regulations. The design manager shall seek to design facilities taking into account these requests and coordinating the opinions of animal experiment researchers, veterinarians and animal caretakers.

When designing outdoor housing facilities, the following shall be taken into account.

#### 1) Purpose of outdoor housing

The potential purpose of outdoor housing includes maintenance of breeding colonies, establishment of a population of NHPs with particular characteristics, maintenance of genetic resources at the levels of subspecies or original troops and undertaking of various types of research. Structural design shall be made with sufficient consideration of these applications.

#### 2) Security considerations

Except for small NHPs such as New World monkeys, most monkey species (e.g. macaques and anthropoids) are designated as either specified animals or invasive alien species. Outdoor housing facilities intended for these laboratory animals shall always be designed with double security measures, whether they are case-type facilities, such as group cages and sunrooms, or wall-type facilities, such as a loose housing yard. Wall-type facilities may be fenced by various types of fences, such as trenches, walls and electric fences. The selection of fence type and construction components shall be determined taking into account the physical capability of the species to be housed and other factors, with the first priority given to the prevention of escape of housed animals outside the loose housing yard and entrance of outsiders into the area. Situating an outdoor housing facility equipped with an air-conditioning system as a resting area during night and holidays is also recommended as a security measure. The structures of wire meshes (e.g. wire size, strength and mesh size) and grids (e.g. grid size, space and material) for outdoor cages and sunrooms shall be designed more carefully than for indoor cages, with consideration given to the doubling of security measures and other aspects. Introduction of systems for detecting animal escaping and invasion is also essential.

#### 3) Structural considerations

The sizes of group cases, sunrooms and loose housing yards shall be determined according to the characteristics of the species to be housed. These characteristics include natural space-use patterns (i.e. arboreal, terrestrial or semi-terrestrial), movement patterns, migration range (or home range) and social structure. On the basis of these characteristics, the optimal area per animal or minimal population shall be determined for each species to allow an appropriate number of animals to be housed at an appropriate density. Outdoor housing facilities should preferably be equipped with such structures as sunshades, wind guards and rain hoods. Measures shall also be taken against the heat during summer, such as water sprinklers and river pools. Adjacent indoor housing facilities required for security management are also useful for protection against the cold temperature during winter.

#### 4) Care management and veterinary management

Outdoor housing facilities shall be designed to allow easy daily care management and health management of animals. Special considerations shall be given to observation, capture of a population or particular animals, measures against contamination of soil, water and structures, waste disposal and water drainage.

#### 5) Behavioral management

When designing outdoor housing facilities, similarly to the case for indoor housing facilities, behavioral management, such as environmental enrichment, shall be taken into account. In addition to preparing appropriate physical environment for animals, such as the introduction of structures that enable use of three-dimensional space depending on the characteristics of species to be housed, as mentioned in 3) above, sufficient considerations shall also be given to the social and feeding environments for animals (see Chapter V).

The following material should be referred to when designing outdoor housing facilities: Code of Practice for the Public Display and Exhibition of Animals (Department of Primary Industries, Victoria, Australia).

## Chapter III: Rearing environment

Maintenance of the rearing environment for laboratory Nonhuman primates (NHPs) is essential for their growth, breeding and health, which ensures collection of valid research data as well as the health and safety of animal experiment researchers and animal caretakers. It is also important in terms of animal welfare. Many factors are to be considered for maintenance of the rearing environment and sufficient knowledge is required regarding specific standards to be met for each factor. To improve the rearing environment, it is important to constantly review conditions and improve them as needed. Animal experiment researchers and animal caretakers must be aware that the rearing environment can be improved by their willingness and ingenuity.

### 1. Cages

#### 1) Requirements for cages

Cages shall be designed and prepared taking into account the behavioral characteristic of laboratory NHPs, with considerations given to the following.

- a. Designed to allow animals to move freely, to keep their natural posture and to rest.
- b. Designed to prevent animal escape.
- c. Designed to permit easy handling of animals.
- d. Designed to allow animals easy access to food and water and to permit easy cleaning and changing of feeding and watering devices.
- e. Designed to permit appropriate ventilation.
- f. Designed to be suitable for the maintenance of normal body temperature, urination, defecation and breeding (as needed).
- g. Designed to permit easy cleaning and prevent water retention.
- h. Designed to prevent injury of animals.
- i. Designed to permit easy monitoring of the condition of animals.
- j. Where cages are adjacent to each other, protection plates or equivalent items shall be placed between cages to prevent interference between animals.

#### 2) Maintenance of environment taking animal welfare into account (see Chapter V)

From the animal welfare viewpoint, providing variety in the rearing environment, such as establishment of a social environment, is an important element in the rearing of NHPs. Although extensive studies have been conducted on environment enrichment (i.e. invention and equipment for improvement) and its effects, it is currently difficult to determine the universally optimal rearing condition. It is thus necessary to make best efforts at this time by actively incorporating various ideas and trials as well as previous experience and knowledge.

#### 3) Housing space

Cage size has a significant impact on captive NHPs. Nevertheless, the optimal calculation method for determining cage size has not been established and cage size is generally determined empirically. The minimum required cage sizes for NHPs of different sizes are summarized in Table 3-1 (NHPs are grouped by size and standard cage sizes are defined for each group; Guide for the Care and Use of Laboratory Animals, NRC, 1996). For studies of metabolism, genetics and behavior, and for breeding, special considerations are required for cage design.

Housing of multiple animals, such as troops and mother-child pairs, requires cages of sufficient size according to the housing space

guideline (Table 3-1). For group housing, animals that get along with each other should be selected to make up each group and cages should have a minimum height of 1.83 m and be equipped with perches and hiding places. Cages for spider monkeys, gibbon, orangutans or chimpanzees should be tall enough such that their each hind foot does not touch the floor when hung from the ceiling with hands stretched.

#### 4) Movement in captivity

NHPs in captivity are more restrained than they would be in the wild. Housing NHPs in cages alters the quality of their movement and limits the amount of their activity. The minimal amount of movement each animal requires shall be determined comprehensively taking into consideration the characteristics, age, rearing history and physical condition of individual animals as well as research details and duration of caging. Measures to encourage physical movement should also be taken; for example, ropes hung from the ceiling, sticks, hard rubber toys and three-dimensional constructs (e.g. perches) are preferred as supportive exercise equipment/plaything. Also see Chapter IV.

Table 3-1: Guideline for determining the housing space of monkey cages

Species	Body weight (kg)	Floor area /animal (m <sup>2</sup> )	Height (cm)
Prosimians, New World monkeys and Old World monkeys *1			
Group 1	- 1	0.15	50.8
Group 2	1 - 3	0.27	76.2
Group 3	3 - 10	0.39	76.2
Group 4	10 - 15	0.54	81.3
Group 5	15 - 25	0.72	91.5
Group 6	25 - 30	0.90	116.2
Group 7	30 - *2	1.35	116.9
Apes			
Group 1	- 20	0.90	139.7
Group 2	20 - 35	1.35	152.4
Group 3	35 - *2	2.25	213.4

\*1: Cages for NHPs of the family Callitrichidae, Cebidae and Cercopithecidae shall be taller than specified by this guideline.

\*2: Additional space shall be given for NHPs heavier than this weight.

## 2. Cage rooms

Rearing environment for NHPs shall be maintained with considerations given to the factors listed below, depending on the characteristics of each species and the history of individual animals. Air conditioning shall be set to maintain cage rooms under negative pressure to prevent the spread of infections and their transmission to humans.

### 1) Microenvironment and macroenvironment

The term microenvironment in animal rearing refers to the environment that comes into direct contact with animals. Practically, this refers to the physical environment inside the cage including specific temperature, humidity and gas components. In contrast, the term macroenvironment refers to the physical environment in the entire cage room. It must be fully understood that these two types of

environment are not the same. The temperature, humidity, gas concentrations (e.g. carbon dioxide and ammonia) and aerosol concentration in each cage are higher than those in the cage room, unless cages are individually ventilated. The difference between the microenvironment and the macroenvironment depends largely on cage design. Based on the understanding that the microenvironment can affect animal care/management and research results, efforts shall be made to prevent environment deterioration and promote improvement of the environment. In addition to regular monitoring of macroenvironment, regular measurement of temperature, humidity and gas concentrations in the microenvironment as well as environment monitoring of microbes and insects shall also be carried out.

## 2) Temperature and humidity

Temperature and humidity are the most important elements of the physical environment surrounding captive NHPs. Both environmental factors affect metabolism and behavior of animals. The temperature range in which oxygen consumption rate is maintained at the lowest level and remains unchanged even when a slight change occurs in temperature, is referred to as the thermoneutral zone. Within this temperature range, animals do not require any physical or chemical mechanism to regulate heat loss/production. When animals are exposed to a temperature outside this range, heat loss via perspiration occurs in higher temperature environments to prevent overheating while heat production occurs in lower temperature environments to prevent decrease in body temperature. In both cases, increased metabolic rate causes physiological stress to animals. Both high temperature and low temperature are physiological stressors to NHPs, and they increase metabolic rate. For optimal growth, comfortableness, responsiveness and adaptability of captive NHPs, temperature shall be set at slightly lower than the thermoneutral zone. Special attention shall be paid to temperature when housing animals that have just been introduced and have not been acclimatized to the new environment. For outside enclosures and group cages, doors that separate the outside and inside shall be movable. Additional considerations may also be required, such as letting animals choose the place according to their preference.

Heat energy comes in and out of a cage through three mechanisms: conduction, radiation and convection. Heat transfer is also induced by moisture (i.e. vaporization heat) and wind. Therefore, the rearing environment shall be established taking these factors into account. For indoor housing, air-conditioning systems are indispensable due to difficulty in introducing indirect temperature control measures, such as planting. Sufficient attention shall therefore be paid to the temperature control capacity of these systems. Table 3-2 summarizes the acceptable range of temperature and humidity for the indoor housing of NHPs. Where an air-circulating air-conditioning system is used, a separate consideration is required for ventilation. Attention shall be paid to excess moisture during the rainy season and to dryness resulting from air heating during winter, which shall be corrected by appropriate dehumidification and humidification, respectively. Special considerations are also required for treatment rooms and housing facilities for younger/aged NHPs.

Table 3-2. Acceptable range for temperature and humidity

Prosimians (e.g. galagos)	24-27°C	40-70%
Small sized New World monkey (e.g. marmosets)	26-27°C	40-70%
Long-tailed macaques	27°C (summer) 23°C (winter)	40-70%
Japanese macaques, rhesus macaques, Formosan macaques, bonnet macaques, stump-tailed macaques, patas monkeys, green monkeys, Papi hamadryas baboon etc.	27°C (summer) 20°C (winter)	40-70%

Chimpanzees, gibbons, spider monkeys, capuchin monkeys, etc.	24-27°C	40-70%
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### 3) Noise

Voice and sound made by NHPs and noise from rearing activities are imperative in animal housing facilities. Consideration shall thus be given to noise control when designing housing facilities. NHPs continuously exposed to a noise level of  $\geq 85$  dB may develop hearing disorder as well as physiological disorders, such as increased blood pressure. When housing NHPs that make especially loud noise, such as chimpanzees and gibbons, special measures shall be taken, such as housing them isolated from other species. Activities that may generate noise should be carried out in a place away from housing areas to minimize stress to laboratory NHPs. Excessive noise can be reduced by training of animal caretakers and workers, attaching cushions to casters and installing dampers to carts. Noise from an outside enclosure and indoor housing facilities will bother other personnel working in and out of animal housing areas, and thus isolating housing areas from personnel areas (i.e. laboratories and offices) is the best way to protect personnel from such noise. Moreover, noise generated from an outside enclosure or indoor housing facilities can be environmental concerns for residents in neighboring areas and should thus be addressed appropriately.

Table 3-3. Acceptable range for environmental conditions other than temperature and humidity

Environmental factor	Acceptable range
Ventilation frequency	10-15 times/hr (100% fresh air)
Airflow velocity	13-18 cm/sec (direct exposure of animals to wind should be avoided)
Lighting	150-300 lux
Noise	No more than 60 dB
Odor	No more than 20 ppm of ammonia

\* Compliant with the NRC guideline (1996)

### 4) Environmental control

Control of environmental factors other than temperature, humidity and noise is also important for the proper care and management of laboratory NHPs. The acceptable range for specific environmental conditions for NHPs is summarized in Table 3-3. The ranges presented in Tables 3-2 and 3-3 are also applicable to outdoor housing. Environmental parameters to be controlled and setpoints shall be determined depending on monkey species, mode of housing and experimental procedures. Lighting shall be controlled by a timer, with 12-14 hours lights on and 12-10 hours off. For nocturnal species, the lighting cycle may be set to simulate a reversed day-night rhythm.

## 3. Food and water

### 1) Feeding

Proper feeding/watering is essential for the normal growth/development of animals and maintenance of health. Special attention should be paid to feeding/watering of pregnant or lactating animals. When food (calorie) and/or water intake needs to be controlled as part of an

experiment, care must be taken to keep it above the minimal acceptable limit (see Chapter VI for more details). The basic metabolic rate per kilogram body weight (per day) for specific monkey species is summarized in Table 3-4. A previous study has demonstrated that feeding with calorie and protein intakes lower than standard values prolongs life expectancy. In addition, some of the dietary components, such as dietary fiber, have been shown to play an important role in digestion process. The amount and quality of feeding shall be constantly re-evaluated based on current research findings.

Table 3-4. Basic metabolic rate for primate species

Primate species and developmental stage	Basic metabolic rate (kcal/kg BW/day)	Reference
Marmoset, 190 g	92	McNab, 1988
Squirrel monkey, infant	300 – 600	Nicolosi et al, 1979
Squirrel monkey, adult	100 - 300	"
Rhesus macaque, 1 month	270	Kerr et al, 1969
Rhesus macaque, 1 year	190	"
Rhesus macaque, juvenile	130	"
Rhesus macaque, adult	40 - 50	Hamilton & Brobeck, 1965
Japanese macaque, adult	48 - 55	Osawa et al., 1980
Baboon, adult (female)	53 - 72	Gilbert & Gilman, 1956
Chimpanzee, juvenile	100 - 120	Hodson et al, 1967
Chimpanzee, adult	50 - 60	"

NHPs shall be fed primarily with commercially available pelleted diets, with fruit and vegetables given as supplementary diets. Table 3-5 summarizes the nutrient compositions of various pelleted diets. Different pelleted diets may need to be selected for different species, depending on their physiological/nutritional requirements. Different supplementary diets may also be given to different monkey species. For example, marmosets and other New World monkeys should be fed with apples, bananas, eggs, mealworms, etc. as supplementary diets. For indoor housing, fluorescent bulbs emitting ultraviolet rays (UV-B) should preferably be used. As UV irradiation may be insufficient even with fluorescent lights, diets supplemented with vitamin D3 shall be given once or twice a week. Food is an important part of environmental enrichment. NHPs shall be fed with a variety of food, not with a consistent type of food. Since NHPs tend to be interested in manipulating things with fingers and mouth, especially when eating food, the installation of feeding devices that require complicated manipulations may also be considered (see Chapter V). CHEMR personnel shall record the types and amount of food given to each monkey and retain the records for health management purposes.

Some animals may develop acute bloat, a condition characterized by gas accumulation in the gastrointestinal tract, abdominal distension and subsequent breathing difficulty. Acute bloat is considered to be caused by changes in food type or rearing environment, abrupt change in feeding time or intake of large amounts of pelleted food and water at a time. In order to prevent the occurrence of such conditions and promote environmental enrichment, multiple feeding is recommended. For group-housed, pregnant or lactating animals, food intake shall be adjusted according to nutritional requirements. Where NHPs are housed in groups or mother-child pairs, it must be ensured that all animals

have access to food. Special attention must be paid to infants.

Table 3-5. Nutritional compositions of pelleted diets for primates

Product name	AS	SPS	CMK-2	TekLad 2050	TekLad 2055	TekLad 2056	5038 Monkey Diet	5040 New World Primate Diet	5048 Certified Monkey Diet
Shape	Biscuit	Short stick	Spherical				Biscuit	Cylindrical	Biscuit
No. of grains per 100 g	≈53	≈640	≈68						
Moisture (g)	7.5	7.4	8.2	10.0	10.0	10.0	10.2	10.1	12.0
Crude protein (g)	27.6	24.1	20.1	20.0	25.6	23.9	15.7	20.1	25.0
Crude fat (g)	9.2	9.9	4.0	5.4	5.9	6.6	5.0	9.5	5.0
Crude fiber* (g)	1.8	2.2	4.2	8.1	3.5	3.1	4.5	3.5	6.5
Crude ash (g)	7.2	5.7	6.3	6.1	6.4	5.0	5.3	5.6	6.0
Soluble nitrogen-free extract (g)	46.7	50.7	57.2	50.4	48.6	51.4	59.3	51.2	47.9
Calorie (g)	380	388	344	280	320	320	405	370	420

AS and SPS are manufactured by Oriental Yeast Co., Ltd. Data are average values in years 1999-2001. CMK-2 and CMS-1M are manufactured by CLEA Japan, Inc. Data are average values in year 2007. TekLad 2050, 2055 and 2056 are manufactured by Harlan Laboratories, Inc. 5038 Monkey Diet, 5040 New World Primate Diet and 5048 Certified Monkey Diet are manufactured by PMI Nutrition International (distributed by Japan SLC, Inc. as the exclusive agent in Japan).

Daily feeding amount varies depending on monkey species and rearing environment and thus shall be determined according to CHEMR's standard operating procedure, which was prepared based on the basic metabolic rates of NHPs presented in Table 3-4 and on previous experience. NHPs do not always eat the entire amount of food given. Feeding amount shall thus be adjusted depending on whether there is leftover food in the feeding device or on the floor.

## 2) Water supply

The Director of CHEMR and CHEMR personnel who are also animal caretakers shall ensure that NHPs have constant access to fresh and uncontaminated water. For this purpose, the following aspects shall be taken into consideration.

- a. NHPs housed in cages in PRI are usually watered by an automatic watering system, which must be inspected on a daily basis to detect possible clogging of a feed-water nozzle. Since NHPs often drink water while chewing pelleted food, sticking of pelleted food to a feed-water nozzle and resulting nozzle malfunctioning can occur. When a large amount of leftover pelleted food is found in a feeding device, the possibility of nozzle clogging and resulting lack of water access shall be considered and the nozzle shall be checked for clogging.
- b. Since water in the piping of the watering system may not be adequately replaced, a drainage valve or equivalent shall be designed at the end of the piping and shall be opened once a week or so to replace water in the piping with fresh water. In the case that the watering

system has no terminal valve, water shall be replaced by holding the nozzle and allowing water to run for a while.

c. If the watering system is equipped with a decompression tank, the tank shall be emptied and refilled with fresh water on a regular basis.

d. Newly introduced animals may need to be trained to use the automatic watering system. NHPs shall be carefully observed for drinking behavior and shall be trained if deemed necessary.

e. Two or more feed-water nozzles shall be available for multiple or group housing.

f. Periodical monitoring of drinking water shall be put in place to detect possible contamination of water with harmful substances or microbes.

### 3) Storage of food, etc.

The following points shall be considered when storing food.

a. Food shall not be placed directly on the floor and shall be stored protected from direct exposure to sunlight, major temperature change during the day and high temperature/humidity. Preferably, food should be stored at  $\leq 15^{\circ}\text{C}$ .

b. Pelleted food that has been sitting for  $>90$  days after manufacture may be deteriorated or contain reduced amounts of nutrients than declared on the label. Therefore, only an amount of food required for keeping animals for a certain period of time shall be stocked. In addition, the date of receipt of food shall be recorded to facilitate scheduled turnover of stocked food, based on the "first-in, first-out" principle.

c. Fruits, vegetables and other perishable food products shall be stored in refrigerators.

d. Bacteriological examination may be required to detect possible contamination of food with pathogenic bacteria.

Given a previous report that chemical pollutants were detected in laboratory animal food, periodic analysis of food contents shall be conducted depending on the research objectives.

e. Food storage areas and corridors in front of animal rooms used for temporary food storage shall be maintained clean, as food powders and fragments fallen on the floor and accumulated at corners may attract cockroaches and rats. Pelleted food that has been opened and exposed to hot and humid atmosphere may develop molds in a few days. Sufficient care shall therefore be taken during use as well as storage of food. Caution shall also be exercised so that food and powders do not accumulate at the bottom of a polyethylene bag or other containers used for temporary storage. Refill of a food container with food remaining at the bottom of the container shall be avoided.

f. Fruits and vegetables received in an unwashed state shall be thoroughly washed with water to remove pesticides and dirt.

g. Used feeding devices, such as buckets and bowls, shall be washed with water in a washing tank in a cooking room and then dried.

## 4. Other

### 1) Individual identification and recording

Laboratory NHPs shall be identified by tattoos, acrylic name plates and/or identification chips. CHEMR must prepare, fill in and retain an animal registry that records basic information for all animals housed in the PRI. At the completion of a monkey experiment, the principal investigator should report the details and results of the experiment to the Monkey Committee and CHEMR without delay (see the "Laboratory Animal Use Report" and "Animal Experiment Completion Report"; for animal experimental procedures, see Chapter VI). The methods for terminal treatment of animals, such as euthanasia, should also be reported to the Monkey Committee and CHEMR (via the "Euthanasia Report"). CHEMR shall record the details of the report in the animal registry and other related documents, and retain the

records. These records must be disclosed as required.

## 2) Management in case of emergency, power outage or holidays

Actions to be taken in case of emergency, as well as during holidays, shall be determined in advance and all personnel should be familiarized with the emergency call network. The Notices of emergency call network shall be positioned in corridors in front of laboratories and cage rooms to facilitate personnel's preparedness for an emergency response. Drills shall be carried out if deemed necessary. All personnel shall read through the Safety and Health Manual carefully and familiarize themselves with emergency procedures.

a. Every effort shall be made to prevent escape of laboratory NHPs. Measures to be taken in case of animal escaping shall be in place to prevent accidents in such cases.

b. Measures to be taken in case of emergency events, such as earthquake and fire, shall be established. In the event of an unusual disaster, these measures shall be implemented to ensure immediate response for the protection of NHPs and prevention of animal escape and accidents (for specific roles and information about the emergency call network, see the "Safety and Health Manual").

c. Measures in case of power outage (including gas supply disruption)

Scheduled power outage for construction or inspection of the electrical system shall be announced in advance and blackout time shall be confirmed. Appropriate precautions shall be taken for the shutdown of freezers, air-conditioning systems and pumps for sewage treatment tanks and water receiving tanks. Drinking water shall also be stocked if water outage is expected.

After resumption of power supply, confirm that there is nothing wrong with the NHPs. For animal rooms in which the lighting cycle is controlled by a timer, the timer setting shall be corrected. During water outage, the valve located at the end of the piping shall be opened since air may enter the piping of the automatic watering system. Alternatively, hold the terminal nozzle for a while to remove air. Make sure that freezers and air-conditioning systems have reverted to normal conditions and are operating properly. Safety confirmation is also required at disruption and resumption of gas supply.

Shutdown of air-conditioning systems due to power outage has significant impacts on NHPs and thus should be detected immediately. Once deemed to have occurred, necessary measures shall be implemented for restoration of the system. Preferably, independent electric generators shall be in place in the case of power outage. In addition, measures to minimize impacts of power outage during the night and holidays should preferably be in place, such as duplication of air-conditioning systems. Guidelines for emergency procedures, such as the operation of independent electric generators, and measures to be taken after restoration of power supply shall be in place and all personnel shall be familiarized with them.

d. Feeding, watering and observation of NHPs and cage inspection for prevention of animal escaping and injury shall be done on holidays as well as regular working days. Any personnel who has found an ill animal or any abnormality in animals shall notify CHEMR and seek veterinary care when deemed necessary.

## 3) Cleaning

Monkey housing areas and experimental rooms in which NHPs are handled shall always be kept clean. Cages in animal rooms and experimental boxes and other items located in experimental rooms shall be cleaned of animal waste and leftover food on a daily basis. Dirt on a washable surface shall be washed off with water and then disinfected. Dirt on a non-washable surface shall be disinfected and wiped off with a cloth or equivalent. For disinfection, alcohol or hypochlorite disinfectants (e.g. Purelox, Antec Virkon S) shall be used. Bedding, such as straws and wood chips, shall be changed 2–3 times a week. Bedding left unchanged for a long time is unhygienic and produces unpleasant odors. Personnel perform cleaning should wear specified clothes and protective equipment, such as a mask, apron, working cloth,

working pants, cap, gloves and face shield. After the completion of cleaning, these items shall be disinfected and sterilized in a specified manner and then washed. Disposable items shall be disposed of. CHEMR is responsible for the washing and management of these items. Disinfection and sterilization should be carried out in accordance with the Disinfection/Sterilization Manual based on the Infectious Disease Law (MHLW, dated January 2004).

The features of commonly used disinfectants are summarized in Table 3-6.

Table 3-6. Features of disinfectants and precautions in their use in animal housing facilities (modified from Kagiya N. et al. Q&A on Diseases and Health of Laboratory Animals. Sheichi Shoin, Tokyo, 1985) Terms in parentheses are trade names.

	Usual concentration	Virus	Bacterial	Acid -fast bacilli	Gram-positive bacteria	Gram-negative bacteria	Fungus	Features/ Precautions
Ethyl alcohol	70-90%	△	×	○	○	○	○	Irritative
Formaldehyde fumigation	15-20 ml/m <sup>3</sup>	○	○	○	○	○	○	Irritative, corrosive, protein permeab Specified chemical substance
Saponated cresol solution	3-5%	×	×	○	○	○	○	Effective in presence of organic substances, corrosive Skin irritation
Surfactants (e.g. Osvan, Hyamine, Welpas)	0.05-0.1%	△	△	×	○	△	○	Ineffective if used with soap Non-irritative
Sodium hypochlorite (e.g. Virkon, Hyporite, Purelox)	100-200 ppm	○	○	○	○	○	○	Reduced effect in presence of organic substances
Iodine (e.g. Isodine, Popiyodon, Prepodyne)	50-100 ppm	○	○	○	○	○	○	Metallic corrosion, equipment Coloration Toxic iodine vapor
Chlorhexidine (e.g. Hibitane, Allcut)	0.1-0.5%	△	△	△	○	○	△	Minimal toxic, but causes conjunctivitis if contact with the eyes
Glutaraldehyde (e.g. Cidex)		○	○	○	○	○	○	Very toxic Use a lidded container Do not inhale vapor

Table 3-7. Animal care equipment disinfection/sterilization procedures

Equipment type	Disinfection/sterilization procedure
Feeding devices Capturing net Cleaning tools	Spray sodium hypochlorite solution (500x concentrated Virkon solution) or 0.1% iodine disinfectant or 3% solution (effective for tubercle bacilli and parasites) over the entire surface and wash off with water after a while. Repeat this procedure twice, if possible.
Individual cages Carrying cages Primate chairs	Spray sodium hypochlorite solution (500x concentrated Virkon solution) or 0.1% iodine disinfectant or 3% solution (effective for tubercle bacilli and parasites) over the entire surface and wash off with water after a while. Repeat this procedure twice, if possible.
Carts	Spray sodium hypochlorite solution (500x concentrated Virkon solution) or 0.1% iodine disinfectant or 3% solution (effective for tubercle bacilli and parasites) over the entire surface and wash off with water after a while. Repeat this procedure twice, if possible.
Working clothes Caps	Autoclave (2 atmospheric pressures, 120°C, 20 min) or immerse well in sodium hypochlorite solution (100x concentrated Virkon solution) and then wash as usual.
Masks	Use disposable masks. Used masks shall be disposed of.

Rubber/vinyl apron and rubber boots	Immerse well in 100x concentrated Virkon solution ( $\geq 12$ h) and then wash as usual.
Rubber gloves	Basically use disposable gloves. Non-disposable gloves, such as safety gloves, shall be immersed well in 100x concentrated Virkon solution ( $\geq 12$ h) and then washed as usual.

Carrying cages, primate chairs and carts shall be disinfected and washed as described in Table 3-7 after use. Tools such as rail nets should also be disinfected/washed in the same manner.

**Disinfection of fingers**

All personnel who handle NHPs must wear rubber gloves or equivalent. After handling of NHPs, fingers shall be chemically disinfected. Chemical agents with a relatively low skin irritating property include quaternary ammonium salts (surfactants), chlorhexidine and iodine disinfectants. Fingers shall then be washed off. After washing, a drier or clean clothes or paper towels shall be used to dry hands. For more details, see the Safety and Health Manual.

The animal care equipment disinfection/sterilization procedures are described in Table 3-7.

4) Disposal of waste

Solid and liquid waste or unwanted substances arising from monkey housing/experiment areas, including garbage, sludge, urine/feces, waste oil, waste acid, waste alkaline and animal carcasses, are subject to the provisions of the Wastes Disposal and Public Cleansing Act. These biohazardous waste materials (i.e. waste materials possibly containing zoonotic agents) shall be disinfected or autoclaved and then immediately disposed of with care taken not to spread infectious disease or cause environmental pollution, according to the procedures enforced by Inuyama Health Committee (i.e. the Garbage Disposal Guidelines) (also see the Safety and Health Manual).

a. Disposal of carcasses: Carcasses of NHPs, except those that have not been autopsied or have been treated with perfusion fixation, and burnable waste contaminated with massive blood shall be sterilized by autoclaving and placed in a black plastic bag. The bag shall be tied at the mouth, with its surface disinfected, and stored in a designated refrigerator located in a storage room (located in the north of Pilotis) in PRI until disposed of by a contract waste disposal service. Carcasses treated with perfusion fixation shall not be autoclaved. These carcasses shall be disinfected by spraying an appropriate disinfectant over the surface, placed in a black plastic bag and disposed of in the same manner. Also see the guidelines established by the Biosafety Committee.

b. Disposal of solid waste materials arising from animal rooms: Feces and leftover food accumulated in the waste collection mesh basket should be placed in a waterproof bag, such as a plastic bag and used food container, to prevent leakage of water and dissipation of waste substances during carriage and disposed of appropriately.

c. Sewage treatment: A sewage treatment tank shall be in place for sewage treatment. Feces and leftover food shall be removed with a mesh basket and disposed of as described in procedure b. When having a contract waste disposal service undertake the disposal of these materials, they must be given adequate directions and instructions. Sewage treatment shall be undertaken in accordance with the Water Quality Pollution Control Act, which mandates periodic testing of drained water and compliance with the water emission standard. Formaldehyde and paraformaldehyde used for perfusion fixation and other applications shall be collected in a tank and not be disposed of as sewage. The collected liquid shall be disposed of according to the chemical disposal procedure.

d. Used metallic, glass and plastic equipment and supplies (e.g. syringes, injection needles, blades, glassware, plastic ware) shall be autoclaved and temporarily stored in a sealed lidded container specified by Inuyama Health Committee (i.e. Medipail) by individual users. The Medipail containers shall be disposed of periodically by a contract waste disposal service.

Other types of waste materials shall be autoclaved, placed in a dedicated cardboard container and stored in a storage room designated by PRI (located in the north of Pilotis) at room temperature until disposed of by a contract waste disposal service.

e. Collected waste shall be periodically or frequently carried out of the institution. When storing waste temporarily, care must be taken to prevent the generation of unpleasant odors and emergence/entrance of pests, such as flies, cockroaches and rodents, in the storage place. These pests shall be eliminated as soon as they are detected. The source of the emergence/entrance of these pests shall be eliminated, such as closing the route of entrance, and pests shall be eliminated by pesticides or rodenticides. These chemicals shall be used after confirmation of the absence of adverse effects on the environment or NHPs.

f. Waste storage places should preferably be designed to be washable and disinfectable.

#### References

1. Guide for the Care and Use of Laboratory Animals (1996). Translated by Kagiya N. and Nomura T., SoftScience, Tokyo, 1997
2. Laboratory Animal Techniques. Edited by Tajima Y., Asakura Publishing Co., Ltd., Tokyo, 1977
3. Descriptions on Standards for Care and Management of Laboratory Animals. Edited by the Study Group on Laboratory Animal Care & Management. Gyosei Corporation, Tokyo, 1980
4. Maejima K. et al. Disinfection and Sterilization for Hygienic Management of Laboratory Animals. SoftScience, Tokyo, 1980
5. Laboratory Animal Handbook. Edited by Nagasawa H., Yokendo Co., Ltd., Tokyo, 1985
6. Wolfensohn S. and Honess P. Handbook of Primate Husbandry and Welfare. Translated by Yoshida H. Showado, Tokyo, 2007
7. Safety and Health Manual. PRI Safety Committee at the Primate Research Institute of Kyoto University, 2007

## **Chapter IV: Veterinary management**

In PRI, all care/management and experimental/research activities involving Nonhuman primates (NHPs) shall be conducted under veterinary supervision by CHEMR faculty and staff members who are qualified veterinarians. Management based on veterinary knowledge and techniques is essential not only for the health management of NHPs but also for the safety of animal experiment researchers and animal caretakers, and plays an important role in obtaining reliable experimental results. These veterinary management activities are performed by CHEMR under instruction from the Monkey Committee and include the following.

- Daily observation of all animals for evaluation of their health status and rearing conditions.
- Prevention, control, diagnosis and treatment of diseases and injury.
- Educating animal experimental researchers and animal caretakers in the proper handling, restraint, sample collection, operation, anesthesia, analgesia, postoperative management and euthanasia of NHPs through training programs, or performing them when necessary.

Any personnel who has noticed any health problems or abnormal behaviors of NHPs shall immediately notify CHEMR veterinarians of the precise information and ask for instructions. Veterinarians who have noticed any problems in the experimental conditions or animal experimental protocol shall notify the Chair of the Monkey Committee, from the standpoints of health management for humans and NHPs and animal welfare. The Chair of the Monkey Committee shall pass on the report to the Monkey Committee members, confirm the reported facts and report to the Director of PRI. The Director provides researchers with advice if deemed necessary. In addition, to facilitate communication between the Monkey Committee and CHEMR regarding the coordination and monitoring of monkey care and use, at least one of the council members affiliated with CHEMR who is a qualified veterinarian shall be included as a member of the Monkey Committee and the Director of CHEMR (or designee) and at least one of the technical staff shall attend each Monkey Committee meeting.

### **1. Preventive medicine**

Disease prevention is the primary objective of veterinary management and various considerations are required to achieve this objective.

#### **1) Precautions for entering/leaving monkey housing facilities and working clothes for handling NHPs**

Since NHPs may have various pathogens that can cause serious disease in humans once transmitted (see Appendix “Infectious Diseases in NHPs”), their handling requires extreme attention.

- a. When entering or leaving animal housing facilities, use only the specified entrance, exit and route.
- b. If deemed necessary, keep a visitor log and mandate measurement of body temperature at the entrance to animal housing facilities as a disease preventive measure.
- c. Animal experiment researchers and animal caretakers shall enter /leave animal housing facilities according to the specified procedures (see Chapter II of the Safety and Health Manual).
- d. When regular procedures cannot be followed for such reasons as equipment carrying into animal housing facilities and construction, sufficient care must be taken and advice shall be given to service persons for the prevention of infection transmission and animal escape (see Chapter II of the Safety and Health Manual).
- e. Personnel who perform animal care/experiment activities shall wear specified working clothes and implement protection measures (against infection and accidents) according to the characteristics of the activity undertaken (see the Safety and Health Manual).

#### **2) Introduction of NHPs**

All NHPs, whether alive or dead, shall be introduced legally (see Chapter I and the “Guidelines for Studying Wild Primates or Using Wild Primates in Research”).

a. Introduction of live NHPs

Anyone who wishes to introduce NHPs into PRI must submit an application to the Monkey Committee within 1 month prior to introduction, in principle. The Monkey Committee consults CHEMR and requests the conduct of quarantine. On the basis of quarantine results, the Committee determines whether the NHPs can be introduced. The introduction of NHPs that have been once moved out of PRI should also meet the specified quarantine requirements, in principle. Animals that have been carried into the infection experiment areas (e.g. P2 experiment/care room) shall not be carried out of the areas alive unless the safety of doing so is confirmed, except when specifically approved by the Director of PRI or CHEMR.

b. Introduction of carcasses or materials derived from live animals

The introduction of carcasses and materials derived from live animals shall be carried out in accordance with separate guidelines.

3) Quarantine and acclimatization

Quarantine at introduction shall basically be carried out in the quarantine room in CHEMR, in accordance with the Monkey Quarantine Guidelines issued by CHEMR. Exceptionally, quarantine duties can be outsourced to an outside organization when approved by the Director of CHEMR.

Acclimatization is a process to allow newly introduced NHPs to become physiologically and nutritionally adapted to a new rearing environment and subsequently become physiologically and behaviorally stable. This facilitates the conduct of reliable animal experiments. Animals that have just been introduced into a new environment may suffer excessive stress from environmental change, which may affect their health and even result in death in severe cases. CHEMR must carefully monitor the conditions of animals that have just been introduced.

4) Separation of animals by species, place of origin and health status

Separating animals in different animal rooms by species and place of origin is recommended for the prevention of interspecies disease transmission and conduct of reliable experiments. For example, NHPs originating from Africa may be infected with monkeypox or simian hemorrhagic fever virus, which may cause clinical symptoms in NHPs from Asia. This can also occur among species from the same geographic region. Squirrel NHPs are often infected latently with *Herpesvirus saimiri*, which may cause malignant lymphocytic leukemia once transmitted to New World monkeys. Thus, NHPs of different species shall basically be housed separately.

## 2. Disease monitoring and control

All NHPs housed in PRI shall be observed on a daily basis by personnel who are trained to identify signs of disease, injury and abnormal behavior. Any personnel who has noticed any abnormalities should immediately notify the principal investigator and CHEMR faculty members and seek instructions from the responsible veterinarian if deemed necessary. Animals found to be ill or injured shall be treated immediately. Animals suspected of having an infectious disease shall be isolated from animal experimental researchers, animal caretakers and healthy animals. When an animal room has been exposed or is suspected to have been exposed to infectious agents, animals housed in the relevant room shall not be transferred to other rooms, but shall be kept in place during the monitoring and control processes.

Since pathogens such as viruses and bacteria can be transmitted via various biological materials, such as tissue and blood, the introduction of cultured cells and other biological materials shall be done with sufficient care. Basically, all materials derived from NHPs shall be handled in

a P2 or P2A-level laboratory, given that NHPs may carry unknown viruses or other pathogens. The same considerations shall be exercised when handling cultured cells and urine/feces derived from NHPs. When these materials are to be carried outside PRI, warning shall be given to the research institution to which the materials are to be sent.

### **3. Surgical procedures and postoperative management**

Surgical procedures must be performed using appropriate anesthetic/analgesic methods, based on preoperative health check-up results and the postoperative management plan. Generally, the conduct of survival surgical procedures involving invasion or exposure of body cavities or causing physical/physiological damage to the animal requires more strictly aseptic techniques. Aseptic surgical procedures shall only be performed using dedicated equipment by trained animal experiment researchers. Where aseptic procedures cannot be performed, operators shall wear sterilized surgical gowns, etc. and use as aseptic techniques as far as possible to prevent infection.

Postoperative management consists of observation of the operated animal until it is completely recovered from anesthesia, administration of replacement fluid, analgesics, anti-inflammatory agents, antibiotics and other agents, and other necessary procedures. Unless expected to affect the conduct of the experiment, a certain recovery period shall be set, during which meticulous monitoring and management shall be performed. Researchers must be well aware of the need for procedures to minimize pain experienced by animals throughout the preoperative, intraoperative and postoperative periods.

### **4. Anesthesia and analgesia**

When a procedure invasive to NHPs is to be performed, appropriate anesthetics, analgesics and sedatives must be selected from scientific and ethical standpoints, depending on the operation time, invasiveness, species, age, general condition, environment in which the procedure is to be performed and other factors. Anesthesia shall only be performed by animal experiment researchers with knowledge and skills in the use of the anesthetics and anesthetic methods to be used. For a lengthy procedure, it is preferable to have a person in charge of anesthesia who is dedicated to the anesthetic management and systemic management of the operated animal. Neither muscle relaxants nor paralytic agents (e.g. succinylcholine and other curarimimetic) are anesthetics. These agents shall not be used alone in surgery, as they have no analgesic effect and do not cause loss of consciousness.

Animals scheduled to be anesthetized shall be fasted from the evening of the day before surgery, in principle, and the operative plan must be designed so that all procedures are completed by 15:00 on the day of surgery at latest. After surgery, the operated animal shall be observed until adequately recovered from anesthesia and not be left unattended while anesthetized. When the need for urgent anesthesia arises, administer preanesthetic medications and check if there is any food in the cheeks of the animal. If there is any food present, it must be removed.

Representative anesthetics and analgesics that have been commonly used in NHPs are summarized in Tables 4-1 and 4-2, respectively, along with their usual doses. Some of these anesthetics (e.g. ketamine), sedatives and analgesics are narcotics or psychotropics and thus shall be managed appropriately in accordance with the Narcotics and Psychotropics Control Act (established March 17, 1953, Act No. 14; revised June 14, 2006, Act No. 69). Several dosing examples are provided below. The types and doses of agents to be used shall be determined taking into account the purpose and nature of the procedure (although the examples below include only ketamine, pentobarbital and isoflurane, which are commonly used in the PRI, other agents may also be used). These dosing examples are applicable only to healthy animals to be anesthetized for research purposes. For aged or diseased animals, further considerations must be given and veterinary advice be sought when deemed

necessary.

Ketamine is commonly used for anesthesia in short and less invasive procedures. When ketamine is to be used alone, anti-cholinergic agents (atropine or glycopyrrolate) shall be administered as premedications to prevent hypersalivation and bradycardia. Ketamine is commonly used with sedatives, such as xylazine and medetomidine, for reduced side effects and better muscular relaxation. When medetomidine is used, its antagonist atipamezole shall be administered at the end of the procedure. Medetomidine cause transient increase in blood pressure (followed by persistent decrease) and thus shall not be used with anti-cholinergic agents. Given its effect on hemodynamic status, medetomidine shall be given at a reduced dose or not be used in aged or debilitated animals. When bradycardia or hypotension induced by medetomidine has caused problems, atipamezole shall be administered immediately. Both ketamine and medetomidine have an analgesic effect, although ketamine is considered to be less effective against visceral pain.

Anesthetics commonly used in lengthy procedures include pentobarbital (a barbiturate), propofol (a GABA agonist) and isoflurane (an inhalation anesthetic). All of these anesthetics have a minimal analgesic effect and shall thus be used with appropriate analgesics if used in invasive procedures. In addition, circulating blood volume shall be maintained via adequate hydration and electrolyte replacement achieved by drip infusion, etc. When an inhalation anesthetic is to be used, special care must be taken to prevent the animal from becoming anoxic. Care shall also be taken to prevent hypothermia and maintain body temperature. If an inadequate anesthetic effect is noted even after the target dose has been reached, check if there are any problems, such as false intubation and leakage from the catheter. It must be remembered that giving additional doses of anesthetics without discretion may result in overdose and even death. This guidance does not provide any specific guideline for the use of propofol due to limited experience of its use. Propofol is characterized by a short duration of action, good controllability and rapid awaking after discontinuation, and should be considered for active use in the future.

In both methods, it must be confirmed, before surgical procedures are started, that the animal is in the stage of surgical anesthesia by the absence of pain response or other approaches. During anesthesia, the animal's physiological parameters, such as respiration, circulation and body temperature, shall be monitored so that anesthetic depth can be adjusted. When an additional dose of an anesthetic is to be given, care shall be taken so that the total dose remains acceptable. The data for physiological parameters shall be retained along with those for time of administration and the amount of the anesthetics given. After anesthesia, the animal experiment researcher shall monitor the animal's condition until it recovers from anesthesia. Since it may take several hours until the anesthetic is completely eliminated from the animal's body and apparent recovery from anesthesia does not necessarily guarantee the absence of residual anesthetic effect on the gastrointestinal system, watering and feeding of the postoperative animal shall be done by the animal experimental researcher after careful evaluation of consciousness.

#### **A. Combination anesthesia with ketamine hydrochloride and pentobarbital sodium**

- 1) Anesthesia is induced by intramuscular injection of ketamine with or without medetomidine or xylazine into the femoral or brachial muscle of the animal.
- 2) Once the animal has been anesthetized and immobilized, transfer the animal to the operation table and intravenously infuse pentobarbital sodium at 20–25 mg/kg. It should be remembered that rapid infusion of pentobarbital sodium may kill the animal. After about half of the total dose has been infused, check the respiratory rate and other physiological parameters and continue infusion at a lower rate thereafter. Since the anesthetic effect may vary depending on animals, anesthetic depth should be evaluated by pain response or other approaches.

#### **B. Combination anesthesia with ketamine hydrochloride and inhalation anesthetics**

- 1) Similarly to the anesthetic method A, anesthesia is induced by intramuscular injection of ketamine with or without medetomidine or

xylazine into the femoral or brachial muscle of the animal.

2) Once the animal has been anesthetized and immobilized, transfer the animal to the operation table and administer an inhalation anesthetic (with isoflurane, 2–4% for induction and 1–2% for maintenance) through a mask or tracheal tube. During inhalation anesthesia, the vaporizer setting should be maintained carefully by a person familiar with its operation. For the safety of the animal experiment researcher, the concentration of inhalation anesthetics in the surrounding environment should be controlled at <2 ppm.

NHPs tend to conduct themselves trying not to display signs of pain as much as possible, which makes pain evaluation very difficult. Thus, personnel should not determine that there is no pain based only on the absence of visible signs of pain. NHPs are genetically and physiologically close to humans and thus are believed to have pain transmission pathways similar to those of humans. Unless contrary evidence is available, it is reasonable to assume that procedures that are painful for humans are also painful for NHPs. Although pain is an important component of the biological defense mechanism, excessive pain causes stress and delays postoperative recovery. Since pain is far more harmful than beneficial, the use of appropriate analgesics is required.

Pain sensation is perceived through reception of a noxious stimulus, transmission by a nerve, modulation in the spinal dorsal horn and projection to the sensory area of the cerebral cortex. Under general anesthesia with pentobarbital or isoflurane, such stimuli are not perceived as pain sensation in the cerebral cortex, as long as anesthetic depth is maintained at an appropriate level. However, since noxious stimuli are transmitted and projected to the spinal dorsal horn normally, repeated input of stimuli can increase the sensitivity of the synapses in the spinal dorsal horn, resulting in increased pain sensation after recovery from anesthesia. This is why the proper use of analgesics is required even under general anesthesia. The starting of pain management in the preoperative period is believed to reduce reception, transmission and hypersensitization and contribute to better postoperative outcomes (i.e. preemptive analgesia).

#### References

Anesthesia and Analgesia in Laboratory Animals, Second Edition, 2008

Editors: Richard E. Fish, Marilyn J. Brown, Peggy J. Danneman, Alicia Z. Karas, pp 335-363, Table 12-2

Zoo Animal & Wildlife Immobilization and Anesthesia, 2007

Editors: Gary West, Darryl Heard, & Nigel Caulkett, pp 367-394

Table 4-1 Representative anesthetics that can be used in NHPs

	Drug name	Macacas	Common marmosets	Chimpanzees	Note
Anti-cholinergics	Atropine	0.02-0.05 mg/kg IM	0.04 mg/kg SC or IM	0.02-0.04 mg/kg IM, IV, SC	Not to be coadministered with medetomidine
Dissociative anesthetic	Ketamine (Ketalar)	5-10 mg/kg IM	15-20 mg/kg IM	5-10 mg/kg IM	Wide safety range, but uncontrollable once administered Minimal effect on the respiratory system May cause increased blood pressure and increased rate Virtually no muscle relaxation Strong analgesic effect
Combination with ketamine	Ketamine (K) Medetomidine (Domitor) (M)	K 2.5 mg/kg + M 0.1 mg/kg or K 5 mg/kg + M 0.05 mg/kg mixed and given IM		K 3.5 mg/kg M 0.035 mg/kg mixed and given IM	Antagonist: atipamezole (Antisedan) (0.25-0.5 mg/kg IM) Peripheral vasoconstriction by medetomidine Transient increase, followed by decrease, in blood pressure May cause decreased heart rate, occasionally arrhythmia
	Ketamine (K) + Xylazine (X)	K 7mg /kg X 0.6 mg/kg mixed and given IM			Ketamine + medetomidine is better Antagonist: atipamezole (or yohimbine at 1 mg/kg, IM)
Intravenous anesthetics	Pentobarbital (Nembutal, Somnopentyl)	20-25 mg/kg IV			Given IV after immobilization is achieved by ketamine with or without medetomidine Uncontrollable once administered May cause respiratory depression May cause decreased blood pressure Muscle relaxation
Inhalation anesthetics	Isoflurane (Isoflu)	1.5-2% (1 MAC=1.28%)	1.0-3.0%	1.0-3.0%	Rapid introduction/recovery and good controllability May cause respiratory depression May cause decreased blood pressure and increased heart rate

	Sevoflurane	2-4% (1 MAC=2%)		2-4%	Faster introduction/recovery than isoflurane Good controllability May cause respiratory depression May cause decreased blood pressure and increased heart rate Nearly odorless
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IM: intramuscularly, IV: intravenously, SC: subcutaneously

Table 4-2 Representative analgesics that can be used in macacas

	Agent name	Dose	Duration of action	Administration route	Note
Nonsteroidal antiinflammatory drugs	Acetaminophen	10-15 mg/kg	6 hrs	PO	May cause gastrointestinal disorder
	Aspirin	12-15 mg/kg	6 hrs	PO	May cause gastrointestinal disorder
	Carprofen (Rimadyl)	2-4 mg/kg	12-24 hrs	PO SC	May cause gastrointestinal and hepatic disorders
Opioids	Buprenorphine (Lepetan)	0.01 mg/kg	8-12 hrs	IM IV	Minimal respiratory depression Acts on the $\mu$ -receptor
	Butorphanol (Stadol, Betorphanol)	0.1-0.2 mg/kg	3-4 hrs	IM	Possible respiratory depression Has an antagonistic effect on the $\mu$ -receptor and acts on the $\kappa$ receptor (reverse buprenorphine and morphine)
	Morphine	1-2 mg/kg	4 hrs	IM IV SC	Respiration and gastrointestinal transit depression Acts on the $\mu$ -receptor

Cited and translated from Joanne Paul-Murphy, 2001 Primate Analgesia, Proceedings of TNAVC 2001

## 5. Euthanasia

The slaughter disposition of laboratory NHPs shall be conducted by euthanasia in accordance with the Guidelines for Slaughter Disposition Method of Animals (Ministry of the Environment, 2006). Euthanasia refers to the practice of putting an animal to death (i.e. cardiac death) as rapidly as possible with minimal pain or distress. During the euthanasia process, specified procedures must be performed courteously and solemnly with respect for the dignity of life. Therefore, euthanasia shall be conducted only by licensed animal experiment researchers who have completed the lectures and practical training programs provided by CHEMR and acquired required knowledge and skills (see Chapter VI). Once having acquired such knowledge and skills, these personnel shall keep trying to improve the quality and rapidity of procedures resulting in death so that pain and distress experience by animals can be reduced and to collect information about new anesthetics and analgesics.

The following points shall be taken into consideration when conducting euthanasia.

- Do not make the animal anxious unnecessarily prior to the initiation of procedures. Care shall also be taken that other animals do not become anxious.

- The procedure shall be performed without causing pain or distress to the animal.

- The time from the initiation of procedures to loss of consciousness shall be made as short as possible.

- The death of the animal shall always be confirmed. Not only respiratory arrest but also cardiac arrest must be confirmed.

- Consideration shall always be paid to surrounding people when carrying out euthanasia procedures.

1) When euthanasia is considered acceptable.

Euthanasia is required in the following 4 cases.

(1) As no improvement in health status is expected due to illness or injury, and severe pain and distress are expected to continue until death, euthanasia is considered the only way to save the animal from the pain and distress.

(2) The animal was found to be or is suspected to be infected with a highly dangerous pathogen.

(3) The animal is unlikely to recover even after the completion of an invasive experiment and is obviously forced to live the rest of life under severe stress.

(4) There is a reasonable research basis for the need for slaughter disposition and there is no alternative method at the moment (i.e. experimental sacrifice).

Euthanasia may be considered in the context of laboratory animal production for the planned adjustment of animal population; however, this should be done after consideration of possible alternatives. For cases (1) to (3), judgment is made based on the concept of humane endpoint and approval by the monkey committee based on a veterinarian's advice is required. For case (4), i.e. experimental sacrifice, approval by the monkey committee is required.

2) Methods of euthanasia

Euthanasia of NHPs should be achieved by overdose of a barbiturate anesthetic (at  $\geq 100$  mg/kg for pentobarbital sodium) or exsanguination under deep anesthesia. Although any barbiturates can be used as long as given IV, pentobarbital sodium is most widely used. Intraperitoneal injection is also acceptable in small animals for which intravenous infusion is difficult to perform. Alternatively, overdose of KCl (intravenously or intracardially) under deep anesthesia can also be used to induce respiratory and cardiac arrest. Sample collection, if

required, shall be performed after confirmation of respiratory arrest, cardiac arrest and pupillary dilatation after sufficient amount of time has elapsed.

Where perfusion is to be performed, the following procedures must be followed, in principle. These procedures are basically the same as those for surgical anesthesia and are followed by the procedures for exsanguination/perfusion. The following examples also describe the use of ketamine, pentobarbital sodium and isoflurane, as they are most commonly used. Other appropriate agents can also be used for euthanasia (see Tables 4-1 and 4-2).

#### **A. Combination anesthesia with ketamine hydrochloride and pentobarbital sodium**

- 1) Anesthesia is induced by intramuscular injection of ketamine with or without medetomidine or xylazine into the femoral or brachial muscle of the animal.
- 2) Once the animal has been anesthetized and immobilized, transfer the animal to the autopsy table and intravenously infuse pentobarbital sodium at a dose of  $\geq 25$  mg/kg. Intraperitoneal injection is only acceptable for small animals for which intravenous infusion is difficult to perform.
- 3) Confirm that the animal is deeply anesthetized by the absence of pain response or other approaches.
- 4) Transect both jugular veins to exsanguinate the animal. When deemed necessary, open the thoracic cage to allow breathing to stop or exsanguinate the heart. Then, perfuse the carcass with saline or fixative solution depending on the research purpose.
- 5) After a sufficient amount of time has elapsed, confirm respiratory arrest, cardiac arrest and pupillary dilatation.

#### **B. Combination anesthesia with ketamine hydrochloride and inhalation anesthetics**

- 1) Similarly to the anesthetic method A, anesthesia is induced by intramuscular injection of ketamine with or without medetomidine or xylazine into the femoral or brachial muscle of the animal.
- 2) Once the animal has been anesthetized and immobilized, transfer the animal to the operation table and administer an inhalation anesthetic (with isoflurane, 2–4% for induction) through a mask or tracheal tube. For small NHPs, a box can be used to induce anesthesia with an inhalation anesthetic, in lieu of step 1) above.
- 3) Perform step 3) and subsequent steps as in case A.

Euthanasia using this method is not recommended for neonates or infants aged 16 weeks or less, as they are resistant to hypoxia. The most recommended anesthetic for these animals is halothane, followed in order by enflurane and isoflurane. During inhalation anesthesia, the vaporizer setting shall be maintained carefully by a person familiar with its operation. For the safety of the animal experiment researcher, the concentration of inhalation anesthetics in the surrounding environment shall be controlled at  $< 2$  ppm. It should be remembered that in case of displacement of a mask or other accidents, the animal may become conscious even after it has been deeply anesthetized with isoflurane, due to the rapid introduction/recovery property of isoflurane.

Sample collection and perfusion shall be carried out after confirmation of the animal being deeply anesthetized. In consideration of psychological effect on people, euthanasia procedures shall be performed in a place only accessible by the person who performs the procedures and other people involved, with additional considerations adopted such as carrying a carcass in an opaque bag to ensure the invisibility of the carcass.

- 3) Principles in conducting experimental sacrifice, sample collection and reporting of the completion of euthanasia

Deeply anesthetized animals should experience no pain or distress, but should still be treated as living entities. In regard to the

experimental procedures performed on laboratory NHPs, only those considered indispensable for the purpose of the experiment shall be performed based on sufficient animal welfare considerations. Sample collection shall be performed only from animals that have been euthanized according to the above steps and confirmed to be dead. Exceptionally, when required for the purpose of the research, sample collection from a live animal is permissible only after review by the Monkey Committee and approved by the Director (see Chapter VI). In this case, the animal shall be euthanized immediately after samples have been collected according to the specified steps. Ethical judgment of the appropriateness of such experiments shall be made with special care. These procedures shall not be performed unless they are imperative for the purpose of the research. At present, even if an invasive procedure is deemed imperative, it is preferable to actively develop alternative methods.

The person who has performed euthanasia must submit a completed Euthanasia Report to the Monkey Committee without delay.

## 6. Disposal of carcasses

### 1) Euthanasia

For euthanized animals, sample collection and other procedures shall be carried out based on the principle of efficient use of carcasses (i.e. multiple utilization). Procedures involving invasion into and exposure of body cavities may allow viruses and other pathogens to exit the carcass. When these procedures are to be performed, appropriate precautions shall be taken in case of increased risk of infection. In this light, after the procedures have been completed, the used instruments, autopsy table and floor shall be washed off and disinfected with appropriate chemicals and carcasses, organs and other waste shall be sterilized and then disposed of appropriately (for more information, see the “Disposal of carcasses” section of Chapter III). These procedures must be performed by and under responsibility of the person who has performed euthanasia. Working clothes and other protective equipment used during these procedures shall be disposed of using the specified methods, with sufficient care taken to avoid infection and other risks. When carrying collected samples to a laboratory, the samples shall be treated as infectious materials and must be carried in a dedicated container with sufficient care and consideration given so that the leakage of blood and body fluid does not occur and the samples are invisible to third parties. Sufficient consideration shall also be given to the traffic line during carriage, including avoiding the use of corridors and elevators in non-restricted areas. When blood or other fluid has attached to the floor or wall, it must be disinfected and washed off immediately.

### 2) Animals that died from causes other than euthanasia

Any personnel who has found an animal to be dead from causes other than euthanasia shall immediately notify a CHEMR veterinarian and ask for instructions if pathological autopsy is deemed necessary for infection control purposes. The veterinarian shall immediately perform pathological autopsy to identify the cause of death, with the possibility of zoonosis in mind. When deemed necessary, appropriate infection control measures shall be implemented. Carcasses shall be disposed of in a safe and reliable manner (for more information, see the “Disposal of carcasses” section of Chapter III). The animal experiment researcher who has used the animal shall submit the Accident Report to the Monkey Committee and CHEMR without delay. The Accident Report shall specify the conditions of the animal before, after and at the time of the accident, autopsy results, possible causes of the accident and preventive measures to be taken. For other procedures, see the Safety and Health Manual.

## **Chapter V: Considerations of the behavior and psychological condition of NHPs**

The recent trend in animal welfare focuses on considerations from the viewpoint of animals, namely, subjective recognition of the animal. The fact that animals are not automatically responding to the environment and that they are able to indicate their intention via behavior has gradually been accepted as scientific knowledge. Thus, when rearing and handling animals, it is essential to give consideration to the behavior and psychological condition of animals.

### **1. Considerations of the behavior and psychological condition**

Improvement of the rearing environment is important in that it leads to better experimental data. In this light, it is necessary to maintain laboratory animals not only “physically” but also “psychologically” and “behaviorally” healthy. Consideration shall be given such that laboratory Nonhuman primates (NHPs) can perform their species-specific behavioral pattern (i.e. types and time allocation) at the maximum level according to their physiological, ecological and behavioral characteristics, within the range that does not interfere with the objectives and methods of the research. The rearing environment and procedures shall also be adjusted so that animals do not exhibit abnormal behaviors related to stress caused by the rearing environment and research use. For this purpose, at least the following five requirements shall be satisfied.

(1) Animals shall be housed in a residential environment that permits species-specific postural maintenance and movement patterns.

(2) Animals shall be given the opportunity to exhibit such behaviors as eating, searching and manipulation of objects, depending on species, age, sex and individual conditions.

(3) Maintenance of species-specific social interactions.

(4) Maintenance of an appropriate relationship with humans.

(5) Reduction of pain and distress.

To achieve these goals, it is necessary to respond flexibly to the actual situation and actively seek to improve the rearing environment. It is also important from the animal welfare standpoint to make maximal efforts to improve the environment for each animal while considering the purpose of the research and animal care/management and realistic possibilities.

### **2. Environmental enrichment**

Considerations of the behavior and psychological condition of NHPs should include the active promotion of “environmental enrichment”, a means of adding various functions to the rearing environment, in addition to satisfying the requirements for cage floor area, cage volume, etc. described in Chapter III. Overall environmental enrichment is required in various aspects of the environment where NHPs live (i.e. physical, social, eating and sensory environments). Improvement of these aspects of the environment will contribute to reduced stress and abnormal behaviors and increased exercise opportunity, and help with the development and maintenance of various physical and social functions. If not all aspects can be improved adequately due to experimental and environmental limitations, improvement of selected aspects may be able to compensate for the loss of other aspects. For example, if there is limited social environment, efforts must be made to enrich the eating and physical environments. However, conducting environmental enrichment without sufficient consideration may result in inadequate effect or even increased injury or conditions related to physiological stress, such as metabolic disorder. Thus, for effective environmental enrichment, it is necessary to enrich various aspects of environment concurrently while giving consideration to animal care/management. Given below are the specific strategies for environment enrichment.

#### 1) Physical environment

In addition to making individual living space as large as possible, it is also important to increase spatial utilization potential in consideration of the behavioral characteristics of each species. This can be done by placing a perch, three-dimensional structures and play equipment. Other equipment/structures that can facilitate physical environmental enrichment include rain/sun shields for outside housing and hiding places and escape routes for housing in social groups.

When installing these equipment/structures, sufficient consideration shall be given to cage position in each room (e.g. face-to-face positioning and the distance between each cage and the room floor or ceiling) and other factors, depending on the characteristics of each rearing environment.

#### 2) Introduction of novelty, unconstantness, selectability and controllability

The rearing environment for laboratory animals is generally monotonous, which is one of the major causes of various problems. It is thus recommended to introduce novelty, unconstantness and systems that allow laboratory animals to make selections and take control in a subjective manner. It is also effective to increase the potential for selection and control of the environment by not only introducing play equipment but also changing them on a regular basis or introducing various types of play equipment that can be manipulated in various ways, as part of physical environment enrichment. In regard to eating environment, NHPs are generally omnivorous and tend to prefer varied feeding to nutritionally adequate but monotonous feeding. NHPs also tend to be interested in manipulating things; they are especially fond of manipulating tools for eating food. To satisfy such desires of NHPs to interact with the environment, it is recommended to introduce variables, such as changing feeding items, installing a feeding device that requires choice and complicated manipulation and placement of structures that require manipulation of an object or physical movement according to individual situations. It should be remembered that these variables should be introduced taking into account the conditions of individual animals, rearing environment and research objectives.

#### 3) Social environment

Since NHPs are highly social animals, actions involving physical contact, such as grooming, and communication via visual, auditory and olfactory senses are important components of their daily life. Thus, they should be allowed to live in a social environment suited to each species. To establish effective groups, however, it is necessary to create physical environment with consideration given to species-specific social structures and behavioral characteristics as well as friendliness among animals and social hierarchy, so that appropriate individual relationships can be established. It should also be ensured that the established groups are socially stable and appropriate social interactions can be maintained among animals. Where NHPs have to be housed individually, adjustments should still be made to allow them to have visual/auditory contact with other animals. Social stimuli are also considered as being able to introduce unconstantness continuously into the rearing environment.

#### 4) Improvement of the relationship of animals with animal experimental researchers and animal caretakers

To reduce the stress experienced by animals and increase the safety of animal experimental researchers and animal caretakers, it is necessary to establish a good relationship between these personnel and animals through routine activities. The establishment of such a relationship will help the personnel notice changes in the behavior of each animal and may even make animals cooperative to stressful procedures required for the purpose of the research, such as restraint and drawing blood. Moreover, if an adequate social environment cannot be provided to NHPs for research reasons, their good relationship with humans will to some extent compensate for the lack of social interactions.

#### 5) Reduction of physical pain and stress.

This is closely related to the anesthetic methods and postoperative management as described in Chapter IV, rather than environmental management. However, since it is obvious that pain and other types of physical stress (e.g. restraining and holding animals) significantly affect the behavior and psychological condition of animals, appropriate measures shall be taken to reduce these factors as much as possible. In relation to 4) above, the positive reinforcement should be leveraged to train animals to be cooperative to stressful procedures (i.e. husbandry training), so that experimental procedures can be performed more smoothly and the stress experienced by both animals and animal experimental researchers can be reduced.

### 3. Implementation and evaluation of environmental enrichment

To maximize the effects of various types of environmental enrichment, attention shall be paid to characteristics that differ among animals, such as species, sex, age and origin. It is the responsibility of animal experiment researchers and animal caretakers to observe the animals as part of routine animal care. Through daily observation, abnormalities such as stereotyped behavior (i.e. repeating the same behavior), abnormal behaviors (e.g. plucking, self-mutilation, regurgitation, coprophagia and fingering feces) and excessive weight gain/loss can be detected at an early stage. Once these abnormal behaviors have been detected, attempts shall be made to identify their causes and establish specific strategies towards maximum improvement of the environment, not only from the standpoint of veterinary management but also from that of environmental enrichment. Daily observation and promotion of environmental enrichment are also important for the prevention of these problems. Every effort shall thus be made to improve the environment even for animals that do not exhibit marked abnormal behavior. In addition to implementing environment enrichment, it is also important to observe and record animals' behavior as closely as possible and evaluate the effect of the implementation. Further environmental improvement should be sought on the basis of the evaluation results. The Monkey Committee shall conduct inspections periodically or on an as-needed basis and report to the Director of PRI on whether there are any problems. When the Director has judged there is a serious problem with the NHPs, he or she may issue appropriate recommendations such as discontinuation of research.

### 4. Research and animal welfare

Any personnel who wishes to conduct an animal experiment shall be aware that animal welfare standpoints are essential in establishing the animal rearing environment and take it into account when preparing an animal experiment protocol.

## Chapter VI: Planning and conducting of animal experiments

When using animals for research/education purposes, sufficient consideration shall always be given not only to the proper care and management but also to the humane handling of animals. The proper handling of animals shall be carried out with the full understanding of relevant laws and regulations, including the Act on Welfare and Management of Animals, Basic Guidelines for Comprehensive Promotion of Measures Concerning the Humane Treatment and Management of Animals, Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain, Guidelines for the Sacrifice of Animals, and Fundamental Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions (MEXT). In addition, the internationally accepted 3R principles of animal experimentation, which is also included in the Act on Welfare and Management of Animals, shall always be taken into consideration. It is requisite in designing an animal experiment to consider the 3R principles, namely, replacement (use of alternative methods; i.e. non-animal research methods or substituting animals), reduction (use of fewer animals while maintaining scientific reliability) and refinement (minimizing pain and distress experienced by the animals subjected to experiments). This chapter describes the necessary procedures for conducting research projects using nonhuman primates (NHPs) housed in PRI and other factors to be considered, such as the characteristics of NHPs as laboratory animals and experiment-related issues.

### 1. Submission and approval of the animal experiment protocol

All research activities using NHPs housed in PRI must be conducted based on a completed Kyoto University Animal Experiment Protocol (hereinafter, referred to as "the animal experiment protocol") that has been reviewed by the Monkey Committee and approved by the Director of PRI, a department director to whom responsibilities are delegated by the President of Kyoto University. The use of NHPs housed in PRI for research purposes is permitted only by those who have completed training programs provided by the Monkey Committee and CHEMR for the proper handling of NHPs, acquired a specified license and submitted a written pledge and results of specified health check-up items (see Chapter I for the prior-to-use allocation of laboratory animals).

The Monkey Committee immediately conducts review of the submitted protocol regarding the appropriateness of the methods and procedures to be used for the achievement of research objectives and that of routine/experimental procedures and post-procedural management/handling, especially whether all efforts have been made to reduce pain and animal number. This review shall be conducted based on the fundamental notion that "procedures that are painful for humans should be considered as also being painful for NHPs, unless contrary evidence is available". The Committee may request the applicant to submit additional materials or make an oral explanation for a more detailed review.

The animal experiment protocol shall be submitted every year and may be submitted with the same content up to 3 consecutive years. It is not prohibited to submit an animal experiment protocol with the same or a similar content after 3 years have passed since the initial submission. In this case, however, a completed Animal Experiment Completion Report (see Section 8 of this Chapter) must be submitted at the end of the third year and the new protocol will be treated and reviewed as a new application. The submitted Animal Experiment Completion Report will be reviewed by the Monkey Committee and the review results will be reported to the Director of PRI. Based on the review results, the Director conducts self-inspection and evaluation. Depending on the review results, the Director may provide the animal experiment researcher with instructions and/or advice.

Pursuant to the Information Disclosure Law promulgated in 1999, animal experiment protocols, animal experiment completion reports and

their review results may be disclosed to third parties at request, except for information whose disclosure may affect personal rights and benefits. Approval by the Director of PRI assures externally that the relevant protocol has been reviewed and implemented in accordance with the guidelines described in this document. Applicants are thus required to prepare their animal experiment protocols without omissions, with the full understanding of the purpose of preparing the protocol.

Protocols for monkey research projects to be conducted by PRI personnel outside PRI (or participated in by PRI personnel) shall also be reviewed by the Monkey Committee and approved by the Director of PRI, in advance.

## 2. Categories of experiments

When an animal experiment is to be conducted based on the 3R principles, criteria for assessing the degree of pain experienced by animals through experimental procedures shall be established to determine whether “refinement” is satisfied. PRI classifies the degree of pain experienced by animals into 5 categories, depending on research details, as described below. The pain categorization system employed by PRI is based on the pain categorization table prepared by the Scientists Center for Animal Welfare (SCAW), an assembly of scientists from across North America (the table is also employed by the Animal Experiment Committee of Kyoto University), and has been modified from the original version taking into account current scientific trends and the environment specific to PRI. It is generally held that pain assessment in an animal experiment shall be made by the person who performs the experiment taking into account the balance between pain for animals and expected research results (i.e. cost-benefit consideration) and that the institutional animal experiment committee should determine the appropriateness of the pain assessment. However, since it is very difficult uniformly to classify the nature of pain or evaluate the degree of pain caused by various experimental procedures in various species, the Monkey Committee is required to determine the degree of pain and assess the appropriateness of the protocol carefully. For category C procedures, additional considerations are required depending on the degree and duration of stress/pain. Animal experimental researchers and principal investigators who are to conduct category D procedures must consider alternative strategies to eliminate or minimize pain experienced by animals. The need for euthanasia as a humane endpoint may also be considered. For experiments involving category E procedures, their appropriateness and necessity shall be assessed more carefully. Such experiments shall not be permitted unless the conduct of the animal experiment protocol is considered indispensable after consideration has been given to the balance between pain for animals and expected research results (i.e. cost-benefit consideration).

Each principal investigator shall prepare an animal experiment protocol with the full understanding of what types of procedures fall under which pain category. In addition to the understanding of the pain categories, the principal investigator and animal experiment researcher must obtain an appropriate license required for the conduct of the protocol to be submitted (see Section 8 of Chapter I and the “Monkey Handling Skill Qualification System”). Submission of the protocol for an experiment using hazardous substances or new chemicals shall be accompanied by separately specified documents.

Table 6-1. Category of biomedical experiments and pain and distress modified from SCAW (1990).

Category	Representative procedures
<p>A</p> <p>Experiments involving no living NHPs</p>	<ul style="list-style-type: none"> <li>- Biochemical study</li> <li>- Microbiological study</li> <li>- Cell culture</li> <li>- Research on tissues obtained from using autopsy</li> </ul>
<p>B</p> <p>Experiments on monkeys that are expected to produce little or no discomfort</p>	<ul style="list-style-type: none"> <li>- Restraining an animal for a very short period of time (<math>\leq 1</math> min)</li> <li>- Restraining an animal in an experimental cage for a short period (<math>\leq 2-3</math> h) without immobilizing its limbs or head</li> <li>- Administration of a relatively harmless substances and simple procedure, such as drawing a small amount of blood</li> <li>- Experiments on completely anesthetized animals and thus associated with no discomfort after completion of procedures</li> <li>- Deprivation of food and/or water for a short periods (a few hours)</li> <li>- Sacrifice animals using appropriate euthanasia procedures</li> <li>- Non-fatal animal experiments (involving infectious agents, etc.) that do not cause serious symptoms in animals</li> </ul>
<p>C</p> <p>Experiments that involve some stress or pain (short-duration pain) to NHPs</p>	<ul style="list-style-type: none"> <li>- Restraining an animal on a monkey chair or other equipment for a short period (<math>\leq 2-3</math> h)</li> <li>- Exposure of blood vessels or choronic catheters with anesthesia</li> <li>- Surgical procedures performed under anesthesia that cause mild post-procedural discomfort</li> <li>- Application of noxious stimuli from which escape is possible</li> <li>- Non-fatal animal experiments (involving infectious agents, etc.) that cause serious symptoms in animals</li> </ul>
<p>D</p> <p>Experiments that involve significant but unavoidable stress or pain (prolonged period, up to several hours or more)</p>	<ul style="list-style-type: none"> <li>- Deliberate induction of behavioral stress in order to test is effect</li> <li>- Major surgical procedures performed under anesthesia that result in significant, persistent post-operative discomfort</li> <li>- Application of noxious stimuli from which escape is impossible</li> <li>- Prolonged period (up to several hours or more) of physical restraint such as monkey chair</li> <li>- Maternal deprivation with substitution of punitive surrogate</li> <li>- Induction of aggressive behavior leading to self-mutilation or intra-species aggression</li> <li>- Causing pain without using anesthesia</li> <li>- Stress and shock research that would result in pain approaching the pain tolerance threshold, the point at which intense emotional reactions occur</li> <li>- Fatal animal experiments (involving infectious agents, etc.) that cause serious symptoms in animals</li> </ul>

<p>E</p> <p>Procedures that involve inflicting severe pain near, at, or above the pain tolerance threshold on unanesthetized, conscious NHPs</p>	<ul style="list-style-type: none"> <li>- Use of muscle relaxants or paralytic drugs such as succinylcholine or other curariform drugs alone for surgical restraint without the use of anesthetics</li> <li>- Severe burn or trauma infliction on unanesthetized animals</li> <li>- Sacrifice by use of using a domestic microwave ovens for domestic kitchens or by or strychnine</li> <li>- Inescapable sever stress or terminal stress</li> <li>- Stress and shock research that would result in pain approaching the pain tolerance threshold or in severe stress, which induce psychotic-like behavior</li> </ul>
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### 3. Health management during animal experiments

Health management of laboratory animals should basically be undertaken by an animal experiment researcher under supervision of CHEMR faculty members who are qualified veterinarians and designated by the Monkey Committee. The Monkey Committee conducts periodical inspections and assessment of the conditions of animal housing facilities and states of animals. On the basis of the inspection results, the Committee may provide advice on the conduct of the research project. The animal experiment researcher shall enter and manage information for all animals used in experiments (e.g. food intake, shape and amount of feces, body mass and other general physical conditions) into the Monkey Management System managed by the Monkey Committee. When deemed necessary by the Monkey Committee based on the content of the animal experiment protocol, it is the responsibility of the principal investigator of the animal experiment to periodically enter information required for evaluation of health status into the system, such as water intake and change in body mass.

On special occasions, such as when a serious health problem has occurred, the Monkey Committee shall conduct a health check-up of the relevant animal and determine the appropriateness of research continuation. When it has been determined that research should be discontinued due to poor health of the relevant animal, the Committee shall notify such decision to the Director of PRI. The Director may recommend the interruption or discontinuation of the research project, even if it is an ongoing project.

Health management-related information obtained in an on-going experiment may be disclosed to third parties at request, except for information whose disclosure may affect personal rights and benefits.

### 4. Conduct of animal experiments involving restrictions

#### 1) Experiments involving restrictions

Restrictions on the intake of water, food or selected nutrients, weight gain, etc. may affect the development and health status of NHPs and thus shall be avoided as much as possible. In addition, for animals that exhibit high-level emotional responses such as NHPs, social restrictions, such as maternal deprivation, and other types of restriction, such as blockage or modification of sensory organs, are known to affect their development and health status. Where these interventions are inevitable for the purpose of the experiment due to the lack of alternative methods, their frequency and duration shall be kept to the minimum levels required and sufficient attention shall be paid to the development and health of the animals. When conducting these interventions, the development and health status of the NHPs shall be evaluated based on the norm of body mass shown later. Special attention shall be paid when using immature or aged animals in experiments involving restrictions, as the health status and physical/mental development of these animals may be significantly affected by these interventions. Frequent observations are required to judge signs of pain and consideration shall be given to the use of a humane endpoint at the earliest stage possible. The plan for an experiment

involving restrictions shall be described in detail in the Animal Experiment Protocol and reviewed by the Monkey Committee. During these experiments, health monitoring by a veterinarian designated by the Monkey Committee shall be carried out when deemed necessary.

Where immature animals are to be used in these experiments, the animals are required to have attained the developmental stage that it is unlikely that experimental procedures will significantly affect their physical and mental development. Although there are no objective criteria for many NHPs species to determine whether the animal has attained this developmental stage, this judgment can empirically be made based on whether deciduous teeth are replaced by permanent teeth (incisors for Japanese monkeys and rhesus monkeys). For Japanese monkeys and rhesus monkeys, those aged around 2.5 years or more and weighing over 4.5 and 4.0 kg, respectively, are generally considered eligible for these experiments.

#### 2) Conduct of experiments involving restrictions and record

Experimental procedures, watering and feeding schedules and restriction levels shall be described in detail in the Animal Experiment Protocol and reviewed by the Monkey Committee. Due to the wide variation in food/water intake between animals, baseline values for each animal's body mass and food/water intake shall be measured, and restriction levels and experimental schedules shall be adjusted based on these norms. During restriction, body mass and water intake shall be measured on a regular basis and entered/recorded into the Monkey Management System. Measurement/recording shall basically be done on all experimental days. The Monkey Committee will evaluate the effects of each experiment based on the norm of body mass and a physical examination report prepared by a veterinarian, as described later, and take appropriate action.

Where social restrictions (i.e. maternal deprivation and isolation from other animals) are to be imposed on animals, health monitoring of each animal and reporting body mass and food/water intake to the Monkey Committee are required.

#### 3) Water restrictions

In an experiment involving water restriction, the minimum water intake shall be set to 30 ml/kg BW, with a target level of 45 ml/kg BW or more. Animals should be given free access to water (i.e.  $\geq 100$  ml/kg BW/day) at least one day in a week. Since insufficient water intake may cause an animal to have difficulty in eating solid food, such juicy supplements as fruits and vegetables shall be given as needed. The Monkey Committee will monitor water intake of laboratory animals on a regular basis and, when water intake is considered insufficient, may provide the principal investigator and animal experiment researcher with instructions.

#### 4) Feeding restrictions

Effects of feeding restrictions on the health of animals, unlike those of water restrictions, do not necessarily become apparent immediately. Since malnutrition may significantly affect the development and health of animals, appropriate recovery periods shall be scheduled depending on the duration and intensity of restriction. Feeding restrictions shall be carried out in principle such that energy intake does not fall below the basal metabolic rate, which is determined as a function of species, developmental stage and body mass (see Chapter III), and with the fasting duration shall be as short as possible (i.e.  $\leq 48$  h).

#### 5) Evaluation of the effects of restrictions and actions to be taken

The health status of NHPs during an experiment involving restrictions can be evaluated by increases/decreases in body mass, change in food/water intake, amount and condition of feces, skin and pelage conditions and abnormal behavior. These changes may be missed unless observed closely by an experienced observer. An inexperienced researcher shall seek instructions from CHEMR faculty members or veterinarians and observe the animals carefully. Any personnel who has found even a slight sign of health problems should notify a CHEMR faculty member who is a qualified veterinarian and seek veterinary advice when deemed necessary. A body mass loss of  $\geq 20\%$  within a few days or  $\geq 25\%$  in 7 days is considered to greatly affect the health of laboratory NHPs. If such changes have been noted, the experiment shall be

immediately discontinued, advice should be sought from the Monkey Committee and the Director of CHEMR, and efforts shall be made to restore the health of the animal. Based on the report of the CHEMR veterinarian, the Monkey Committee will judge whether the animal experiment can be continued. Even if it has been determined that the experiment can be continued, the Committee may provide the principal investigator and animal experiment researcher with advice and instructions for improvement of the experimental and/or rearing methods when deemed necessary. The need for euthanasia as a humane endpoint shall also be considered depending on the situation. When a particular animal experimental researcher has been found to have repeated violation of any rule, the Monkey Committee shall notify the Director of PRI and the Director may take appropriate action, such as the interruption of the research project or revocation of the license of the animal experimental researcher.

#### 5. Blood sampling and biopsy

The total amount of blood drawn from an animal in a 2-month period shall not exceed 10% of the total blood volume. For Japanese monkeys, no more than 5 ml/kg BW of blood shall be drawn in a 2-month period. The maximum blood volume that can be drawn from each animal shall be determined for each species and sex. For species other than Japanese monkeys, the maximum volume shall be calculated according to Table 6-2.

Table 6-2. Allowable blood sampling volume

Species	Blood volume (ml/kg BW)	Total blood volume of healthy adult monkey (ml)	Range of blood volume can be drawn safely at a (ml)*	Usual amount of blood drawn at physical examination	Maximum total volume of blood to be drawn in a 2-month period at PRI (ml/kg BW)
Marmoset	70	21–24.5	2.1–2.4	0.5	7
Squirrel monkey	70	Male: 39-77 Female: 24.5-52.5	Male: 3.9 – 7.7 Female: 2.4 – 5.2	0.5	7
Rhesus monkey	55-80	Male: 420-770 Female: 280-630	Male: 42 – 77 Female: 28 – 63	1-2	5
Cynomolgus monkey	50-96	Male: 280-560 Female: 140-420	Male: 28 – 56 Female: 14 – 42	1-2	5
Baboon	62-65	Male: 1430-1950 Female: 715-975	Male: 143-195 Female: 71 – 94	1-5	6

Modified from Wolfensohn & Lloyd (1998) Handbook of Laboratory Animal Management and Welfare, 2<sup>nd</sup> edition

\* 10% of total blood volume

Tissue sampling from a live animal (i.e. biopsy) should basically be performed within a range that does not affect the normal living functions of monkeys. The Monkey Committee will review the appropriateness of the amount and procedure of biopsy, according to the Animal Experiment Protocol and taking into account the target tissue and the invasiveness of the procedure.

## 6. Animal experiments using hazardous substances

When an animal experiment is to be conducted using biological, chemical or physical hazardous substances (e.g. RI, radiation), including the use of X-ray apparatus, PET, MRI and other imaging equipment, various types of equipment and systems must be in place to protect animal experiment researchers, animal caretakers, the general public and laboratory animals from exposure to these substances and prevent environmental pollution.

The conduct of an experiment using hazardous substances requires dedicated equipment. Such equipment shall be installed in areas away from laboratory animal housing facilities and laboratories and appropriately indicated as a hazardous area. Personnel who are to use such equipment are required to obtain the necessary licenses for handling of the relevant hazardous substances and must be familiar with risk management procedures in case of accidents and other procedures (see the Safety and Health Manual).

For the care of laboratory animals; process of animal waste and carcasses; storage, use and management of hazardous substances; and clearly defined, safe operating procedures and sufficient education and training programs shall be provided to protect working personnel from hazards. All personnel shall be well familiarized with the properties of the hazardous substances they are to handle and with the required protection measures. Possibly contaminated equipment and waste shall be disposed of appropriately in each facility to prevent the leakage of hazardous substances out of the hazardous area (waste disposal procedures shall be followed the regulations enforced by Inuyama Health Committee). Moreover, all accidents that occur during the handling of laboratory animals must be immediately reported to the Monkey Committee (using the “Report of Laboratory Animal-related Accidents” form).

When entering an area in which hazardous substances are handled, personnel shall wear protective equipment, such as a protective gear and gloves, to protect them from contamination. When entering an area in which hazardous aerosols and vapors are possibly present in the air, personnel shall wear a mask or other appropriate equipment to protect the respiratory tract from exposure to these substances. When leaving the area in which hazardous substances are handled, personnel shall dispose of protective gears and other possibly contaminated items within the area and not take them out of the area. They will also try to remove all substances attached to the body by showering or equivalent facilities.

Researchers who wish to conduct an experiment using biohazardous materials, such as microbes, or chemical hazardous materials must submit an Animal Experiment Protocol to the Disease Control Committee, Biosafety Committee, Radiation Safety Committee, Chemical Substance Control Committee and other relevant committees for approval before submitting it to the Monkey Committee. For an experiment using other types of hazardous substances, the Monkey Committee, along with other relevant committees (e.g. Disease Control Committee, Biosafety Committee, Radiation Safety Committee and Chemical Substance Control Committee), is responsible for approval and monitoring of the experiment. Approval is granted in accordance with the criteria established by the government (i.e. relevant ministries, such as MEXT, MHLW and MAFF) and Kyoto University.

## 7. Animal experiments using recombinant DNA

Animal experiments using recombinant DNA are carried out using animals, including NHPs, in accordance with the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (MEXT, dated February 2004). When conducting these experiments, the principal investigator (i.e. a faculty member) must submit an application to the relevant authority or an application for minister's approval, in accordance with the Act, and obtain such approval before starting the experiment. A graduate student, research fellow or cooperative research fellow conducting an animal experiment using recombinant DNA in PRI shall submit an application under the name of his or her supervising or corresponding faculty member as the principal investigator and obtain approval. Those who are conducting an animal experiment using recombinant DNA must obtain approval from the Biosafety Committee and the Monkey Committee at PRI before submitting an application to Kyoto University and MEXT, as described above. After approval by the Biosafety Committee, the application shall be submitted to the Monkey Committee together with prior written approval and other required documents (e.g. sequences for donor nucleic acid, nucleic acid donor, vector and host DNA, safety manual, etc.). The Monkey Committee will then review the application focusing on animal welfare-related issues during the experiment and safety of the rearing of animals. When deemed necessary, the Committee will hold a joint meeting with the Biosafety Committee examine these issues.

Animal experiments using recombinant DNA shall be carried out in an experimental area approved by PRI.

## 8. Euthanasia notification and reporting/verification of completion of experiment

After completion of an experiment, the outcome of the animal experiment must be reported to the Monkey Committee using a form designated by the Committee (i.e. Animal Experiment Completion Report). If it is deemed necessary to further continue the animal experiment that has already been carried out under the same research project for 3 years, the Animal Experiment Completion Report must be submitted to the Monkey Committee in the final experimental year to have the Committee evaluate the conduct of the animal experiment. In this case, an Animal Experiment Protocol with the same content can be submitted as a new application in the following year. If any problems have occurred during the experiment, the Monkey Committee will request documents and/or materials from the principal investigator and animal

experiment researcher to explain about the problem. The Committee will then report the problem to the Director of PRI. The Director may, when deemed necessary, recommend the implementation of remedial action for recurrence prevention. The submitted Animal Experiment Completion Report shall be retained by the administration office.

When conducting euthanasia as the final step of an animal experiment, the principal investigator and animal experiment researcher must comply with the guidelines described in this Guideline (section 5 of Chapter V) and notify the Monkey Committee and CHEMR of the conduct of euthanasia within 2 weeks or as soon as possible if this is impossible, in compliance with the multiple utilization principle. The person who has carried out euthanasia must submit a Euthanasia Report to the Monkey Committee.

The Director of PRI must conduct self-inspection/evaluation of all animal experiments conducted in the year. For this purpose, the Monkey Committee will conduct an evaluation based on the submitted Animal Experiment Completion Report. On the basis of the evaluation results, the Director shall conduct self-inspection/evaluation of animal experiments and make its details public via a website or other media. The principal investigator and animal experiment researcher must submit additional documents as required for evaluation purposes. Failure to submit these documents or to retain necessary data may result in questioning of the appropriateness of research continuation.

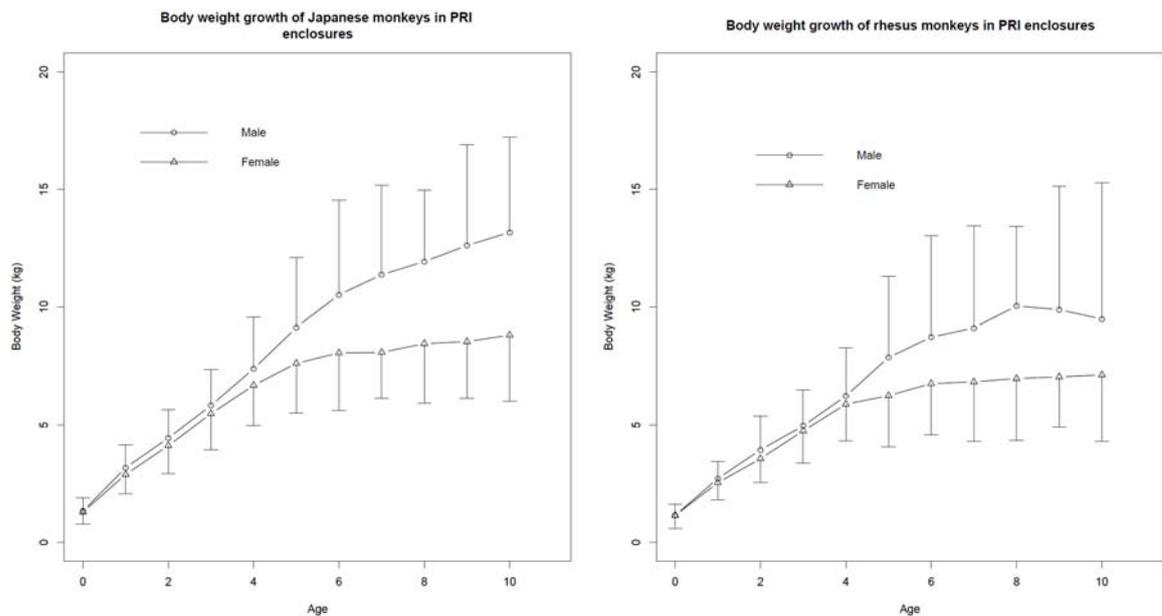


Figure 6-1. Standard body mass curves (mean and  $\pm 2$  standard deviation) for Japanese monkeys (*Macaca fuscata*, left) and rhesus monkeys (*Macaca mulatta*, right) housed in the corral cages at PRI

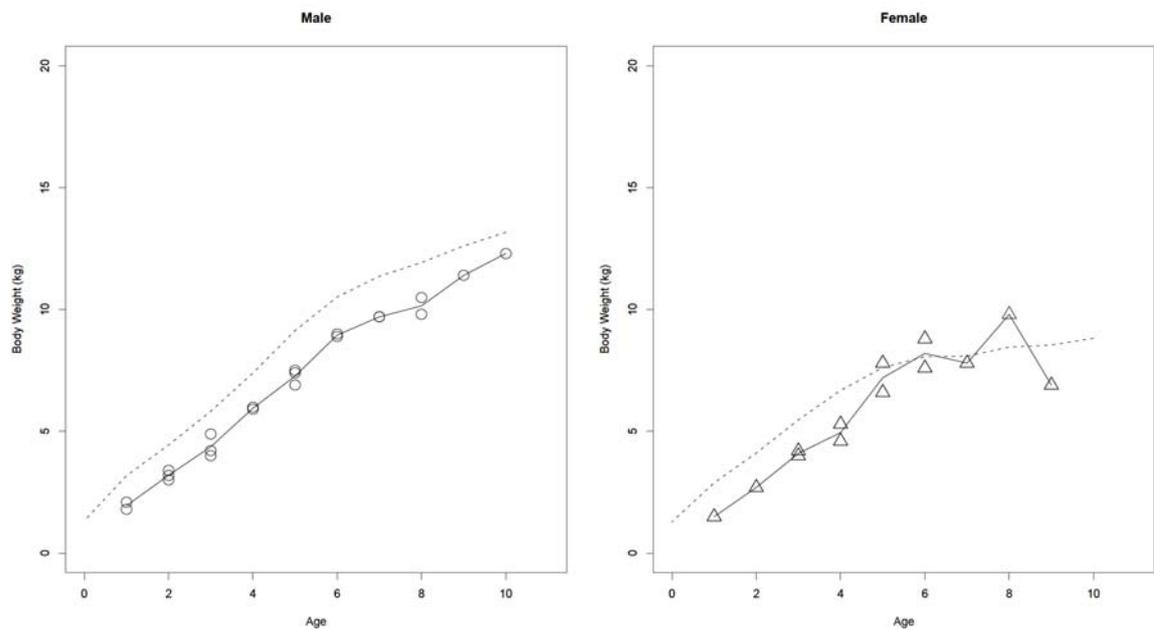


Figure 6-2. Body mass growth of the Japanese monkeys reared in individual cages at PRI. The data were derived from a small population of 3 male and 2 female monkeys. Solid line denotes the mean  $-2sd$ ; dotted line denotes the mean values presented in Figure 6-1 . When the dotted line is regarded as the minimum acceptable standard body mass of monkeys kept in a corral cage, the result indicates that the body mass of Japanese monkeys kept in individual cages is comparable to or about 1 kg lower than the minimum. Therefore, it must be taken in consideration that individually housed monkeys, even with normal growth/development, do not have comparable mass increase with corral cage housed ones.

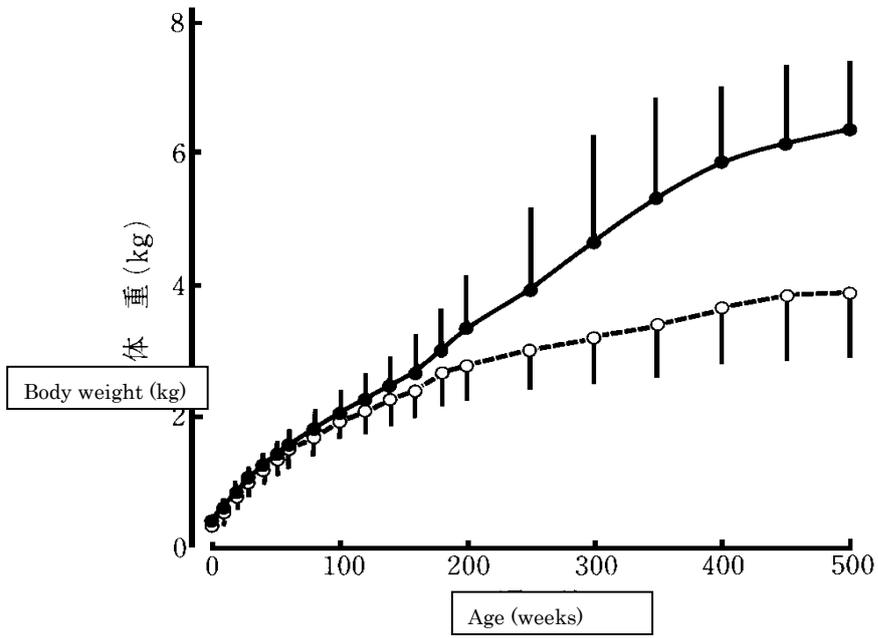


Figure 6-3. Body mass of cynomolgus monkeys (*Macaca fascicularis*). ● and ○ denote mean values for male and female monkeys, respectively.

Vertical lines indicate standard deviations for each mean value (modified from Yoshida, 1994).

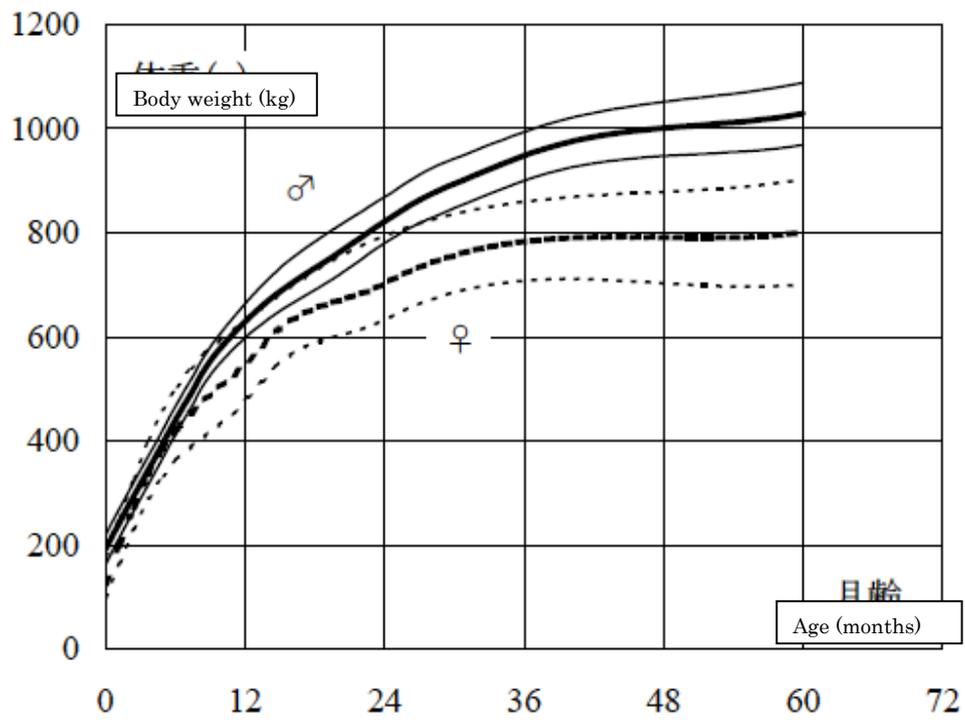


Figure 6-4. Body mass curves for squirrel monkeys (*Saimiri sciureus*) (modified from Long & Cooper, 1962).

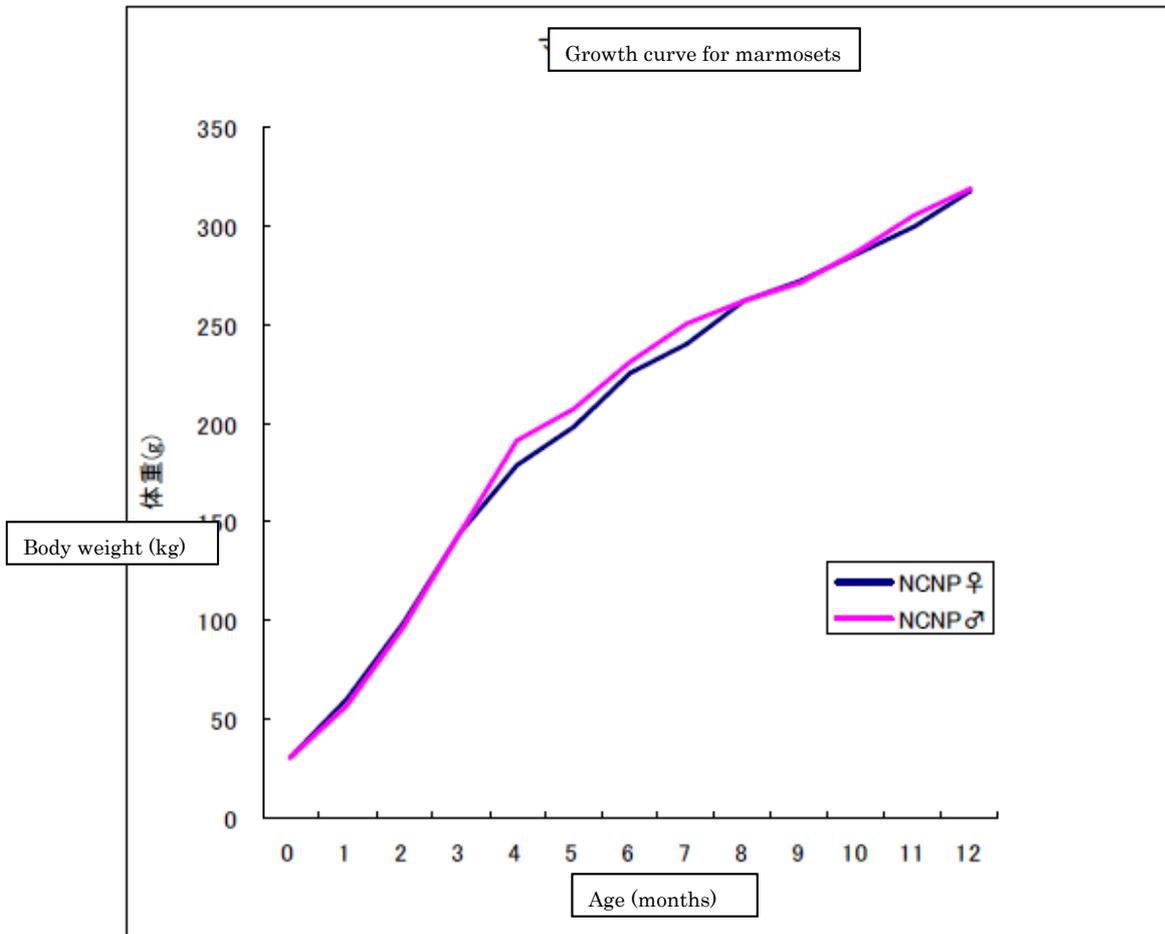


Figure 6-5. Average body mass growth curves in marmosets (*Callithrix* spp.). Adopted from the Guidelines for the Care, Management and Use of Primates, Ver. 2. National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Ministry of Health, Labour and Welfare.

## Postscript

In 1986, the Primate Research Institute of Kyoto University (hereinafter, “PRI”) established guidance for the care, management and use of Nonhuman primates (NHPs) for research purposes for the first time in Japan (“Guidelines for the Care, Management and Use of NHPs”, April 1986). This guidance does not simply describe the philosophy but also refers to various other issues concerning the use of NHPs for research purposes, including the structure of housing facilities, cage size and recommended anesthetics, and has been consulted by many researchers who use NHPs in experiments. The guidance has significantly contributed to the widespread conduct of NHP research in Japan. Since then, the attitude to animal handling has gradually changed, as symbolized by the amendment of the “Law Concerning the Protection and Control of Animals” (Law No. 105 dated October 1, 1973) to the “Act on Welfare and Management of Animals” (amended on December 21, 1999). In fact, the purpose of this Act is specified as “to engender a spirit for animal welfare among citizens and contribute to the development of respect for life and sentiments of amity and peace by providing for the prevention of cruelty to animals, the proper handling of animals and other matters concerning animal welfare”. It also states that “no person shall kill, injure, or inflict cruelty on animals without due cause, and every person shall treat animals properly by taking into account their natural habits and giving consideration to the symbiosis between humans and animals”. In line with the changing times, the PRI guidelines have also incorporated the concepts of animal welfare, experimental ethics and environmental enrichment. The overall amendment of the guidance incorporating these concepts was made possible 16 years after the release of the first version (“Guidelines for the Care, Management and Use of NHPs, Version 2”, May 2002).

Thereafter, in 2006, a significant event occurred concerning animal experimentation in Japan: the Act on Welfare and Management of Animals (Ministry of the Environment) was amended in 2005 (amendment finalized on June 2, 2006). A particularly significant change was the inclusion of the so-called “3R principles” into the Act, as it requires researchers to consider the use of alternative methods (“replacement”), use of fewer animals while maintaining scientific reliability (“reduction”) and minimization of pain experienced by animals subjected to experiments (“refinement”) when preparing an experimental protocol. In particular, pain reduction is also referred to in the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Ministry of the Environment, dated April 28, 2006) and should basically be evaluated according to pain categorization systems, such as the SCAW, which is adopted by the Japanese Association of Laboratory Animal Facilities of National University Corporations. Several related ministerial guidelines were also established on June 1, 2006, including the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (MEXT), Basic policies for the Conduct of Animal Experiments in Research Institutions under the Jurisdiction of the Ministry of Health, Labour and Welfare (MHLW) and Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan). Since the establishment of these guidelines, it has become a necessity to specify through internal regulations that the director of each research institution takes all responsibility for animal experiments conducted at his or her institution and that animals experiment cannot be carried out without approval from the Director of each institution.

As 7 years have passed since the previous amendment of PRI guidelines without incorporating these major acts and guidelines concerning animal experimentation, we finally decided to amend the guidelines in 2009. PRI’s guideline is a model guidelines for the use of animals in experiments conducted at other research institutions. During the present amendment, special consideration was given to the following points: 1) ensure the guidelines incorporate the spirit of the 3R principle, specify the reason for the use of NHPs in the experiment and allow for self-inspection of whether such efforts have been made to minimize the number of animals to be used; 2) reconsider the types of anesthetics to be used and indicate the anesthetic methods that are currently considered best choices for the reduction of pain in animals; and 3) include

outside experts and non-researchers in the experimental protocol review process for fairer review, which was not put into practice in the version 2 guidelines.

As PRI was established more than 40 years ago, the monkey housing and experimental areas are not completely separated from other areas inside the building. This problem must be solved as soon as possible, in view of the recent spread of infectious diseases caused by various types of viruses and the concept of biosafety to protect humans and NHPs from these diseases. It is also important to make continuous efforts to improve the rearing environment for NHPs, rather than being satisfied with the current situation. Although there remain a number of issues to be improved upon, the present guidance is what is believed to be most appropriate at present by PRI personnel, who have more than 40 years of experience in NHP experimentation. We hope that this guidance will help a wide range of people who use NHPs in experiments with safer, proper and smooth conduct of research activities.

The amendment process was substantially completed within half a year. This was made possible partially because I, as Chair of the Committee, pushed the committee members participating in this work, knowing that I was asking much of them, as I considered it important to finish this work with minimal delay. If this resulted in any deficiencies in the content of the guidance, I am the one at fault. If you notice any incorrect descriptions or other errors to be corrected, please contact me.

The following members participated in the amendment of the guidelines and wrote each chapter:

Katsuki Nakamura (Chapter I), Masaki Tomonaga (Chapter II), Takao Oishi (Chapter III), Takako Miyabe (Chapter IV), Masaki Tomonaga (Chapter V), Hiroki Koda (Chapter VI), Akihiro Hosokawa and Kazuyo Ono (editors). I would like to take this opportunity to thank them all. I would also like to thank staff members at the Center for Human Evolution Modeling Research and graduate students for their valuable opinions.

**The primary responsibility for the wording of this document lies with Katsuki Nakamura**

## Appendix

### Infectious Diseases in Monkeys

Infectious diseases that affect both humans and animals are referred to as zoonosis. As monkeys are phylogenetically close to humans, many zoonoses have been identified in monkeys.

Although not affecting humans, several monkey-specific infectious diseases that cause serious symptoms in monkeys have also been identified.

Major infectious diseases in monkeys

Viruses	Bacteria	Protozoa
Monkeypox *	Dysentery *	Malaria
B-virus disease *	Salmonellosis *	Amebic dysentery *
Simian varicella virus infection	Yersiniosis *	<i>Balantidium coli</i> infection *
Herpesvirus saimiri infection	Campylobacteriosis *	Giardiasis *
Herpesvirus tamarinus infection	Tuberculosis *	
Simian AIDS (SV)	Atypical mycobacterial infection *	
Type D simian retrovirus (SRV/D) infection	Hansen's disease *	
Simian T-cell leukemia	Tularemia *	Parasites
Ebola hemorrhagic fever, Marburg disease *	Melioidosis *	Helminthic infection *
Hepatitis (A and B)	Leptospirosis *	Filariasis *
Measles *		
Rabies *		
Yellow fever *		

\* Zoonoses transmittable from NHPs to humans

Even if infected with pathogens, animals may not show any symptoms during the asymptomatic phase or in case of occult or latent infection.

Adopted from "Health management of cynomolgus monkeys at Tsukuba Primate Research Center"

Tsukuba Primate Research Center and The Corporation for Production and Research of Laboratory Primates