

Guidelines for proper conduct of animal experiments by the Science Council of Japan

Hideaki Karaki

Science Council of Japan
Office 301, 3-5-6-301 Honkomagome, Bunkyo-ku, Tokyo 113-0021, Japan
karaki@gakushikai.jp

Abstract

In 1980, in response to a recommendation submitted by the Science Council of Japan (SCJ), the Ministry of Education issued a notification to related institutions. Based on this notification, research institutions established policies for more appropriate conduct of animal experiments. In 2004, the SCJ issued a proposal entitled "Promotion of public understanding of animal experimentation". On receipt of this proposal, the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labor and Welfare compiled basic policies for the conduct of animal experimentation. The two ministries requested the SCJ to prepare detailed guidelines to serve as a reference material or a model when research institutions compile their own specifications for animal experimentation, and SCJ submitted the guidelines in 2006.

Keywords: guideline, animal experiments, 3Rs, Science Council of Japan

The major part of Guidelines for Proper Conduct of Animal Experiments, submitted by the Science Council of Japan on June 1, 2006, is as follows:

Preface

The necessity of basic considerations for the handling of laboratory animals in Japan had been based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1973) and Standards Relating to the Care and Management of Experimental Animals (Notice No. 6 of the Prime Minister's Office 1980).

Under these conditions, the rationalization of animal experimentation was based on administrative guidance rather than laws and regulations because of its importance in the advancement of scientific research. The Science Council of Japan submitted a recommendation to the government in 1980 entitled "Establishment of Animal Experimentation Guidelines." In response to this recommendation, the Ministry of Education issued a notification to related institutions entitled "Animal Experimentation in Universities, etc." (Director General, Science and International Affairs Bureau, 1987). Based on this notification, research institutions established policies for more appropriate conduct of animal experiments and Institutional Animal Care and Use Committees, and applied them in detail. As a result, it became possible to conduct highly creative scientific research in a free and open manner and Japanese medicine and life sciences made remarkable progress on an international level.

For progress in life science, it is recommended to have a voluntary system of animal experimentation under the responsibility of researchers who best understand the necessity of such experimentation. There are also calls for the exercise of government authority in animal experimentation. Therefore, establishment of guidelines on animal experimentation became an urgent necessity and Subcommittee 7 of the Science Council of Japan issued a proposal entitled "Promotion of public understanding of animal experimentation" in 2004.

On receipt of this proposal, the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labor and Welfare compiled "Fundamental guidelines for proper conduct of animal experiment and related activities in academic research institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology" and "Basic policies for the conduct of animal experimentation in the Ministry of Health, Labor and Welfare." The two ministries requested the Science Council of Japan to prepare detailed guidelines to serve as a reference material or a model when research institutions compile their own specifications for animal experimentation in accordance with the above fundamental guidelines and basic policies.

Handling of laboratory animals is influenced by the religion and culture of each country. The so-called North American model specifies voluntary management of animal experimentation without relying on legal restrictions on scientific procedures, while Japan favors the establishment of a system

based on Japanese customs.

With such a system, it is always hoped that animal experimentation will be promoted appropriately with the understanding of the people and will contribute to advances in life science research.

Basis and objectives

Animal experiments are indispensable in medical and life science education, research and testing. They should be managed and conducted voluntarily under the responsibility of each research institution. The researcher must draft the animal experiment protocol based on scientific rationale and also should consider the welfare of the animal. The researcher must have the Institutional Animal Care and Use Committee review the suitability of the proposed animal experimentation protocol when conducting an animal experiment.

These Guidelines were prepared with the objective of appropriate implementation of animal experiments from a scientific standpoint in accordance with policies on the conduct of animal experiments formulated by government organizations with jurisdiction over institutions conducting animal experiments (Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labor and Welfare, etc.) ("Fundamental guidelines for proper conduct of animal experiment and related activities in academic research institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology" Notice of the Ministry of Education, Culture, Sports, Science and Technology dated June 1, 2006 and "Basic policies for the conduct of animal experiments in research institutions under the jurisdiction of the Ministry of Health, Labor and Welfare," Notification of the Ministry of Health, Labor and Welfare dated June 1, 2006). The handling of laboratory animals is specified in "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain" (Notice No.88 of the Ministry of Environment dated April 28, 2006).

These Guidelines consist of chapters on the responsibility of institutions concerning voluntary management and the Institutional Animal Care and Use Committee at the beginning followed by chapters on animal experiment protocol; drafting and experimental procedures and selection of laboratory animals. These are followed by care and management of laboratory animals, laboratory animal health management, facilities and safety management, education and training of personnel and others including in-house inspections and assessment and information disclosure.

Each institution should formulate voluntary in-house regulations for proper scientific conduct of animal experiments based on these Guidelines.

No. 1 Definitions

(Abbreviated)

No. 2 Responsibilities of the director of the institution

The director of the institution bears the final responsibility for all experiments conducted in his or her institution. The director of the institution prepares the facilities considered necessary for proper care and management of the laboratory animals and proper and safe conduct of the animal experiments, appoints the manager and appoints a person with knowledge and experience related to laboratory animals as the laboratory animal manager. The director of the institution also provides education for related persons including the researchers and animal technicians with the cooperation of the manager and laboratory animal manager to inform them of the related laws and policies.

In each institution, in-house regulations including the authority and responsibilities of the director of the institution, standard operating procedures (SOP) for the conduct of animal experiments, proper care and management of laboratory animals and methods of maintenance and management of facilities should be established based on the policies.

An Institutional Animal Care and Use Committee should be established in each institution. The director of the institution requests the Institutional Animal Care and Use Committee to review the animal experiment protocols submitted by principal investigators based on scientific rationale and in consideration of animal welfare. The director of the institution then approves or does not approve the protocol based on the report of the Institutional Animal Care and Use Committee. After completion of the animal experiment, the director of the institution examines the results obtained and instructs the principle investigator and manager to make improvements based on advice of the Institutional Animal Care and Use Committee.

The director of the institution retains the animal experiment protocols, results obtained from the animal experiments and the minutes, etc. of the meetings of the Institutional Animal Care and Use Committee; assures transparency of the animal experiments and publishes the results within a range that does not interfere with research or corporate activities in consideration of protecting private information and research information. The director of the institution should take the necessary measures to provide education and training to improve the quality of laboratory animal managers, researchers and animal technicians.

No. 3 Institutional Animal Care and Use Committee

The Institutional Animal Care and Use Committee

objectively reviews and inspects animal experiments at an institution to assure that they are planned and conducted properly. To achieve this, the Institutional Animal Care and Use Committee should be established independently from any organizations involved in administration of the facilities. The role and organization of an Institutional Animal Care and Use Committee are indicated below.

1) Roles of the Institutional Animal Care and Use Committee

Following consultation with the director of the institution, the Institutional Animal Care and Use Committee reviews from the standpoint of scientific rationale the animal experiment protocol submitted by the principal investigator in consideration of the "Law for the Humane Treatment and Management of Animals", "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain" and policies, and reports the results of the review to the director of the institution. The Institutional Animal Care and Use Committee also receives the results of implementation of the animal experiment protocol from the director of the institution, and examines the actual conditions at the facilities, etc., as required before reporting back to the director of the institution and providing advice.

The Institutional Animal Care and Use Committee obtains details of the situation regarding the education and training of laboratory animal managers, researcher(s) and animal technicians, and offers the director of the institution advice. The Institutional Animal Care and Use Committee may also participate in education and training as required. Items discussed by the Institutional Animal Care and Use Committee are recorded as the meeting minutes that must be maintained and retained. Institutional Animal Care and Use Committee meeting minutes include the items below.

- (1) Day, time and location of meeting
- (2) Names of members who participated in the meeting
- (3) Details of items discussed at the meeting (details of questions from committee members and answers from principal investigators, etc.), and the results of discussions.

2) Institutional Animal Care and Use Committee organization

The Institutional Animal Care and Use Committee is composed of members appointed by the director of the institution. To assure that committee members possess the knowledge required to fulfill the role of the Institutional Animal Care and Use Committee, those appointed are researchers conducting animal experiments, laboratory animal specialists and other persons of knowledge and experience.

The number of committee members is decided taking into consideration factors such as the size of the institution, the scope of the research, and the number of animal experiment protocols to be submitted. A committee member should not participate in the review of an animal experiment protocol for an experiment for which he or she is principal investigator.

No. 4 Animal experiment protocol drafting and experimental procedures

When conducting animal experiments, the significance of the research and the reasons why animal experiments are required must be explained. Animal experiments must be conducted based on scientific rationale. At the same time, they should be conducted in compliance with the internationally accepted 3R principles of animal experimentation as clarified in amendments of the "Law for the Humane Treatment and Management of Animals" (Law No.105, 1973; the latest amendment on June 22, 2005), namely Replacement: the application of alternative methods that do not require the use of animals within limits that allow scientific objectives to be achieved, Reduction: the use of as few animals as possible within limits that allow scientific objectives to be achieved, and Refinement: the application of methods that do not distress the animals or subject them to pain within limits required for use. These 3R principles are the ideology behind both animal experimentation and the handling of laboratory animals. Consequently, within the limits required to achieve the objectives of research, they should be taken into consideration and applied appropriately when conducting animal experiments.

1. Drafting of the animal experiment protocol

In accordance with the above principles, the principal investigator should prepare an animal experiment protocol recording the necessary items in the form (2 below), and submit it to the director of the institution for approval. The director of the institution requests the Institutional Animal Care and Use Committee to review the protocol content from a specialized standpoint. The Institutional Animal Care and Use Committee promptly reviews the protocol and immediately reports the results of the review to the director of the institution. The animal experiment can begin only after the principal investigator has received approval from the director of the institution.

The principal investigator conducts the animal experiment in compliance with the protocol approved by the director of the institution. If changes to the protocol are required that go beyond the approved scope of the experiment, procedures stipulated in the in-house regulations should be followed. After completion of the experiment, a report to that effect

should be submitted to the director of the institution in compliance with the inhouse regulations. If improvements indicated by the director of the institution are to be implemented, the principal investigator should confer sufficiently with the laboratory animal manager as required.

Below are examples of items that the principal investigator should consider when preparing a protocol, together with details of the animal experiment protocol form.

1) Items requiring consideration when drafting an animal experiment protocol

- * The objective and necessity of the animal experiment
- * Whether or not the animal experiment is unnecessary repetition
- * Whether an in vitro experiment could be conducted or the animal could be replaced by a phylogenetically lower species (use of alternative methods)
- * Whether a change could be made to a less invasive animal experimentation method.
- * The species of laboratory animals used and the genetic and microbiologic quality
- * The number of laboratory animals used
- * Educational and training experience of the researcher(s) and animal technicians.
- * Reasons why special cages and rearing environment are required
- * The anticipated disorders, symptoms and severity of pain resulting from experimental procedures
- * Measures to alleviate pain when it is anticipated that the laboratory animal will suffer severe pain
- * The use of sedatives, analgesics and anesthetics
- * Whether major surgical procedures should be repeated
- * Postoperative management methods
- * Terminal treatment of laboratory animals (method of euthanasia, etc.)
- * Whether the animal experiment could possibly affect people or the environment. If so, required measures and procedures
- * Issues concerning the occupational health and safety of the researcher(s) and animal technicians.

Concerning new animal experiments on as yet unrecognized research subjects, determining the experimental method and number of animals to use may pose problems. In such cases, attempts should be made to prepare a final protocol after conducting preliminary experiments to ascertain possible appropriate methods and number of animals. For protocols that entail unavoidable, severe pain for the animals, the principal investigator should conduct literature searches to determine whether alternative methods are available. If there are no alternative methods, in cases where the relief of pain through the use of measures such as anesthetics and analgesics is thought to be difficult, it is desirable that advice

be obtained from a laboratory animal specialist as required. When this is necessary, it should be clearly noted in the protocol.

2) Animal experiment protocol form (Abbreviated)

2 Experimental procedures

When conducting animal experiments, pain suffered by the laboratory animals should be reduced as much as possible within limits that allow the scientific objective to be achieved. Since scientific requirements differ for each animal experiment, the principal investigator should describe specific experimental procedures and the anticipated severity of pain in the animal experiment protocol and receive approval from the director of the institution after review by the Institutional Animal Care and Use Committee.

The principal investigator should retain test reagents, drugs and laboratory equipment appropriately. In particular, the laws and ordinances concerning the storage of controlled substances such as narcotics, poisons and deleterious substances must be observed. When conducting experimental procedures researchers should bear in mind the following points.

- * Acquisition of skills in restraining laboratory animals, administering drugs, obtaining samples and other techniques.
- * Acquisition of skills relating to surgical procedures (prolonged operative procedures such laparotomy, thoracotomy, craniotomy, orthopedic surgery and other procedures should be conducted under the guidance of a specialist with sufficient knowledge and experience of those procedures.)
- * Pain relief procedures for laboratory animals.
- * Observation of experiment discontinuation and completion criteria (humane endpoint).
- * Acquisition of knowledge and skills related to euthanasia procedures.

1) Laboratory and laboratory equipment (Abbreviated)

2) Animal restraint

Physical restraint refers to localized or general restriction of the normal movements of laboratory animals manually or with devices for examinations, sample collection, dosing and treatment. Restraining devices (restrainers, etc.) should be an appropriate size and easy to use, and should cause laboratory animals as little discomfort and injury as possible. When using restraining devices, training of laboratory animals is required to enable them to become accustomed to the devices and researchers. With dogs, cats and monkeys, if they are conditioned for aggressive restraint, their limbs extend outwards and they assume an immobile posture for short experimental procedures in many cases.

Restraint for a prolonged period in a monkey chair or other device should be avoided unless it is essential for achieving the research objective. Light restraint such as a leash for restraining monkeys or other devices that do not interfere with the natural posture of the animal is applicable within the range of experimental purposes. Items that should be considered concerning restraining devices are indicated below.

- * A restraint period only as long as that required to achieve the research objective.

- * Frequent observation of the condition of laboratory animals.

- * Release from the restraining device of laboratory animals suffering from trauma or poor physical condition due to restraint.

- * Restraining devices should not be considered as rearing devices.

- * Restraining devices should not be used as convenient tools for rearing management.

3) Food and drinking water restrictions (Abbreviated)

4) Surgical procedures (Abbreviated)

5) Analgesic procedures, anesthetics and postoperative management

The alleviation of pain in laboratory animals is important not only from the standpoint of animal welfare but also to assure the reliability and reproducibility of animal experiments.

- * Analgesic procedures should be initiated when symptoms of pain are perceived in a laboratory animal. When an animal feels pain, species-specific behavior includes vocalization, depressed behavior, abnormal expressions or posture and lack of movement.

- * To be able to perceive an abnormality, it is important to understand the behavioral, physiological and biochemical characteristics of that species (or individual) when at rest and at ease.

- * To select analgesic and anesthetic methods that do not interfere with the objective of the research, advice should be obtained from a physician, veterinary surgeon, pharmacist or other specialist as required.

The degree of observation of animals required during the postoperative recovery period depends on the animal species and the contents of the surgery. Attention should be focused on environmental temperature control, monitoring of cardiovascular and respiratory function and postoperative pain, with particular attention paid to symptoms of recovery from anesthesia.

- * To deal with unexpected situations, advice should be obtained from a laboratory animal health management specialist.

- * Monitoring items include depth of anesthesia and

physiological functions as well as evaluation of clinical symptoms and general condition.

- * Maintaining normal body temperature is effective for preventing cardiovascular and respiratory disorders caused by anesthetics.

- * During the recovery period after anesthesia, laboratory animals should be kept in a clean location at an appropriate temperature and humidity, with their condition monitored frequently.

- * Consideration should be given to parenteral infusions to maintain the water/ electrolyte balance, and to administration of analgesics and other agents for management of the surgical field.

6) Humane endpoint

The humane endpoint refers to the timing of termination of an experiment (in other words, the timing of the application of euthanasia procedures) to release a laboratory animal from severe pain and suffering. It is a term used in contrast to "death" as an endpoint that is used in protocols of animal experiments where the experiment continues until the animal's death.

- * As a rule, euthanasia procedures should be available for termination of animal experiments.

- * At the final stage of an animal experiment or when analgesics, sedatives or other agents do not provide relief, euthanasia procedures should be performed to release the laboratory animal from pain and suffering (one pain relief method).

- * Indications of when humane endpoint is applicable include food and water intake difficulties, moribund symptoms (self-injurious behavior, abnormal posture, respiratory disorders, vocalization, etc.), abnormal appearance over a prolonged period with no visible indications of recovery (diarrhea, bleeding, soiled genital area, etc.), weight loss (20% or more over several days), and marked increase in tumor size (10% or more of body weight).

- * Reference should be made to pertinent international guidelines for details concerning determination of the humane endpoint.

- * When conducting animal experiments in which the degree of pain and suffering is high, such as lethal toxicity studies, infection experiments and radiation experiments, the principal investigator should examine setting of the humane point in the planning stage of the animal experiment.

7) Euthanasia procedures

When disposing of laboratory animals on completion of the experiment in accordance with the animal experiment protocol or due to the laboratory animals being subjected to severe pain and suffering during the course of the experiment when anesthetics and analgesics can not be used in the research, the researcher(s) should conduct euthanasia.

Selection of the agent and method used for the euthanasia procedure depends on the animal species and the objective of the experiment. In general, a chemical method (overdose of a barbiturate anesthetic, administration of a non-explosive inhalation of anesthetic or carbon dioxide gas) or a physical method (cervical dislocation, decapitation, exsanguination under anesthesia, etc.) is used. However, from the standpoint of animal welfare, the principal investigator should seek the advice and guidance of a laboratory animal specialist as required since there are slight international differences on what are judged to be appropriate methods of euthanasia for laboratory animals.

* Euthanasia procedures refer to procedures resulting in the rapid loss of consciousness and then death of a laboratory animal not associated with pain or suffering. In addition to Guidelines on Methods of Sacrificing Animals (Notice No.40 of the Prime Minister's Office, July 4, 1995), international guidelines should be taken into consideration.

* Euthanasia should be performed by methods that do not cause distress to other animals in the laboratory. This requires careful attention because until animals lose consciousness they can vocalize and release pheromones.

* A person who has acquired the skills required for handling a particular animal species should conduct euthanasia procedures, and the death of the animal should always be verified.

9) Reports of animal experiment results (Abbreviated)

Only contents are shown hereafter. Full text can be obtained at;

<http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-k16-2e.pdf>

No. 5 Laboratory animal selection and receipt

- 1) Introduction of laboratory animals
- 2) Quarantine and acclimatization
- 3) Transport
- 4) Provision of information on delivery and receipt of laboratory animals

No. 6 Care and management of laboratory animals

- 1) Fundamentals of care and management
- 2) Cage environment and animal room environment
Housing space; Environmental temperature and humidity; Ventilation; Lighting; Food; Water
- 3) Retention of records

No. 7 Laboratory animal health management

No. 8 Facilities

No. 9 Safety management

- 1) Understanding and dealing with risk factors
- 2) Prevention of injury by laboratory animals
- 3) Measures when laboratory animals escape
- 4) Dealing with emergencies
- 5) Maintenance of the living environment

No. 10 Education and training

No. 11 Others

Additional provisions

Revision of these guidelines

References

Appendix