

Supplementary information

**Guideline for the Care and Use of Laboratory
Animals in Iran**

In the format provided by the
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Supplement 1. Guideline for the Care and Use of Laboratory Animals in Iran

1. Chapter 1: Responsibility of Persons and Organizations

1.1. General Rules

1.1.1. Any intervention on laboratory animals can only be performed in the context of a project approved by a relevant ethics committee. Before receiving approval from the relevant ethics committee, no action regarding laboratory animals, such as placing animal purchase orders, should be initiated.

1.1.1.1. Performing pilot experiments is subjected to the same rules and regulations governing ethical considerations of original research. To avoid the prolongation of the ethical review of pilot experiment proposals, the ethics committees should initiate the processing of these proposals at the first available time following submission of the proposals.

1.1.1.2. For projects that are approved by the ethics committee outside the Islamic Republic of Iran (IRI), or any joint project with other countries, if any part of the project requires using animals in Iran, the project must be reviewed and approved by a relevant ethics committee in IRI.

1.1.1.3. If any part of the projects that are approved by the ethics committee outside IRI (or any joint project with other countries), require the use of animals outside of Iran, the principal investigator of the project must present a copy of the ethical approval, issued by the ethics committee of the relevant country/countries, to the ethics committee in IRI. Not receiving the

aforementioned copy by the relevant ethics committee in Iran leads to automatic ethical rejection of the project from the perspective of the Iranian body. Further, the relevant ethics committee in the IRI has full authority to decide on the ethical approval/rejection of a foreign-based project, and reject an approved project, if said project is noncompliant with the content of the current guideline or other pertinent Iranian regulations.

1.1.1.4. Cooperation in projects that are mutually performed with institutions not under the Ministry of Health and Medical Education, requires ethical approval of the project by an ethics committee that is accredited by the National Committee for Ethics in Biomedical Research.

1.1.1.5. Any cooperation of the persons or organizations in laboratory animal projects without proper approval from a Competent Scientific Council and a relevant animal ethics committee, which is approved by the Ministry of Health and Medical Education, is considered as a research misconduct.

1.1.2. Laboratory animals can only be used for the following purposes:

1.1.2.1. Basic research;

1.1.2.2. Practical research, or translational research aiming to transfer the results of basic science to practical use with the goal of improving the health and wellbeing of human or other living beings. The aim of this research could be:

- a) Prevention, diagnosis, or treatment of diseases or any abnormal condition or complication, in humans, animals, or plants;
- b) Evaluation, diagnosis, regulation, or improvement of the wellbeing of humans, animals, or plants;

- c) Welfare of animals, and improvement of farm animals' production and welfare.

Note 1) Using laboratory animals for the development or production of drugs, food, and other materials is conditional to the availability of scientific justification(s) for the absolute necessity of using animals for these purposes.

Note 2) Using laboratory animals for testing of the quality, and/or efficacy, and/or safety of drugs, food, and other materials is conditional to the availability of scientific justification(s) for the absolute necessity of using animals for these purposes.

- 1.1.2.3. Environmental protection with the aim of preserving the health and welfare of humans or animals;
- 1.1.2.4. Research aiming conservation of animal species;
- 1.1.2.5. Using animals for education, specifically for higher education, continuous education, apprenticeship, educational workshops, improving professional skills, and alike [when it's impossible to use replacements or alternative methods for these scientific purposes];
- 1.1.2.6. Using animals for the production of biological materials;
- 1.1.2.7. Research in forensic medicine.
- 1.1.3. All interventions should only be performed at the location designated in the ethics committee approval.
 - 1.1.3.1. The ethics committee may exempt some projects from this article, according to proper scientific justifications. By doing so, all biological, ethical, and scientific consequences that could arise from the performance of the

intervention outside of a designated institution must be considered. The principal investigator and the exempting ethics committee share responsibility in this regard.

1.1.3.2. No intervention should be performed in animal holding places and there should always be a standard physical barrier or distance between animal holding places and procedure rooms. When the performance of the project outside the animal holding place is essentially impossible due to certain project requirements, this issue must be reviewed and decided by a relevant ethics committee.

1.1.4. All interventions in a project must be performed according to four basic principles, named as 4Rs: Responsibility, Replacement, Reduction, and Refinement:

1.1.4.1. Responsibility principle: Animals are capable of perceiving unpleasant experiences such as pain, suffering, hopelessness, depression, anxiety, and fear, and they are also capable of experiencing pleasant feelings such as welfare and comfort. Therefore, all individuals involved in the production, care, and use of laboratory animals are responsible to respect the sanctity of the animals' lives and welfare, while aiming to achieve valid scientific results. This principle is based on religious beliefs, human conscience, and ethics. Accordingly, all individuals should implement professional ethical criteria and scientific principles and standards at their research.

1.1.4.2. Replacement principle: Whenever possible, using replacement methods rather than live animals should be prioritized. Various methods may be used in this regard; such as using non-sentient or significantly less-sentient living beings (e.g., plants, microorganisms, protozoa, and parasites), using the carcasses of animals that are euthanized according to certain veterinary clinical matters, computer methods, statistical and mathematical calculations,

animal simulators, *in vitro/ex vivo* experiments, organ-on-chip or body-on-chip methods, microdose experiments on humans, and other valid methods. Nevertheless, if there is a replacement method available to run an experiment, the use of animals in that experiment is forbidden. According to the high cost and complexities of animal studies, and the extensive prerequisites that are required for performing proper animal research, whenever there is a replacement method available for achieving research or educational goals (such as performing student theses or alike), no real person or legal entity may demand the use of animals for that purpose.

- 1.1.4.3. Reduction principle: The number of animals used in a project should be reduced to the minimum possible. However, reducing the number of animals should not cause an increase in the pain and suffering of the other animals being used. Also, reducing the number of animals should not negatively affect the scientific validity of the project. In other words, if the number of animals used in an experiment would be lower than a certain amount, the results of the experiment would not be statistically relevant and this may necessitate repeating the experiment and therefore increasing the total number of animals being used. An improper number of animals may also lead to misleading results that could harm scientific integrity and eventually humans and other living beings. There are various methods suggested for proper reduction of the number of animals in a project, such as statistical methods, proper design of the experiments (e.g., using factorial design or other appropriate designs), using *in vitro* screening tests before advancing experiments on laboratory animals, reducing the experimental error by using more accurate techniques and more advanced devices, proper selection of the animal species according to the type of the study, and other relevant methods.

1.1.4.4. Refinement principle: Researchers should provide the best possible condition for animal care and use, in order to reduce the pain and suffering of animals to the minimum possible. Various methods for the improvement of animal welfare include: proper learning of theoretical and practical principles of working with laboratory animals, applying the best techniques when working with laboratory animals, designing a sound methodology for the experiments, and using appropriate animal species in a project. The principal investigator must select animal species with the least possible capacity to perceive pain, suffering, discomfort or lasting harm. Preferably animals in the lower evolutionary classification of the nervous system, which their results are relevant to the target species of the project (e.g., human), should be used. It is necessary to avoid using the spontaneous death of the animals as an experimental endpoint, because this may entail severe pain and suffering before the death of the animals.

1.1.5. The method of performing interventions is determined according to the species, body weight and size, sex, age, clinical condition of animals, type of the interventions, and other relevant criteria. In general, said method should provide the maximum possible welfare for animals, and should be designed according to the technical principles and methods provided in the valid and up-to-date scientific resources. When choosing between various interventions, the one that is more likely to produce reliable scientific results and can accomplish all of the below criteria more than the others, is preferred:

1.1.5.1. Using fewer animals;

1.1.5.2. Choosing the animal species recommended by the latest and valid scientific resources. Where different species of animals could be used, the principal investigator is responsible for selecting the animal species with the least

possible capacity for perceiving pain, suffering, distress, or lasting harm (such as the animals lower in the evolutionary development of the nervous system);

- 1.1.5.3. Causing the least possible amount of pain, suffering, distress, or lasting harm.
- 1.1.6. Research and educational activities, specifically those that may cause pain or distress for the animals, should be performed as quick as possible. However, the shortness of the duration of pain and suffering cannot justify performing very painful or very distressful projects, and cannot justify the performance of any project that is ethically prohibited according to the content of the current guideline.
 - 1.1.6.1. In particular, animal restraining using physical, chemical, or any other methods should be accomplished in the shortest time possible. It is also required to avoid unnecessary confinement of animals for prolonged periods. In projects where prolonged restraining or confinement of animals are essential (such as using metabolic cages), necessary precautions should be undertaken to meet the biological needs of the animals and to prevent their behavioral and psychological disturbances. It is also necessary to use proper environmental enrichments for the animals under prolonged confinement, and to maintain standard environmental conditions at the animal holding place, according to the technical principles provided in the up-to-date and valid scientific resources. These animals should also be regularly evaluated by a designated veterinarian with knowledge in caring of animals under prolonged confinement, and due actions should be undertaken to improve the animals' welfare.
- 1.1.7. Performing any intervention, which is classified with an actual severity score of 'severe' according to the Ethical Guide for the Severity Scoring of Interventions on Laboratory Animals, and also any other interventions that may lead to severe pain,

suffering, and/or distress and these conditions are likely to be prolonged and could not be alleviated, is prohibited.

1.1.8. If a research proposal with actual severity scoring of ‘moderate’, requires no action to be performed for alleviating the pain, distress, and suffering of animals (e.g. due to the belief of the researchers that using analgesics may interfere with the results of the project), and a relevant ethics committee concludes that this is not compliant with the ethical and/or scientific principles, the ethics committee must reject the issue. If the ethics committee cannot reach to a consensus decision in this regard, the committee should confidentially investigate the ethical and scientific opinions of at least two experienced laboratory animal researchers that are independent of the project, do not have conflicting interests with the project, and are unaware of the identity of the persons involved in the project:

1.1.8.1. If the decision of both reviewers or one of them requires the alleviation of the pain, distress, or suffering, then the project proposers must undertake all required actions to alleviate the pain, distress, or suffering caused by the intervention. Otherwise, the ethics committee is obliged to reject the research proposal;

1.1.8.2. If the decisions made by both reviewers indicate no necessity for alleviating the pain, distress, or suffering caused by the intervention, then the ethics committee should perform a harm-benefit analysis for the project to evaluate the likely benefit of the project against the pain and suffering caused for the animals, and finally decide on the approval or rejection of the project. A condition for approving such projects is that it is scientifically justifiable that achieving the goal of the proposed project is not feasible by using any kind of analgesics and the goal of the project has such an importance that not

performing the project, would cause substantial harm to the wellbeing of humans or animals.

1.1.9. If there is an animal that has been used in one or several interventions/projects, and there is another animal that has never been used in any intervention/project but could have been used otherwise, the former animal can only be re-used in a new project when all the below criteria are met:

1.1.9.1. The maximum actual severity of the previous projects/interventions on the former animal, had a level of 'mild' or 'moderate' according to the Ethical Guide for Severity Scoring of Interventions on Laboratory Animals;

1.1.9.2. It is demonstrated that the general welfare, health, and psychological state of the former animal are completely restored;

1.1.9.3. The maximum actual severity level of the future projects/interventions that are supposed to be performed on the former animal would have a severity level of 'mild' or 'moderate', or to be 'non-recovery'.

1.1.9.4. The re-use of the former animal is performed under the direct supervision of the designated veterinarian and according to the veterinarians' recommendations. In this regard, the clinical history and the health condition of the reused animal during its life should be considered.

1.1.10. Medications, tools, and equipment used for working with laboratory animals should have relevant standards, proper functionality, and their expiry date should be observed. Equipment and devices should be in good functional order, and this issue is specifically important for the equipment that their improper function could cause pain and distress to animals (such as the dull tip of the injection needles, or the blunt guillotine blades).

1.1.11. Eating, drinking, and smoking in laboratory animal facilities are prohibited.

1.1.12. Any animal-related injuries to staff working with laboratory animals, regardless of the extent of the injury, must be immediately reported to the principal investigator of the project or his/her legal representative. The injured person should receive proper medical care, and if needed, should be immediately transported to the nearest medical facility.

1.2. Rules Pertinent to organizations

1.2.1. Breeders, suppliers, or users of laboratory animals must have permissions related to their activity from a relevant ethics committee [by registering on the Website of the National Committee for Ethics in Biomedical Research].

1.2.1.1. The activity permission can only be issued to breeders, suppliers, and users of laboratory animals if they have received all required permissions from other relevant organizations, and in general, their activity must not entail any legal prohibition.

1.2.1.2. Granting the activity permission to breeders, suppliers, and users is subject to their compliance with the current guideline and any other relevant documents or guidelines. The activity permission has a fixed-term validity, and a renewal request should be submitted for its continuation. The validity period of the activity permission is determined by the ethics committee according to certain circumstances but it should not exceed five years. In this regard, the ethics committee should ensure that during the whole course of the activity permission's validity, all ethical standards of working with laboratory animals would be maintained, and no deviation from the proper condition of performing projects or maintaining animal welfare would occur. Some criteria that may be considered for determining the validity period of the activity permissions are: condition of the animal holding places or procedure rooms of the breeders, suppliers, or user facility, the competence level of individuals performing the procedures in the facility, and the history of the compliance of the facility's real persons and legal entity with the content of the current guideline.

1.2.1.3. Any significant change(s) in the structure or function of the breeder, supplier, or user organizations that may have negative consequences on the welfare of

animals, require granting new activity permission by the relevant ethics committee. The ethics committee or any other relevant entities may determine whether the change(s) of the structure or function pertinent to the current article are significant; however, proving that the change(s) are not significant, is in the solemn authority of the relevant ethics committee.

1.2.1.4. The activity permission granted by the ethics committee should designate a real person as the 'responsible person' for ensuring the compliance of the performance of the breeder, supplier, or user organization with the content of the current guideline. The aforementioned person should have successfully completed all the relevant theoretical education and practical training according to his/her responsibilities in the organization and possesses the necessary theoretical and practical competence and licenses.

1.2.2. In general, a user facility requires the below spaces (items marked with asterisks are mandatory). The characteristics of these spaces should be in accordance with the valid and up-to-date scientific resources:

1.2.2.1. Quarantine room for incoming animals and isolation of sick animals*;

1.2.2.2. Animal holding places*;

1.2.2.3. Temporary caring place for animals that have been used in the experiments, before returning to the main animal holding places*;

1.2.2.4. Place(s) for performing specialized experiments, postmortem examinations, radiography, formulation and preparation of feed, clinical treatment of sick animals, performing diagnostic tests;

1.2.2.5. Place for performing interventions*;

- 1.2.2.6. Places for receiving and storage of feed, bedding, medications, and biological materials;
 - 1.2.2.7. Places for storage or use of equipment for sanitation, disinfection, and sterilization*;
 - 1.2.2.8. Freezer (-20 degrees centigrade) for storage of carcasses and/or a freezer for the temporary storage of wastes before disposal*;
 - 1.2.2.9. A place for animal care personnel, technicians, general staff, and academics*;
and
 - 1.2.2.10. Bathroom.
- 1.2.3. Any required items or conditions for animal care, restraining, and use, specifically those that are important for the safety and health of personnel or animals, must be available according to the animal species in a facility. In case of the lack of these items in a place, the projects that require them must not be performed in that place. Any injury to the personnel or animals that may happen due to the lack of required items, apart from its legal liabilities, would be considered as an ethical misconduct.
- 1.2.4. All laboratory animal facilities are required to perform a risk assessment according to each species of animal in the facility, medications being used or stored in the facility, and equipment and devices in the facility. Laboratory animal facilities should then provide a list of potential dangers and appropriate protocols for preventing and managing them. This list should be readily accessible to all persons in the facility.
- 1.2.4.1. The responsible person at places where potentially dangerous medications and equipment may be used, should ensure the safety and security of these medications and equipment. These items should be out of the reach of unauthorized persons. This article is specifically important regarding medications and equipment that may be used for euthanizing animals.

- 1.2.4.2. Laboratory animal facilities should have standard first aid kits and other therapeutic means, according to the potential risks in the facility. First aid kits and other therapeutic means should be visible and accessible by all relevant persons.
- 1.2.4.3. Depending on the types of risks in a facility, it may be necessary to have at least one onsite medical doctor and/or a person with training in first aid and rescue. This person should be familiar with relevant first aid techniques according to the potential risks in the facility and should have appropriate legal permissions for performing these techniques.
- 1.2.4.4. It is necessary to identify the nearest medical facilities that are equipped with necessary means for managing potential dangers of the animal facility. Their address and phone number should be readily available to all persons and there should be a proper means available for transferring injured persons to these medical facilities. For example, if in an institution, venomous animals are kept or strong opiates are routinely used, it is necessary to identify the nearest medical facilities which have specific anti-venom or proper antidotes. The fastest possible route to each medical facility should be determined in advance. It is also necessary to determine alternative medical facilities and alternative routes to each facility.
- 1.2.4.5. All laboratory animal facilities should have a safe and secure area in which temporary visitors (such as delivery persons) could carry out their tasks, and leave the facility. Entering other places of the facility (beyond the above-mentioned area) requires induction of the incoming person by designated personnel of the facility. If the person requires to stay in the facility for more than one day, the induction should include familiarity with different places of the facility, and knowing the security and safety rules of working in the

facility. If the person needs to stay for a day or less in the facility, the induction should include familiarity with the security and safety rules of working in the relevant places in the facility. All inducted persons should sign a formal document stating that they have received primary information regarding the facility and the security and safety rules of working in the facility.

1.2.5. All breeders, suppliers, and users should have at least one designated veterinarian or trained technician with competence in laboratory animal medicine. This person, as far as possible, should have no conflict of interest with projects he/she oversees, and should not be a principal investigator or co-investigator of those projects. This person is named as a 'designated veterinarian' or 'competent technician' throughout this text. He/she has the following responsibilities and should always be accessible:

- 1.2.5.1. Prevention, diagnosis, control, and treatment of the diseases of laboratory animals;
- 1.2.5.2. Prevention, alleviation, or treatment of the pain and suffering of laboratory animals;
- 1.2.5.3. Providing necessary means and conditions for proper performance of the projects; such as, proposing methods of performing laboratory animal projects, preparation of the information (e.g., normal biological values), and performing special tasks (e.g., anesthesia, analgesia, routine surgeries, post-surgical care, intensive care for sick animals, and/or euthanizing animals according to the proper scientific and ethical principles);
- 1.2.5.4. Development and management of programs in reproduction, husbandry, transportation, and working with laboratory animals;

- 1.2.5.5. Development and updating of regulations regarding the use of laboratory animals in projects and other scientific activities in the institution;
 - 1.2.5.6. Management of the design and maintenance of the required spaces and facilities for breeding, husbandry, transportation, and use of laboratory animals;
 - 1.2.5.7. Control and management of the physical environment related to laboratory animals (such as controlling temperature, humidity, required space area, air quality, lighting, noise, environmental enrichment, and other appropriate matters);
 - 1.2.5.8. Providing consultation and recommendations according to the content of the current guideline, relevant regulations, acceptable sources of information, and other standards pertinent to the welfare and health of the animals used in projects;
 - 1.2.5.9. Practical training for the care and use of laboratory animals;
 - 1.2.5.10. Selection and execution of animal models in projects;
 - 1.2.5.11. Cooperation in the design and management of scientific projects using laboratory animals.
- 1.2.6. Conditional to all relevant permissions being granted by relevant authorities, persons working at laboratory animal facilities should be able to attend at their workplace, any day of the week and at any required time to attend to tasks related to the care or use of laboratory animals (such as feeding and watering animals, providing veterinary care, and other relevant matters). By considering all state rules, institutional regulations, and any other rules and regulations, the relevant authorities should ensure that the commute of the person(s) to/from their workplace and their presence at their

workplace within the required times would not breach any regulations and their safety and security is maintained.

1.2.6.1. No person should work alone in animal holding or use places, as far as possible. The person responsible for the laboratory animal facility should be aware of the entrance and leaving times of persons in the facility and the reason for their presence in the facility. To record the identity of persons and their entrance and leaving times, a booklet or other appropriate methods could be used.

1.2.7. All animals that are supposed to be used in interventions, regardless of being group-housed or solitary-housed, should receive unique permanent identification marks as soon as possible after birth. The marking method should cause the least possible amount of pain.

1.2.7.1. Erasure marking or not marking the animals by breeders, suppliers, or users would interfere with the supervisory tasks of the ethics committees and would be considered a violation of the rules. In specific cases where it is required not to identify an animal, justifying reasons should be provided to the ethics committee and approval should be sought for not identifying certain animals. Even then, it is still necessary to have a proper method of identification and propose this method to the ethics committee. In this regard, methods such as identification by superficial body signs, hair color, specific pattern of the hair colors, specific characteristics on the body (e.g., the pattern of the mucous membrane colors of dogs), or photo taking could be considered.

1.2.7.2. When a dog, cat, or primate is transferred between breeders, suppliers, or users before marking, it is necessary for the receiver to keep a record of the

animal's history, specifically its parents, until the marking of the animal is performed.

Note: For small rodents or species of animals that individual marking is not always possible, identification marks and the above-mentioned information could be registered for a group of them.

1.2.8. Breeders, suppliers, and users must create an individual record sheet for each animal upon its arrival into the facility. The record sheet should include the following information:

1.2.8.1. The identification number of the animal, name of the animal, species, sex, birth date (hour, day, month, and year), place of birth, information about the parents of the animal (including the identification number of the parents), birth weight, trend of weight change, veterinary medical history, genetic identity, information about the previous holding places of the animal, date of death (hour, day, month, and year), cause of death, place of death and other relevant information;

1.2.8.2. It is necessary to determine if the animal is essentially bred for being used in research interventions, or is it acquired from other sources;

1.2.8.3. In case of transferring the animal to another place, it is necessary to provide the recipient with a copy of the above-mentioned record sheet.

Note: When having a separate record sheet for each individual animal is not always possible (such as rodents), it is necessary to provide the record sheet for each group of the animals.

1.2.9. All breeders, suppliers, and users are required to keep a record of the following information regarding the function of their institution:

- 1.2.9.1. The number and the species of the animals that are bred, supplied, used, returned to nature, adopted by the public, or transferred to farms;
- 1.2.9.2. The dates in which animals are bred, supplied, used, returned to nature, adopted by the public or sold;
- 1.2.9.3. Source of the animals, and information about if they are specifically bred to be used in interventions or not;
- 1.2.9.4. The place(s) where animals are sourced from;
- 1.2.9.5. Name and address of the recipient of the animals;
- 1.2.9.6. The number and the species of animals that have died or were euthanized in the institution. For dead animals, it is necessary to mention the cause of death- if diagnosed;
- 1.2.9.7. User institutions should record the details of the projects that animals were used in.

Note: The hard copy of the above-mentioned records should be kept for at least five years and should be provided to the ethics committee upon their request. Soft copies of the records should be kept forever and should be provided to the ethics committee upon their request.

- 1.2.10. Breeders of laboratory animals should provide the animals' identification data, health certification, and genetic certification to the receivers. The minimum required identification data of animals are: identification number, species, birth date (hour, day, month, and year), birth weight, and identification numbers of its parents. The minimum required information in the health certification of the animals should identify the presence or absence of specific pathogen(s) in them. The minimum required information in the genetic certification of the animals should determine if

they are inbred or outbred, and should provide users with specific genetic information regarding the animals.

1.2.10.1. In case of observing an unfavorable characteristic in the bred animals, the breeder must endeavor to find the reason(s) and to perform appropriate actions to prevent similar occurrences.

1.2.10.2. The breeder is required to handover the animal(s) to the authorized transporting person identified in the project approval or to a legal representative of the supplier/user institution.

1.2.11. The environmental condition in the breeding and holding place of animals should provide the maximum possible welfare for the animals and should be set according to the principles provided in the valid and up-to-date scientific resources.

1.2.11.1. The breeding of animals should be performed according to ethical and scientific principles. It is necessary to avoid the breeding of animals with [unfavorable] genetic deficiencies or to avoid excessive breeding of animals that may cause harm to breeder animals.

1.2.12. The animal user institutions should provide the possibility of sharing organs and tissues of animals that are euthanized. The aim is to reduce the number of animals used in the projects and to prevent unnecessary killing of animals merely for acquiring their organs and tissues. This could also encourage cooperation between projects requiring similar animal models, with the aim of increasing the efficiency of using resources. To protect the intellectual properties of the sharers and users of the shared organs and tissues, a formal agreement must be signed before initiating any sharing or cooperation. A copy of the agreement should be submitted to the relevant ethics committee.

1.3. Rules pertinent to ethics committees

1.3.1. From the issuance date of the current guideline, the animal ethics committees are the responsible body for reviewing and approving all projects performed on laboratory animals. In terms of the structure, members, and responsibilities; the animal ethics committees follow the contents of the Directive for Establishment, Performing Methods and Tasks Description of Research Ethics Committees. The aforementioned animal ethics committees should be accredited by the National Committee for Ethics in Biomedical Research in the Deputy of Research and Technology in the Ministry of Health and Medical education, after approving by their respective parent institutional/regional research ethics committees.

1.3.1.1. Animal ethics committees should follow the standard structure defined by the National Committee for Ethics in Biomedical Research, in which the committee is consisted of the following members for evaluating research proposals concerning laboratory animals: veterinarian or technician with substantial experience in working with laboratory animals; lay person or a representative of a formal animal protection organization in the country; a scientist with substantial experience in working with laboratory animals; a person non-professional in laboratory animal science which has never performed research or educational activities on animals but interested and active in animal welfare matters, as a representative of the general public.

1.3.1.2. If any of the above-mentioned persons could not attend a [decision making] meeting of the ethics committee [regarding certain research proposal(s)], their consultation could be sought using referable communication media such as email, letter, or facsimile. In this regard, appropriate information should be provided to these persons, their comments should be received in due time,

and their comments should become available to all members of the ethics committees attending the meeting.

1.3.1.3. The National Committee for Ethics in Biomedical Research selects senior lecturers from a pool of competent candidates to train other lecturers in laboratory animal science courses. The National Committee for Ethics in Biomedical Research also provides senior lecturers with appropriate teaching facilities.

1.3.2. Even if the total amount of pain, suffering, distress, or lasting harm of a certain project seems to be below the minimum pain threshold, the principal investigator is still responsible for submitting a formal research proposal for ethical review by the ethics committee. The project can only be initiated upon receiving formal ethical approval from the relevant ethics committee.

1.3.3. Applying any minor, major, or essential amendments to the approved research proposals, or any deviation from the approved research protocol in the course of the study, is conditional on proper informing of the relevant ethics committee, and seeking approval from the committee. If the ethics committee disapproves any of the proposed amendments, no changes should be made in the approved research proposal in that regard.

1.3.3.1. Minor amendments are considered modifications to the project that would not cause any added pain or suffering for the animals. It should be noted that applying several minor amendments may eventually lead to increased pain and suffering of the animals and in this case, the amendment would be classified as 'major'.

1.3.3.2. Major amendments include modifications that may cause increased discomfort, pain, or suffering for the animals during the course of the project.

- 1.3.3.3. If performing several minor and/or major amendments are anticipated in a project, the amendment would be classified as a 'major' or 'essential' at the discretion of the ethics committee.
 - 1.3.3.4. Essential amendments are modifications that could alter the ethical or scientific essence of the project, and may introduce new ethical matters to the project in comparison with the previously approved project.
 - 1.3.3.5. Performing minor or major amendments to an approved project requires the submission of the amendment request.
 - 1.3.3.6. Performing essential amendments requires the submission of a new proposal. In other words, performing essential amendments in an approved research proposal revokes the prior approval and requires that the research to be redefined for the ethics committee within a new research proposal.
 - 1.3.3.7. Reviewing, approval, or rejection of minor amendment submissions could be performed by the secretary of the ethics committee in cooperation with the designated veterinarian and the lay person. Review, approval, or rejection of research proposals requiring major or essential amendments should be performed by a full ethics committee with all members present.
 - 1.3.3.8. The maximum permitted number of performing minor or major amendments in an approved project is determined by the relevant ethics committee; so that performing several amendments would not cause an essential change in the project.
- 1.3.4. Appeals to the decisions of the ethics committee regarding research proposals would be reviewed by a higher ethics committee, and finally by the National Committee for Ethics in Biomedical Research in the ministry of health and medical education. The

decision of the National Committee for Ethics in Biomedical Research is final and non-appealable.

- 1.3.5. The animal ethics committees have the following capacities regarding laboratory animal projects, and these are in addition to the committees' regular competencies according to the Directive for Establishment, Performing Methods and Tasks
- Description of Research Ethics Committees:

1.3.5.1. Supervision of the function of the breeders, suppliers, and users of laboratory animals according to the content of the current guideline; issuing or revoking the permissions for the function of these institutions according to their compliance with the content of the current guideline;

1.3.5.2. Planning and supervising the performance of educational courses regarding the care and use of laboratory animals: the competence of the lectures of the educational courses must be approved by a competent scientific body and a relevant ethics committee. The educational courses must be performed according to the content of the Ethical Guide for the Use of Animals for Educational Purposes. Each course should be formally registered by completing a proposal for ethical review of the use of animals for educational purposes. The lecturers of these courses must possess a superior ethical, scientific, and practical competence in working with laboratory animals;

Note: Since the certificates of these courses have a validity period, the holder of the certificate should attend a valid continuing education course before the expiration of his/her certificate. The expired certificates could not be used for granting permission to use laboratory animals.

1.3.5.3. Approval or cancelling permission to use laboratory animals for anyone who may be involved in working with laboratory animals during the interventions. Approval or cancelling the permission would be decided according to the

scientific and technical competence of the persons involved in working with laboratory animals, valid educational courses undertaken, not having a history of ethical or scientific misconduct in working with laboratory animals (at the discretion of the relevant ethics committee), and their compliance with ethical standards of the current guideline.

Note: Permissions to use laboratory animals have a maximum validity of five years from the date of issuance.

- 1.3.5.4. Ensuring that all personnel involved in working with laboratory animals, have access to information related to their type of work and the species of laboratory animals they are working with; all personnel are sufficiently educated; possess practical competence; receive continued education; and work under the supervision of a competent person until they are deemed competent.
- 1.3.5.5. Ethical review and approval of submitted research proposals;
- 1.3.5.6. Assigning auditors for reviewing [laboratory animal related] records and reported events, and performing prearranged or unannounced inspections of places where laboratory animals are used. The auditors are required to evaluate the quality of animal care and use, and report the level of compliance of the facility with the content of the current guideline. The Ethics committee reviews the received reports of the auditors in a meeting and undertakes appropriate actions. All required modifications, and requirements for improving the welfare of animals should be reviewed by the members of the ethics committee, and appropriate actions should be taken accordingly;
- 1.3.5.7. Providing annual reports to the National Committee for Ethics in Biomedical Research concerning the number of animal projects and total budget

dedicated to the projects involving laboratory animals; the number and species of animals used; and the number of projects classified with severity scoring of ‘mild’, ‘moderate’, ‘severe’, and ‘non-recovery’;

- 1.3.5.8. Preparing annual reports (concerning the activity of the committee and its subordinate committees) regarding projects involving laboratory animals, for presenting to the National Committee for Ethics in Biomedical Research;
 - 1.3.5.9. Supervising on animal welfare and the state of care and use of laboratory animals in related facilities;
 - 1.3.5.10. Providing feedback to the relevant superordinate ethics committee, or the National Committee for Ethics in Biomedical Research, regarding any action that improves the ability of the related university/institution to better comply with the content of the current guideline.
- 1.3.6. The basis of performance for related ethics committees are the current guideline and all relevant guides or regulations issued from superordinate ethics committees. Any doubt about the meaning of the content of these documents should be inquired from the relevant superordinate ethics committees.
- 1.3.6.1. In case of rejection of a research proposal by an ethics committee, the ethics committee must inform the scientific body that has approved the scientific aspect of the research proposal. The ethics committee must also inform the granting body(ies) of the project.
- 1.3.7. Any real person or legal entity could report any ethical misconduct related to laboratory animals in a project. The report should be sent to the head of the ethics committee that has approved the project. The ethics committee is responsible for performing appropriate actions while considering the confidentiality of the received information and identities of the persons involved. If necessary, the ethics committee

should report the issue to the relevant competent authority(ies) for further action. If a violation of the rules is proved, the perpetrator(s) would be subject to the content of the Directive for Persecution of Research Misconduct in the Field of Medical Science Research.

1.3.7.1. The competence of all persons involved in an [animal] project according to their role in the project should be approved by the ethics committee.

1.3.7.2. If the ethics committee concludes that a breeder, supplier, or user does not comply with the current guideline, or the ethics committee finds that ethical principles of working with laboratory animals are breached in any stage of a project, even after finishing the project and publication of the results, the ethics committee is required to take appropriate disciplinary actions according to the level of non-compliance and its effect on the animal welfare. The disciplinary action could be in the form of verbal notification, written admonition, commanding certain corrective actions, suspending the project, cancellation of the project, or cancellation of the ethical approval for continuation of the project. The ethics committee must, as soon as possible, inform its decision to the scientific body -that has approved the project- and the granting body(ies) of the project.

Note: Before suspending or cancelling the projects, the ethics committee should carefully consider the fate of the animals related to the project, and ensure that by suspension or cancellation of the project, their welfare would not be affected unfavorably.

1.3.7.3. Publishing the results of the interventions performed on laboratory animals must follow the relevant guidelines, especially Ethical Guideline for Publications in Medical Sciences.

- 1.3.8. The ethics committee must ensure that, whenever possible, proper scientific methods or alternative strategies are used instead of performing interventions on live animals.

Note 1: Due to extensive advancements in toxicity testing methods, the users of laboratory animals must apply the most advanced methods of toxicity tests with the aim of the replacement, reduction, or refinement of the use of laboratory animals in their projects. If using the most advanced methods is not feasible in a certain project, the principal investigator is required to demonstrate his/her awareness of the available methods to the ethics committee, and inform the ethics committee why he/she is unable to use these methods in a certain project. If it would not be possible to provide the necessities of using the most advanced methods in the short run, the ethics committee should perform a cost-benefit analysis on the research proposal and decide on approving or rejecting it.

Note 2: The ethics committee should, as far as possible, provide the prerequisites of using the replacement methods for their relevant scientific community.

- 1.3.9. The ethics committee should ensure that the minimum acceptable number of animals that could develop valid scientific results would be used in projects.

1.3.9.1. An estimated morbidity and mortality count of animals in a project should be reported to the ethics committee before starting the project.

1.3.9.2. If the actual rate of morbidity and mortality of animals during the project exceeds the estimations, and the principal investigator requests increasing the number of animals in the project, the ethics committee should investigate the issue as soon as practicable and decide on the approval or rejection of the request by considering the facts and the technical justifications produced by the principal investigator.

- 1.3.10. Ethics committees must ensure that appropriate actions are taken to refine the breeding, husbandry, transportation, and use of laboratory animals with the aim of eliminating or alleviating pain, suffering, distress, or lasting harm to the animals.
- 1.3.11. The ethics committee must ensure that there is enough equipment, human resources, and knowledge for executing the content of the current guideline when performing certain projects in certain facilities; otherwise, the ethics committee must reject [the performance of] these projects [in the certain facilities].

1.4. Rules pertinent to animal users

- 1.4.1. The principal investigator or the proposer of a laboratory animal project, should possess expertise in the subject of the project, the project's methodology, and principles of working with laboratory animals in the project, so that he/she could develop a novel and appropriate research question or hypothesis, and could design a proper method for examining it. If he/she does not have enough expertise for proper designing of any part of the project's methodology, or methods of working with laboratory animals, he/she should engage other competent expert(s) in the project and apply their recommendations in designing and performance of the project. The question or hypothesis for the project should not be a repetition or duplication of prior projects. Performing minor changes in the methodology of previous projects, without proper scientific justification(s) or important effects, is not acceptable. Acknowledging scientific properness of the justification(s) [is at the discretion of the ethics committee and] should be performed according to valid scientific data. The benefit(s) of the project should be weighed against its costs that may include pain, suffering, and harm to the animals, required financial and human resources, and the time resources of the researchers. The cost-benefit analysis should be performed by the ethics committee. In general, the question or hypothesis of the project should have a reasonable probability to add a value to the current knowledge of the human, and/or could be effective in resolving the problem from human society or the life of other living beings.
- 1.4.2. To request an ethical review of a project, the principal investigator must complete an Application Form for the Ethical Review of Research on Laboratory Animals and submit it to the relevant ethics committee.

- 1.4.2.1. Ethical review of laboratory animal projects in ethics committees should be performed following scientific approval of the project by the relevant scientific authority (such as a university/faculty scientific research council).
- 1.4.2.2. When publishing the results of a project, the author(s) are responsible for providing, in the publication, the ethical approval number of the project along with the name of the issuing ethics committee.
- 1.4.3. The principal investigator is required to determine the real severity score of the project according to The Ethical Guide for Severity Scoring of Interventions on Laboratory Animals and this should be reflected in the ethics application form.
- 1.4.4. The highest responsibility regarding the performance of the content of the current guideline, all matters relevant to the welfare of laboratory animals, and ethics of using animals in a project, is with the principal investigator of the project that has submitted the research proposal of the project to the ethics committee and has accepted its implementation. However, this does not mean to relieve the responsibilities of other persons involved in the project. All involved persons according to their roles in the project have certain responsibilities regarding animal welfare and ethical rules of working with laboratory animals. The principal investigator of the project is responsible for defining each person's task description and responsibilities in the project and ensuring that this is well understood by everyone involved.
- 1.4.5. During the performance of the project, the principal investigator is responsible for:
 - 1.4.5.1. Immediately ceasing any unnecessary pain, suffering, distress, or lasting harm to the animals when performing interventions;
 - 1.4.5.2. Ensuring that the project is being performed in compliance with the relevant ethical approval and any other decisions of the ethics committee. In case of

non-compliance, he/she should ensure that appropriate rectifying actions would be performed immediately, and the results would be recorded;

1.4.5.3. Inform the ethics committee regarding any proposed minor or major amendments in the approved research proposal, by using the form Application for Amendment in the Research Proposal Approved by the Ethics Committee. Approval of the proposed amendments should be granted before making any change to the project.

1.4.6. The principal investigator is responsible for ensuring that everyone involved in working with laboratory animals in a certain project (specifically students), has attended valid educational courses and have received permission to use laboratory animals from a relevant ethics committee. He/she must ensure that these persons have received proper theoretical and practical training before entering an animal holding/use place or performing any action related to the animals.

1.4.6.1. Ease of access, low cost, old age, or surpluses of animals cannot be a justification for letting incompetent/inexperienced persons to work with them under no supervision. Moreover, having a relevant certificate would not merely justify the competence of a person for performing every technique on laboratory animals. If the person is performing a technique for the first time, it is necessary for him/her to cooperate with, or work under the supervision, of an experienced person until he/she is deemed competent in performing that technique.

1.4.6.2. Researchers who design the methods of interventions and projects, should be familiar with the methodology of animal studies, and possess relevant information and knowledge pertinent to the scientific field of the project. If they do not possess the above-mentioned qualifications, they should

cooperate with experts who can bring these to the study. In this regard, consultation with veterinarians or expert technicians is recommended.

1.4.7. The principal investigator is responsible to ensure that all the persons involved in working with animals in a project have received necessary instructions about the zoonotic disease of the animal species being used, methods for preventing the transmission of the disease of animal origin, the type of danger of each animal (such as clawing, biting, stinging, kicking), and methods for management of the dangers caused by the animals. All persons should be able to use these instructions to protect themselves in a certain project.

1.4.7.1. Everyone involved in working with animals should receive necessary vaccinations (such as tetanus, rabies, and/or hepatitis vaccinations, when appropriate) according to the recommendations of the relevant occupational health officer. The health of the persons involved in working with laboratory animals should be evaluated periodically by a relevant health officer. In this regard, allergic reactions or asthma symptoms should be specifically monitored. In case of needle stick injuries, animal bite injuries, certain human diseases that may endanger the health of animals, or any other circumstances that have a potential hazard for transmission of diseases between humans and animals, the health of the persons and animals should be thoroughly evaluated and appropriate actions should be taken to protect the health of the persons and animals.

1.4.8. Upon request of an ethics committee, the principal investigator must produce a final project report, following termination of the project.

1.5. Rules pertinent to animal care personnel

- 1.5.1. Animal care personnel should be ethically responsible, be patient when working with animals, and not react with anger to some normal - but potentially irritating - behavior of animals. They should perform animal handling and restraint according to proper methods.
- 1.5.2. Animal care personnel should be aware of the importance of the project. They should understand the importance of their actions, their role in preserving the health and welfare of animals, and the success of the project. They should fully understand the difference between working in a laboratory animal facility and other places of animal husbandry and breeding. Specific requirements of working with laboratory animals should be taught to them.
- 1.5.3. Animal care personnel should have passed the necessary education according to the animal ethics committee's approved curriculum. New personnel must work under the supervision of more experienced persons until they are deemed competent in their tasks.
- 1.5.4. Animal care personnel should recognize the [normal and abnormal] behavioral signs of the animal species they care for. They should specifically be able to recognize the signs of pain, disease, weakness, depression, or incompatibility of animals. They should regularly inspect animals and control their health. Animal care personnel are required to report any abnormal condition to the principal investigator or other relevant persons.
- 1.5.5. Animal care personnel should be aware of the health and safety issues regarding working with [laboratory] animals and apply them. For example, they should use personal protection equipment (such as gloves, headcover, masks, shoe covers, and clean laboratory coats) in animal holding places. Following exiting from a dirty place, and before entering into a clean place, they should remove and discard all single-use

personal protection equipment or they should sanitize and disinfect dirty multiple-use personal protection equipment. They should wash their hands before entering and after the exit of animal holding places. They may need to take a shower in certain circumstances.

2. Chapter 2: Principles of Working with Laboratory Animals

2.1. The source of animals used in procedures

2.1.1. Only those source(s) of animals that are approved by a relevant ethics committee, could be used in a project

2.1.2. Animals can only be used in interventions if they are essentially bred for this purpose. Wildlife and free-ranging animals may experience extreme stress and suffering from capturing. This not only is an ethical challenge but also could confound the results of the projects performed on these animals. Moreover, the health records and genetic history of these animals are unknown, and this could lead to health problems for the personnel, or may increase data scattering and lead to invalid scientific results in projects.

2.1.2.1. Free-ranging animals, wild species of domesticated animals, or wildlife in captivity could only be used in interventions if a relevant ethics committee concludes that there is an essential need for performing research on the health and welfare of the same species of animals; or the use of these animals is essential for research related to the serious environmental hazards; or the use of these animals is essentially required for the studies concerning serious hazards to the wellbeing and life of humans. There must be clear scientific justification, demonstrating that the above-mentioned projects could only be performed on free-ranging animals, wild species of domesticated animals, or wildlife in captivity. However, according to the intense international sensitivities regarding the use of these animals in research and teaching (specifically dogs, cats, and non-human primates), the ethics committees and users must consider probable adverse consequences of performing and

publishing such projects [due to probably more intense regulations in the international level, rather than what is outlined in the current article].

2.1.2.2. Non-human primates can only be used in interventions if a relevant ethics committee concludes that these interventions are aimed at preventing, diagnosing, or treating a debilitating condition, or potentially life-threatening clinical condition, for humans or the same species of non-human primates. There should be a scientific justification that the aim of these interventions could not be achieved by other species.

2.1.2.3. Non-human primates should not be used in basic research. Great apes must not be used in interventions, unless based on enough justifications, the ethics committee concludes that performing certain intervention(s) on great apes is essential for preserving their species, or performing certain intervention(s) on great apes is essential for controlling an unanticipated dissemination of a life-threatening clinical condition, or debilitating condition for humans. In the above-mentioned situations, and conditional on that the aim of the interventions could not be achieved by any alternative methods or using other species rather than great apes, the ethics committee can temporarily and exceptionally grant ethical approval for conditional use of these species in interventions that have at least one of the aims described in the current article. However, according to intense international sensitivities regarding the use of these animals in research and teaching, the ethics committees and users must consider probable adverse consequences of performing and publishing such projects [due to probably more intense regulations in the international level, rather than what is outlined in the current article].

2.1.2.4. Endangered species, protected species defined by the Department of Environment, and all animal species with legal concerns could only be used

in projects or interventions, if 1) all pertinent legal permissions are acquired from relevant authorities; 2) the ethics committee concludes that these projects or interventions are being performed with the aim of diagnosis or treatment of a debilitating or potentially life-threatening condition for humans or animals of the same species; 3) there is scientific justification that the aim of these projects or interventions could not be achieved by using other species of animals; and 4) there is no alternative research method that could replace the use of these species of animals. However, according to intense international sensitivities regarding the use of these animals in research and teaching, the ethics committees and users must consider probable adverse consequences of performing and publishing such projects [due to probably more intense regulations in the international level, rather than what is outlined in the current article].

2.1.3. Common laboratory animal species must be acquired from an accredited breeder and/or supplier. If an unfavorable feature is found in the received animals, the recipient should inform the breeder or supplier about it.

2.1.4. Trapping wildlife should only be performed by experienced persons and using methods that cause the least possible amount of pain, suffering, distress, or lasting harm to the animals.

2.1.4.1. If an animal is found injured or in ill health during trapping or after trapping, it should be examined by a veterinarian, and appropriate action must be taken to alleviate the pain and suffering of the animal.

2.1.4.2. Trapping animals during reproduction/lactation season(s) is prohibited.

2.1.4.3. From the time of trapping an animal until its transportation and arrival in the animal facility or the intended laboratory, it is necessary to keep the animal in a proper environment, pertinent to its species-specific requirements.

2.2. Transportation of laboratory animals

- 2.2.1. Only those methods of transportation of animals that are approved by a relevant ethics committee could be used in a project.
- 2.2.2. Transportation of animals must be performed according to the principles described in valid and up-to-date scientific resources and should provide the maximum possible welfare for the animals. The issue that some animals would be euthanized for research or educational purposes upon arrival to the destination, could by no means justify improper transportation or suffering of animals.
- 2.2.3. To issue permission for transporting animals, a designated veterinarian or experienced technician is required to examine and certify the following matters:
 - a. The health condition of the animal(s) is suitable for transportation;
 - b. The transportation vehicle is suitable according to the principles discussed in valid and up-to-date scientific resources; and
 - c. The driver is properly trained regarding the principles of transporting animal(s). For air or sea transport, it is necessary to ensure that the transporting company has competence and requirements for proper transportation of animals.
- 2.2.3.1. Permission for transporting animals will be issued to one of the persons involved in the project, which is designated as the “responsible person for transporting animals”. This person should have received necessary training for transporting animals. The breeder must only deliver the purchased animals to this person or a supplier organization. The ‘responsible person for transporting animals’ should provide necessary training regarding the transportation of animals to any other persons involved in the transportation procedure and should supervise their operation.

- 2.2.3.2. Responsible persons for transporting animals may delegate some tasks regarding animal transportation to other real persons or legal entities under certain mutual agreements. However, the responsible person for transporting animals assumes the ultimate responsibility for the organization, performance, and finalization of the transportation.
- 2.2.3.3. For transporting animals between each region of the country, importing animals into the country, or exporting animals from the country, it is necessary to adhere to all regulations and rules pertaining to animal transportation; such as current regulations of the Iranian Veterinary Organization; animals-related quarantine rules; current regulations of the country regarding interprovincial transportation of animals; current regulations on the international transportation of animals in the countries of the origin, intermediate, and destination of the travel; current regulations of the International Air Transport Association; rules and regulations of the Iranian Department of Environment; and other pertinent rules and regulations.
- 2.2.4. All persons involved in the transportation of animals should receive proper training relevant to their tasks.
- 2.2.5. The transportation vehicle should have proper temperature and ventilation for animals. It should also have required safety standards in terms of accidents, fire, and similar events, and should have all required legal licenses for transporting animals. The environmental condition during transportation should be as similar as possible to the environmental condition of the animals at the original institution.
- 2.2.5.1. Transportation could cause severe distress to animals. Unfamiliar noises, harsh vibrations, separation of the animals from original stable groups, motion sickness or vertigo due to transportation movements (specifically for

those animals that are vulnerable to motion sickness), could cause anxiety, fear and ill health for animals. Therefore, it is necessary to dedicate enough time for acclimatization of the animals with the condition and temperature of the transportation container before initiating the transportation of animals. It is also necessary to consider the comfort, suitability, and proper ventilation of the transportation container.

2.2.5.2. The condition and duration of the transportation should be adjusted so that the transportation would have the least detrimental effect on the health and welfare of animals. The transportation route should be planned so that the duration of the travel -from the time of the entrance of the animals to the transportation container, until the exit of animals from the transportation container- remains at the minimum possible. Delays should be avoided as much as possible. Briefly, before initiating the transportation and during the transportation, the animals should be held in a species-specific suitable environment. It is necessary to avoid sudden movements and excessive noises or vibrations during travel. The transportation container should let the animals to rest. The proximity of the prey and predators, even when they are in separate cages, could lead to excessive stress in the animals and must be avoided. The transportation container of animals should be designed so that the animal could be monitored visually by the transportation personnel. The transportation container should not have sharp edges, so that it does not cause injury to the animals by sudden movements of the transportation vehicle. The transportation container should be designed according to the requirements of the animal species and should be escape-proof. The transportation containers/cages should be fastened to their place with straps. The transportation containers should be safe for the personnel and the animals and should have proper conditions for resting and sleeping of animals. Sudden

movements of the vehicle, frequent changes of the climates [during the course of the travel], and fast airflow [in the container] should be avoided. If appropriate, the transportation container should be made as a containment space for microorganisms, or it should minimize the transport of microorganisms between the inside and outside of the container; while letting proper visual inspection of animals without affecting their microbiological status.

2.2.5.3. Feed and water should be provided to the animals during transportation, according to the age, growth stage, and the [species specific] needs of the animals. In long-distance journeys, necessary stops and animals feeding should be considered according to the species-specific nutritional needs of the animals.

2.2.5.4. For animals with high levels of cognitive capacities (such as non-human primates, equids, canines, and felines), it is necessary to consider their psychological needs during transportation, and appropriate actions should be taken in this regard.

2.2.6. The animals must be examined by a veterinarian before and after transportation. Appropriate preventive strategies or treatments modalities should be undertaken according to the pathological reactions of animals to transportation. In this regard, diseases related to transportation stress and motion sickness should be specifically considered.

2.2.7. The person or institution receiving the animals must make necessary arrangements for the receipt and care of the animals, in advance to the initiation of transportation. For example, it may be necessary to apply for certain permissions; to prepare the quarantine room, the animal holding room, water, feed, animal care personnel; to set

up proper temperature and humidity; and to arrange other issues related to the standards of animal holding places.

2.2.7.1. The newly arrived animals must never be used in any intervention. It is necessary to consider enough time for acclimatizing animals with persons and their new environmental condition. The acclimatization time should be set according to the technical recommendations of valid and up-to-date scientific resources.

2.2.7.2. The distress of transportation could depress the immune system of animals and leave them susceptible to [infectious] diseases. Therefore, newly arrived animals should be quarantined for a certain period following their arrival, even if they do not demonstrate signs of ill health. During the quarantine period, these animals should be isolated from other animals in the recipient facility, and should be separated from the materials and devices that have come in contact with these animals. The duration of the quarantine period could be considered as part of the acclimatization time. During the quarantine period, it may be necessary to take certain preventive or treatment measures such as the use of immunostimulant medications or to perform specific adjustments to the temperature, humidity, light, animal drinking water, feeding, and other relevant factors.

2.3. Laboratory animal care

- 2.3.1. Only those animal care routines that are approved by a relevant ethics committee could be applied in a project.
- 2.3.2. During the design of the animal facility, one should consider the requirements of each animal species, the necessities of projects, and the ergonomics of the staff. Ideally, the animal holding rooms should be designed so that their functionality could be changed to meet the needs of other animal species in the future. The paints used in animal holding places should not cause toxicity to the animals, in case they are digested. The cages, walls, floors, ceilings, and other parts of the building should be made of safe and durable materials and should be washable and disinfectable. The building materials should be waterproof, resistant to fire, and should have an intact surface without gaps to provide a properly sanitized condition at animal holding places. Surfaces should be highly resistant to detergents, corrosive agents, high-pressure sprays, flame, and other agents that may come in contact with them. Details of the corridors, plumbing, waste water pipes, electrical connections, doors of the animal rooms, external windows, flooring, waste management system, walls, ceilings, interfaces of various regions of the building, [biocontainment] barriers, anti-shocks, shields, surface plumbing, ducts, personal protective equipment, and other necessary equipment in the laboratory animal facility should be in accordance with the standards provided in the up-to-date and valid scientific resources.
- 2.3.3. The animal holding rooms should be in a place with limited traffic inside and outside of the rooms. The movement of animals, cages, waste materials, and other materials and equipment of the animal holding rooms, should be minimized in the public corridors and elevators. These items should not be moved through high traffic areas of the facility. Access to animal holding places is prohibited for unauthorized persons, specifically children. Persons and equipment should not be displaced

between places with different microbiological status (such as healthy animal holding rooms, sick animal holding rooms, and quarantine area) without proper disinfection. Following the displacement of dirty materials, appropriate actions should be taken for cleaning and sanitizing the displacement route.

2.3.3.1. To reduce the traffic within the animal facility, it is necessary to place frequent access rooms in areas close to the facility's entrance. In this regard, animal holding rooms must be completely separated from the procedure rooms.

2.3.4. The health status of animals should be under continuous monitoring of the designated veterinarian. Performing vaccination(s), administering any medication, and/or taking any required intervention for prevention of diseases, should be tailored according to the species of animal, current regulations of the Iranian Veterinary Organization, instructions of the relevant ethics committees, national and international standards, epidemiology of the diseases in a specific region, and other legal or valid scientific resources.

2.3.5. Environmental conditions have a very important role in determining the biological condition of animals and could significantly affect the results of the scientific projects. Therefore, the environmental conditions at the animal holding places should be adjusted to provide the maximum possible welfare for the animals, and should ensure that the results of the interventions are, as much as possible, reliable and valid. These conditions must be set up according to the technical principles provided in the valid and up-to-date scientific resources.

2.3.5.1. Animals should be kept in specifically designed animal holding places. No animal should be kept in laboratory spaces merely because of the lack of facility or ease of use. If an ethics committee approves holding animals in a laboratory space, it is necessary to set up the animal holding conditions at the

laboratory space similar to the conditions of animal holding rooms. It is also necessary to provide all required measures to preserve the welfare of the animals, and to prevent any possible threat to [the safety of] personnel or animals in the laboratory space.

2.3.5.2. Animals that are moved from their original holding place to other place(s) (such as laboratories, surgical rooms, diagnostic imaging labs, or other areas), or come in contact with different persons (such as using animals in educational programs) could only be returned to their original holding place if all the below criteria are met:

- a) All displaced animals have come from the same original holding place;
- b) If all displaced animals have not come from the same original holding place, the places and equipment that come in contact with these animals, are disinfected between displacing different groups of animals to these places;
- c) If animals from various original holding places would come in contact with people at a new place, people would observe aseptic principles such as washing hands; and wearing new gloves, masks, gowns, and caps before the entrance of each new group of animals;
- d) Returning animals to the original holding place would not transmit contamination or microbial agents to the original holding place and would not interfere with the aim(s) of the project(s).

2.3.6. The housing type of animals (solitary vs. group housing) should be selected accurately and in this regard various factors such as species-specific needs of animals, behavioral characteristics of each animal, project necessities, the health of animals,

available equipment in the holding place, and other factors affecting the welfare of animals and the result of projects, should be considered.

2.3.6.1. The animals that may suffer from solitary housing should not be housed such; unless solitary housing is inevitable, such as certain pathophysiological or clinical conditions of an animal, aggressive behavior of an animal towards other animals of the same species, or requirements of the project. Regarding these animals, certain veterinary principles related to the solitary housing of animals must be observed and the solitary housing of the animal must be approved by the ethics committee. The duration of isolation of the animal should be kept at the minimum possible. For many species of animals, a solitarily housed animal must be able to, at least, see or hear other animals of the same species, or it should be able to feel their proximity using olfactory sense. In general, it is necessary to consider the interspecies and intraspecies relationship of animals. Further, it is of utmost importance that the housing place of animals not be a plain and empty environment. Rather, it is necessary to undertake species-specific environmental enrichment practices to prevent suffering consequences of solitary housing in a plain environment; such as stereotypical behaviors.

2.3.6.2. For group housing of animals, it is necessary to consider certain precautions to prevent aggression, specifically for territory, feed, or mating. The number of animals in holding places should be adjusted according to the criteria and standards described in valid and up-to-date scientific resources, and should meet the specific environmental requirements and social needs of animals. To prevent aggression and distress in a [stable] group of animals, it is necessary to avoid alteration in the grouping or power hierarchy of animals in the groups. Some alterations that may lead to distortion of groups or destabilization of the power hierarchy in the groups, and could result in

aggressive behaviors of animals are: adding a new animal to a stabilized group, removing an animal from a stabilized group, removing an animal from a stabilized group and returning the same animal to the group after a relatively long time, or adding a new animal with different sex to a group of same-sex animals. If performing any of the abovementioned alterations is absolutely necessary, it is required to consult with the designated veterinarian or the competent technician before making any alterations.

- 2.3.6.3. Group housing places of animals should have suitable shelters for subdominant animals to protect themselves from the aggression of dominant animals. Further, there should be a suitable space for animal resting and a secondary space for free movement. If there is not enough space for free movement of all animals in the animal holding place, it may be possible to designate a limited space in the animal holding room for free movement of animals. The animals could be rostered to be placed in the free movement space.
- 2.3.6.4. When the animals from a stabilized group are gradually removed from the group (e.g., euthanizing animals at the end of the study), it is necessary to provide certain precautions before removing the next to the last animal to prevent sudden [distressful] isolation of the last animal. This is specifically important for the animals with a higher level of sentience, for which sudden isolation could lead to severe anxiety and suffering.
- 2.3.6.5. The living environment of all animals, even those that are being group-housed, must not be a plain environment. It is necessary to undertake environmental enrichment practices to prevent the physical and behavioral dysfunction of animals [due to the lack of mental stimulation]. Specifically, these methods should contribute to the prevention of captivity-related

stereotypical behaviors, and induce motivations for living. This is specifically more important about animals with [relatively] higher levels of sentience. It is also necessary to enable animals to exercise their species-specific instinctive behaviors such as resting, exploring, self-grooming, and social interactions with other animals of the same species. Methods of environmental enrichment and other appropriate methods of refining environmental conditions, should be performed according to the valid and up-to-date scientific resources.

2.3.6.6. The number of cages in a holding room, or the number of animals in a cage, would be determined according to the species of the animal and should comply with the technical principles presented in the valid and up-to-date scientific resources. Cages must have enough space for animals to rest and it should be possible to see the animals inside the cages. The animals must not be kept in visual, auditory, or olfactory proximity to their predators. There should be proper measures in place to prevent the entrance of any pests into the animal holding rooms. Only one species of animals should be held in a certain animal holding room. Holding various species of animals in the same place could lead to problems regarding biosafety or [welfare matters such as] incompatibility of animals (e.g., keeping noisy species of animals in proximity to calm and noise-sensitive species).

2.3.7. When holding animals in open fields, it is necessary to provide a shelter to protect them from precipitation, strong winds, sunshine, and other unfavorable atmospheric conditions of an open field. When holding animals in closed spaces, it is necessary to set up proper environmental conditions according to the technical criteria presented in up-to-date and valid scientific resources. In this regard, issues should be considered such as adequate floor [or cage] area according to the species of animal, adequate area for [the performance of] animal care personnel, air temperature and humidity,

light intensity, duration of light/dark cycles, necessity or unnecessary of windows [for animal holding rooms], and air quality. Excessive noises that may cause animal suffering must be eliminated from the environment or at least should be reduced. For certain species of animals, it might be appropriate to avoid absolute silence in the environment by using harmonic white noises, such as the low volume of a radio channel, which could also contribute to the environmental enrichment of animals. In this regard, one should refer to valid and up-to-date scientific resources.

- 2.3.7.1. There should be a proper solitary nest for pregnant animals so that they could deliver their litters [undisturbed] and care for them.
- 2.3.7.2. If a project requires an animal to be kept in an abnormal environmental condition, the issue must be [evaluated] by the ethics committee. If the ethics committee approves the issue, enough time should be given to the animal for gradual adaptation to the new abnormal environmental condition.
- 2.3.7.3. To remove the smell of ammonia and other unpleasant odors from the environment, it is necessary to remove the source of these materials and/or use proper ventilation. Air fresheners or perfumes must not be used to cover the unpleasant odors.
- 2.3.7.4. To reduce the confounding factors of the project, the environmental condition and the density of animals in animal holding places should be kept unchanged during the course of the project. Variations of light, temperature, airflow, and other environmental conditions could cause animal suffering and may lead to significant unwanted effects on the results of the studies. It is also necessary to use proper statistical methods (such as random allocation of animals to cages and/or random arrangement of cages in animal holding rooms), blocking methods, and other pertinent methods to reduce the effect of environmental confounding factors. Circulating cages in the animal holding

place and frequent displacement of cages could be stressful to many species of animals and are not recommended.

2.3.7.5. In case of a sudden change in the environmental condition of the animal holding room, it is necessary to avoid any research intervention on these animals until they are fully adapted to the new environmental condition.

2.3.8. Clean drinking water with proper temperature should be available to animals. There should be adequate reservoir of drinking water according to the number of animals in an institution.

2.3.8.1. It is necessary to ensure uninterrupted access of all animals to drinking water. In this regard, malfunctioning of the nozzles of the water bottles or automatic watering systems may lead to inability of animals to drink, while there is enough water in the system. Therefore, daily control of the remaining water in the drinking bottles of each cage, or controlling the nozzles of the automatic watering system, or other appropriate methods for ensuring the access of animals to drinking water must be a part of the daily routines of the animal care personnel.

2.3.8.2. Watering systems should be designed and used so that they provide enough amount of quality water to animals. There should be enough drinking bottles in an animal holding place. If automatic watering systems are used, their function needs to be regularly monitored, so that probable accidents such as water blockage, water leaking, or spread of contaminations would be avoided. These systems should be regularly serviced and ideally equipped with failure alarms. Water pooling in cages must be avoided.

2.3.8.3. Water can transmit microorganisms, and therefore watering equipment should be made of materials that minimize the risk of contaminations. The equipment should be sanitized and/or disinfected at certain time intervals.

Regarding animals with specific conditions, such as immunocompromised models, it is necessary to consider certain precautions such as sterilization of the drinking water.

2.3.8.4. The resistance of various species of fish, amphibians, and reptiles to water temperature, acidity, chlorine content, and concentration of other chemical materials, are very different. Therefore, the water source for aquariums and fish tanks should be selected according to the necessities of the projects and the resistance of the related species.

2.3.9. Sufficient amounts of healthy feed should be provided to the animals. There should always be enough storage of feed according to the number of animals in an institution.

2.3.9.1. Feeding formulation of an animal would be determined according to the animal's species, weight, body condition score, sex, age, growth condition, normal physiological needs (such as growth, reproduction, parturition, and lactation), certain necessities related to the specific conditions or pathologies of animals (such as fever, pain, infection, and other pathological processes), or according to the types of the intervention (such as performing surgery or inoculating infection). The feed should be formulated according to the technical principles discussed in valid and up-to-date scientific resources. Feed shape, nutritional content, and the method of feeding should be in accordance with the behavioral and nutritional needs of the animals. Food searching opportunities should be given to certain species of animals. The feed should be palatable and free of contaminations. Proper means should be considered for reducing chemical, physical, and microbiological contaminations of the feed during the course of the selection of raw materials, production, preparation, and feeding to the animals. There should be proper

labeling on feed packages that provide clear information about the specifications of the product, nutritional content, and its production date. It is recommended that animal facilities randomly sample their feed packages and determine the nutritional content of the samples via an independent feed analysis laboratory. The expiry date of the feed should be determined by its producer and be printed on the bag. Roughages are an important part of the feeding regime of certain species of animals (such as rabbits) and they could also meet certain behavioral needs of these animals.

2.3.9.2. Packaging, transportation, and storage of feed should be such that prevent the contamination, spoilage, and damage of the feed materials. Storage places should be dry, cold, and dark, and should be protected from vermin. Perishable foods such as vegetables, plants, fruits, meat, and fish should be held in a cold room, refrigerator, or freezer. Feeding equipment, watering places, or other utensils used for the preparation and distribution of feed/water should be regularly cleaned and, if necessary, decontaminated. If wet feeds are used, or dry feeds may be moistened by water, feces, or urine, then it is necessary to regularly clean the feeding and watering places. Regarding animals with specific conditions (such as immunocompromised models) it is necessary to consider specific precautions such as sterilization of feed materials.

2.3.9.3. All animals should have access to feedstuff. The feeding place should have enough space and proper shape so that it prevents feed-related competition and aggression between animals.

2.3.10. Air conditioning systems, ventilation systems, and other facilities should be positioned in certain places in the animal facility so that they cause minimum

disturbance to the animals and their maintenance services could be performed without needing to enter into animal holding places or other places with biosafety risks.

2.3.11. Bedding of animals should be pertinent to their species and should provide a suitable place for them to rest. Bedding should absorb the humidity and odor of urine and feces and should facilitate the sanitization process. The bedding should enable animals to exercise their species-specific activities, such as searching for food, digging, and tunneling. It should also provide a comfortable and safe place for animals to sleep. The bedding should enable animals to make nests for reproduction. Since using only one type of material could not meet all the above-mentioned expectations, it is necessary to use a combination of different materials for bedding. These materials should be dry, moisture absorbent, dust-free, non-toxic, and free from infectious agents, vermin, or other types of contaminations. Wood-derived materials that are produced by or covered with chemical agents, or may contain natural toxins should not be used as bedding materials. The same also applies to materials with unknown content. The underlying surface of the animal holding place should be even, comfortable, and suitable for animals to rest. The place where animals sleep should be kept dry and clean.

2.3.12. Collection, storage, and disposal of waste, and cleaning of the animal holding places should be performed according to the relevant standards and the species of animals. Standard odorless disinfectants should be used for disinfecting and/or cleaning the environment and equipment. At the end of the cleaning procedure, it is necessary to thoroughly remove the chemical materials such as soaps, moisturizers, detergents, solvents, and disinfectants from the surfaces that may come in contact with animals; unless a credible producer has officially declared that these materials are harmless to animals.

- 2.3.12.1. The cleaning process of the animal holding places should avoid causing distress for the animals. Regarding some species of animals, or during certain stages of the animal's life it is necessary not to collect all the soiled bedding of a cage during the cleaning process; rather, it is necessary to leave some soiled bedding to retain the olfactory environment of the animals.
- 2.3.12.2. Collection, storage, and disposal of waste materials should be performed according to the relevant guidelines of the Department of Environment, such as Criteria and Methods for Practical Management of Medical Wastes and other Related Wastes.
- 2.3.13. Emergency protocols should be developed for the power outage, water outage, fire, malfunction of the air conditioning systems, dissemination of contagious diseases, aggression-related injuries to animals, escape of animals from animal holding places, and any other failure in proper caring of animals, specifically during holidays. Emergency protocols should be provided in a written format and should be visible and accessible by all relevant persons in the facility.

2.4. Using animals in procedures

2.4.1. Only those interventions and methods of using animals that are approved by a relevant ethics committee could be applied in a project.

2.4.2. Before initiating the project, researchers should consider all necessary arrangements for preventing or alleviating the pain and distress of animals, and they should undertake these arrangements during the course of the project. Some of the arrangements may include:

- a) Selecting the most humane, less invasive, and quickest method for performing the project;
- b) Ensuring that all persons involved in working with animals have received proper education, have enough technical dexterity and competence for working with animals;
- c) Estimating the amount of pain and distress caused by the project and determining proper methods for alleviating them;
- d) Regular monitoring and evaluation of the animals for discovering signs of pain and distress during the performance of the project and after the project - if the animals are kept alive;
- e) Selecting the best anesthetic and analgesic protocols according to the animal species, type of surgery and the amount of pain it may cause, age of the animal, dexterity of the surgeon/anesthesiologist, and other relevant factors. In this regard, one should act according to the anesthetic/analgesic protocols presented in the most up-to-date and valid veterinary resources;
- f) Determining the most humane endpoints criteria so that an animal would not be used further if its welfare condition is severely deteriorated and could not be

improved, or the condition of the animal is such that it does not provide valid scientific results. In this regard, the humane endpoints criteria should be defined according to valid and up-to-date scientific resources, and these criteria should be reported in the research proposal;

g) Determining the humane methods of terminating the work with laboratory animals, according to the criteria outlined in the current guideline.

2.4.2.1. If the aforementioned preparations for preventing or alleviating the pain and distress of animals are not feasible, proper scientific justification along with the anesthesia/analgesia protocol should be presented to the ethics committee. The committee should be convinced that the total amount of pain and distress of the animals would not exceed the allowed limit according to the content of the current guideline.

2.4.2.2. For animals that may experience pain following surgery/anesthesia, it is necessary to consider preemptive analgesia and post-operative analgesia, or other proper methods of preventing or alleviating the pain of the animal. In general, it is necessary to undertake all possible actions to minimize the pain and distress of animals, once the goal of the intervention is achieved.

2.4.2.3. Tranquilizers, sedatives, hypnotics, or inhalation anesthetics (except nitrous oxide) are mainly effective for calming, immobilizing, and/or inducing sleep in animals and do not have analgesic effects. Nitrous oxide exceptionally induces a limited amount of analgesia. Thiobarbiturates such as pentobarbital and sodium thiopental, do not induce analgesia and should not solely be used for surgical operations. Alpha-2 agonists [such as xylazine], if used alone, do not have a considerable analgesic effect. Further, a combination of ketamine and xylazine (although commonly used), does not have enough analgesic effect for performing major surgeries on many species of laboratory animals,

specifically mice, and rats. Therefore, using these drugs alone is not acceptable for anesthesia/analgesia. Using ketamine alone in some species of animals does not induce considerable analgesia. The combination of ketamine and diazepam is mainly useful for inducing general anesthesia and performing non-painful procedures. Moreover, ketamine alone in many species of animals does not induce visceral analgesia or may induce weak visceral analgesia. To use veterinary medications, the proposal must refer to valid and up-to-date veterinary resources.

2.4.2.4. Using ether as an anesthetic agent or as a means of inducing unconsciousness, hypnosis, muscular relaxation, and the like is prohibited.

2.4.3. Considering the ethical complexities of pain research or projects with ‘pain’ as their inseparable part, these types of research are not exclusively covered in this guideline. For research in this area, it is necessary to apply the ethical principles defined in valid and up-to-date scientific resources. In general, it is necessary to define humane endpoints according to the ethical and scientific criteria, so that a maximum duration and intensity of the perceived pain -conditional to the approval by the ethics committee- is defined for the project. As soon as the expected results of the project are achieved, working with animals should be terminated.

2.4.4. Recognition of pain, suffering, and distress of many species of animals may be a complicated task for individuals with no prior experience in this area. These individuals should consult with a veterinarian or competent technician in this regard. In case of any doubts in determining the amount of pain or distress of a certain procedure, it is necessary to act according to the anthropomorphism principle; so that it would be assumed that any intervention which may cause pain or distress for human, would do the same in animals, unless -based on the sound rationales- proved otherwise. If the anthropomorphism method could not resolve controversies, the

benefit of the doubt should be given to the welfare of the animal and protecting it from probable pain or distress.

- 2.4.5. The type of medications, methods of administration, and the dose of medications should be selected according to the valid and up-to-date veterinary pharmacological resources, pertinent articles, or other relevant resources. If there is no prior data regarding a certain medication, it may be possible to calculate the dose of the medication according to the interspecies dose extrapolation methods. The calculated dose may be administered after ensuring that the calculated dose is safe and functional. In this regard, it is necessary to consider certain contraindications and prohibition of the use of certain medications for certain species of animals.
- 2.4.6. Regarding methods of injection, selection of vessels, body regions that could be used for injection in each species of animals, permissible injection volume, and permissible needle size for various injection methods, one should act according to the up-to-date and valid resources.
 - 2.4.6.1. Cardiac puncture for injection or blood sampling is strictly forbidden in conscious animals or any animal capable of perceiving pain and one must consider other alternative methods for these purposes. When proper anesthesia and analgesia are used for intracardiac injection or cardiac blood sampling, the animal must be euthanized while being fully anesthetized and having proper analgesia, without regaining consciousness.
- 2.4.7. Fluid therapy should be performed according to the technical principles presented in the valid and up-to-date scientific resources. The type of fluid, method of administration, rate, the temperature of the fluid, and other fluid therapy parameters should be selected according to the veterinary principles.
- 2.4.8. Sick animals whose spontaneous diseases are not part of a project, should not be used in interventions. These animals should only be considered as veterinary patients and

they should be either treated or decisions made about them according to the humane endpoint criteria of the project. It is emphasized that no sick animal should be left on its own. It is also necessary to undertake proper [immediate] actions for treatment and/or prevention of the same condition for other animals in danger.

2.4.8.1. If the spontaneous disease of an animal is per se the aim of the intervention and there are sufficient scientific and ethical justifications for performing this intervention, the ethics committee can perform a case-by-case assessment. If the ethics committee approves the project, the diseased animals could be exceptionally used in this project conditional to that all relevant aspects of the disease of the animal are thoroughly reviewed by the investigators, and any attempt is undertaken to reduce the pain, suffering, distress, and lasting harms of the animal. Scientific justification in this article means that the ethics committee is satisfied that the project essentially requires the use of such animals, and there are valid scientific reasons that performing said project could only be achieved by using such animals, and a project has such importance that not performing the project could cause major impairment to the health of the humans or animals.

2.4.9. Animals with specific conditions, such as pregnancy or lactation, should not be used in interventions; unless the ethics committee ensures that the nature of the intervention essentially needs using such animals, there are valid scientific justifications that said interventions could only be performed by using animals under such conditions, and the performance of the project is so important that not performing it, could lead to major impairments to the health of the humans or animals.

2.4.9.1. If lactating or pregnant animals are being used, it is necessary to ensure that using these animals does not cause pain and suffering to the infants of the

lactating animals (e.g. due to the lack of milk) or the fetus of the pregnant animals. Fetuses of pregnant animals concerning this article include:

- a) Mammalian fetuses that are capable of perceiving pain;
- b) Mammalian fetuses that are not capable of perceiving pain at the time of the performance of the experiment; however, they will be kept alive, and it is possible that in future that they become capable of perceiving pain, they would experience the pain, suffering, distress, or lasting harms caused by the previous intervention on the mother or fetuses; and
- c) Any fetal or infantile form of life that is dependent on their mother to stay alive.

2.4.10. Surgery, vivisection, or other interventions with pain intensity similar to surgical pain, must not be performed on an animal that is not properly anesthetized, and/or has not received analgesia, or has only received pain masking agents but is still capable of perceiving pain. This article is not exemptible under any circumstances.

2.4.10.1. It is necessary that the principal investigator and/or other persons involved in the surgery/anesthesia/use of the animal, to ensure that the animal is not perceiving pain, specifically when using succinylcholine, pancuronium, vecuronium, atracurium, and any other curariform agents; nicotine, potassium chloride, or any neuromuscular blocking agents, or any agent with muscular relaxant or muscular paralyzing activity; strong sedatives; or any agent that could mask the common signs of pain in animals or may cause complexity in the management of anesthesia or analgesia of the animals.

Regarding the projects requiring the use of neuromuscular blocking agents or muscular relaxants or paralyzing agents, the principal investigator must seek

specialized consultation from a veterinary surgical/anesthesia specialist regarding the anesthetic protocol of the project. The anesthesia of the animals in these projects should be directly supervised by a veterinary surgical/anesthesia specialist.

2.4.10.2. The type of analgesic medication(s), and the duration of its use should be determined according to the anticipated amount of pain that may arise from a certain intervention, the type of the pain, and the duration of the pain. In this regard, one should seek information from up-to-date valid veterinary resources. As a standard practice, analgesia should be established by various methods (multimodal analgesia). The most effective time of administering analgesic medications is before the pain initiates (preemptive analgesia). When pain perception is initiated, treatment for pain requires using a larger amount of medication, takes longer, and complete resolution of the pain can be more difficult to achieve.

2.4.11. Each episode of anesthesia is considered a stressful event for the animal. Therefore, it is required to reduce the number of anesthetic episodes to the minimum possible. The permissible number of anesthetic events in certain time periods could be determined according to the type and amount of medications, the condition of the animal (age, sex, pregnancy status, the amount of injuries to the animal during the course of the project), and other related parameters.

2.4.11.1. Anesthesia/analgesia protocol for each intervention is unique, and selecting a certain protocol should be performed according to the scientific criteria and technical principles defined in valid and up-to-date scientific resources.

2.4.12. Before performing any surgical operation, it is required to prepare a detailed plan according to valid and up-to-date scientific resources. The plan should cover the below issues:

- a) Ensuring that suitable animal(s) are available for surgery;
- b) Selecting the suitable animal(s) for the [upcoming] surgery;
- c) Evaluating the health condition of the animal(s) before surgery;
- d) Preparing the required materials and equipment for pre-, intra-, post-surgical periods;
- e) Sterilization of the instruments, equipment, and other required items;
- f) Determining the persons required to perform the surgery;
- g) Preparing animal(s) for the surgery;
- h) Determining the required management practices for the post-surgical period or probable emergencies;
- i) Preparing a written protocol determining the details of the anesthesia and surgical procedures;
- j) Recording pre-, intra-, and post-surgical events; and
- k) Estimating the time for beginning and conclusion of surgery; such that the anesthetic recovery time does not coincide with the after-hours or weekends.

2.4.13. The environmental condition of the surgical suite should be in compliance with the standards defined in valid and up-to-date scientific resources.

2.4.14. The necessity for pre-surgical fasting (i.e., water/feed deprivation) would be determined according to various criteria; such as the species of the animal; the age of the animal; the type of the surgery; pre-, intra-, and post-surgical medications; and other criteria defined in valid and up-to-date scientific resources. It should be noted that in many cases, water deprivation is not required to be longer than 30-60 minutes before surgery.

- 2.4.15. The use of pre-, intra-, post-surgical antibiotics should be consulted with the designated veterinarian. Regarding the choice of antibiotics, it is necessary to consider the species-specific sensitivity of animals to certain antibiotics, as well as the general principles governing antibiotic therapy in surgery.
- 2.4.16. Person(s) responsible for surgeries should evaluate their available equipment, and their competence for [successfully] performing surgical techniques using the available equipment. They should only undertake a surgery if they are reasonably sure that they can perform it properly. Before initiating a surgery, these person(s) should collect the required information regarding the anatomy and physiology of the surgical site, and determine the best approach to perform the surgery. In this regard, practicing the surgical technique on animal carcasses that have died due to other issues, or are euthanized for reasons such as veterinary medical issues, is recommended.
- 2.4.17. The least possible invasive methods should be used in surgeries and other invasive interventions. Tissue manipulation and tissue trauma must be kept to the minimum possible. Prevention of bleeding should be performed appropriately. All exposed tissues should be kept moistened. The least amount of foreign bodies should be left in the body. The dead space(s) should be eliminated as much as possible. The intervention should be concluded in the least possible amount of time.
- 2.4.17.1. For all surgeries and invasive interventions on any species of animal (including rodents), aseptic techniques should be observed regarding the operating room, surgical equipment, surgical site, and involved persons.
- 2.4.17.2. For closing defects or incisions, it is necessary to consider proper apposition of the tissues; avoid excessive tension on suture lines; and leave the least possible amount of foreign bodies in the tissues. For those species of animals that do not tolerate suture materials on their skin, it is necessary to consult

with a veterinarian or competent technician during the designing stage of the project and to select the best alternative method that could safely close incisions, while not inducing self-mutilation behaviors in animals.

2.4.18. No animal should be left on its own following termination of the surgery or during unconsciousness.

2.4.18.1. In most species of animals, it is necessary to monitor the anesthetic recovery of the animal until it can hold a sternal position, have positive righting reflex, and is able to drink water. To prevent injuries caused by other animals during anesthetic recovery, it is necessary to place an unconscious or semi-conscious animal in a solitary cage.

2.4.18.2. The bedding of the anesthesia recovery place should be prepared in accordance with the technical principles available in valid and up-to-date scientific resources. In general, it should provide a proper condition for the anesthetic recovery of the animal and prevents injury to the animal. Sawdust bedding, or other similar beddings with small particles, should not be used during the anesthetic recovery of rodents.

2.4.18.3. The normal body temperature of an animal should be maintained from the induction of anesthesia until full anesthetic recovery of the animal, when it can stand on its feet and feed and drink spontaneously. In this regard, necessary precautions should be taken to avoid hypothermia or hyperthermia. Specifically, placing an animal directly on a cold metallic table should be avoided. Prevention of hypothermia is specifically important for small animals, or any animal with a higher ratio of body surface area to body volume.

2.4.18.4. Hypoglycemia should be prevented in anesthetized animals. In general, postoperative nutritional management of animals should be performed

according to the relevant veterinary principles. Nutrition may be provided to the animal by appropriate methods, such as oral administration, nasogastric intubation, parenteral administration, or a combination of oral and parenteral routes.

2.4.18.5. Anesthetic recovery and surgical recovery should be managed to reduce the pain and suffering of the animals. These management practices should be performed according to the technical principles available in valid and up-to-date scientific resources and should include evaluation and taking proper actions regarding the below issues:

- a) breath rate, breath quality, and the amount of oxygenation;
- b) heart rate and cardiac function;
- c) pain and distress;
- d) body temperature;
- e) color of the mucous membranes;
- f) healing status of incisions and surgical related injuries;
- g) cleanliness of the environment and the animal's body;
- h) dryness of the coating of the animal's body;
- i) suture line dehiscence;
- j) pus formation or surgical site infection;
- k) [abnormal] excessive inflammation at the surgical site;
- l) fluid intake and hydration status of the animal;
- m) blood glucose level;

- n) feed intake;
- o) amount of urination and defecation;
- p) pre-surgical and post-surgical body weights;
- q) pre-surgical and post-surgical body condition scores;
- r) bandage condition (bandaging should not be used for some species of animals) ;
- s) occurrence of self-mutilation;
- t) necessity for performing post-surgical rehabilitation; and
- u) any other pertinent issues according to the species of the animal and type of anesthesia/surgical operation.

2.5. Termination of working with animals

- 2.5.1. Only those humane endpoint criteria and methods of termination of working with laboratory animals that are approved by a relevant ethics committee could be used in a project.
- 2.5.2. Termination of working with animals does not necessarily mean ‘killing’ of the animal, and it is required to avoid unnecessary killing of animals. However, keeping animals alive should not cause unmanageable pain and suffering for them. Reusing animals for other interventions/projects is conditional on certain criteria that are discussed in detail in other parts of the current guideline.
- 2.5.3. The principal investigator is responsible for defining the humane endpoint criteria of the project and reporting it to the relevant ethics committee before starting the project. Humane endpoint criteria should be defined according to the technical principles available in valid and up-to-date scientific resources. In other words, it is necessary to determine -before the start of the project- under which conditions the project would be deemed finalized. Further, it should be defined under which condition based on the ethical grounds, no further work will be performed on animals even if the project is not finished; such as severe deterioration of the animals’ condition, or reaching the pain and suffering of the animals to or above an acceptable level. The method of termination of working with animals should be determined according to the principles available in valid and up-to-date scientific resources and should provide the maximum possible welfare for the animal. Said method of termination should be approved by the ethics committee before initiation of working with animals in a project. The following issues should be considered when reporting the humane endpoints criteria to the ethics committee:

- a) The criteria should be reported in detail and should be quantified;

- b) The method of evaluation and the intervals of evaluations during the course of the project should be determined;
- c) The persons responsible for evaluating the criteria should be defined, and the method of teaching this person should be determined (it should be noted that evaluating some criteria by different persons may lead to different results); and
- d) For each humane endpoint criteria, it should be determined what actions would be performed if a criterion is met.

2.5.3.1. Decisions on keeping an animal alive or killing the animal when humane endpoint criteria are met, should be made by a competent person. This competent person should be aware of the nature of the interventions and harms inflicted on the animals, and should have a thorough knowledge regarding the ethical and scientific principles of working with laboratory animals. This person may be a competent veterinarian, which preferentially should not have conflicts of interest with the project, or it could be any other competent person(s) with above-mentioned qualities.

2.5.4. An intervention is deemed final when:

2.5.4.1. No further observations are considered for that intervention; or

2.5.4.2. Evaluation of an animal's condition reveals that using this animal would no longer produce valid scientific results; or the amount of pain and suffering of the animal is so high and long-lasting that although keeping the animal alive may lead to the collection of valid scientific results and may not cause interference with the project, it is against the principles of humane experimentation, religious, moral beliefs, or legal principles, and there is no

means available for elimination or alleviation of the pain and suffering of the animal.

Spontaneous death of animals should not be used as a humane endpoint criterion, as far as possible. Instead, other criteria that are more humane and can sooner determine the proper humane endpoints should be used. In other words, the time should not be wasted so that an animal goes into a moribund state because of the suffering and inflicted injuries. It should also be noted that, although an animal in the moribund state is in a coma and may not perceive significant suffering, it has endured a lot of suffering before reaching that state. Therefore, the moribund state could not be used as a humane endpoint criterion.

2.5.5. If it is likely that an animal remains in a state of moderate to severe pain, suffering, distress, and lasting harm and there is no resolution for this condition, it is necessary to euthanize the animal by a humane method and according to the content of the current guideline. In other situations, under the supervision of a competent veterinarian or technician, it may be possible to use other [terminating] methods such as letting the animal be adopted by the general public, returning the animal to its natural habitat, or sending the animal to the farming system, only if the below criteria are met:

2.5.5.1. The health status of the animal allows this;

2.5.5.2. There is no risk to the environment, public health, and the health of other animals;

2.5.5.3. Proper precautions are undertaken to preserve the welfare of the animal in the new place, which may be the [home of the] animal caregiver, the natural habitat, or the farming system; and

- 2.5.5.4. Doing so does not have any legal prohibitions.
- 2.5.6. If an animal is kept alive at the end of a project, it should receive proper care and husbandry according to its health status. Before an animal can be adopted by the public at the end of an intervention, a competent veterinarian or technician should evaluate the health status of the animal and ensure that the animal is compatible with humans. Regarding wildlife, rehabilitation programs should be performed before returning the animal to its natural habitat, to ensure that the animal is capable of sustaining its life. Wildlife animals that were born and raised in captivity are not generally prepared to live on their own in their natural habitats and specifically require said rehabilitation programs. In this regard, one may approach environmental protection agencies of the country, accredited animal protection groups, or other relevant authorities with professional knowledge and facilities for animal rehabilitation.
- 2.5.7. Euthanizing an animal is a specialized intervention regardless of the life stage of the animal, which could be fetal, larval, infantile, adult, or other biological forms. Euthanasia could result in an easy and painless death without suffering, and provide valid scientific results, only if being performed properly by a competent person. Therefore, it is essential that all persons performing euthanasia, to have proper training in this regard, and to practice physical methods of euthanasia on animal carcasses that died due to other reasons. Following the achievement of valid training certifications, these persons are required to perform euthanasia under the supervision of other competent persons until they demonstrate practical competency.
- 2.5.8. [If an animal is planned to be euthanized], it should have maximum possible welfare and should be kept in a standard place until the completion of euthanasia. For example, if an animal is in pain due to any reason and it is planned to euthanize the animal within the next hour, the impending euthanasia must not interfere with the

proper care and analgesic treatment of the animal. If animals are to be kept in a place for more than three hours before euthanasia, there should be water and feed available to them at this place. For infants or other small animals, the above-mentioned time interval would be in a range of 1 to 2 hours. Infant animals that are planned to be euthanized, should remain with their mothers until all preparations of euthanasia are done.

2.5.9. The euthanasia method must be chosen in exact accordance with the criteria and technical principles available in valid and up-to-date scientific resources and must be approved by the ethics committee before being performed. Any intervention that is not in exact compliance with said criteria and technical principles; methods of animal killing that lack valid scientific support; or experimental methods or methods that their painlessness and humaneness are not proved by valid scientific research; are categorized as unacceptable methods of euthanasia. Using unacceptable methods of euthanasia will be considered as a violation of research [ethics]. Briefly, proper methods of animal euthanasia according to the specific condition of a certain project should meet the following criteria:

- 2.5.9.1. Cause a minimum possible amount of pain, suffering, and distress for the animal, and the induction of unconsciousness and death is accomplished without pain, distress, anxiety, and fear;
- 2.5.9.2. Are compatible with the requirements and aims of the project, and do not interfere with research tests, further experiments, and use of animal tissues;
- 2.5.9.3. Rapidly induce unconsciousness;
- 2.5.9.4. Are reliable so that performing euthanasia on several animals, by different performers, could always lead to an easy death of the animal, and would not cause controversial results;

- 2.5.9.5. Are safe for people;
 - 2.5.9.6. Are non-recoverable; in other words, performing euthanasia should certainly kill the animal and there is no probability of animal recovery following euthanasia;
 - 2.5.9.7. Have minimal emotional and psychological effects on the viewers or personnel performing euthanasia;
 - 2.5.9.8. Are selected according to the relevant guidelines and with consideration of the species, age, size, and health status of the animal;
 - 2.5.9.9. The equipment used for euthanasia constantly having proper functionality;
 - 2.5.9.10. The carcass of the euthanized animal would not be accessible by predators or scavenger animals;
 - 2.5.9.11. The euthanasia method has a minimal environmental impact;
 - 2.5.9.12. The place of performing euthanasia is away from other animals; so that other animals would not be able to directly or indirectly see, hear, or smell (specifically, the smell of the blood and pheromones) of the animal being euthanized.
- 2.5.10. Administration of injectable euthanasia agents, should not be performed via intramuscular, subcutaneous, intrathoracic, intrapulmonary, intrathecal, or intra-arterial routes. This article may be excluded for using very potent opiates, such as etorphine or carfentanil. These opiates may only be used in very rare circumstances and their use is subject to acquiring all relevant legal permissions.
- 2.5.10.1. Intrahepatic, intrarenal, and intrasplenic injections of euthanasia agents are unacceptable in conscious animals. However, intracardiac, intrahepatic, intrasplenic, and intrarenal injections may be performed in anesthetized

animals that do not perceive painful stimuli. As an exception, when intracardiac injection is the only known method of vascular access in some ectotherm animals (such as snakes and some other reptiles), it may be possible to use intracardiac injection for injecting euthanasia agents in conscious animals. However, if there is an alternative humane euthanasia method that would not need intracardiac injection in these animals, the alternative method should be used.

2.5.11. Unacceptable methods of euthanasia must never be used for killing animals. This article cannot be exempted under any circumstances.

2.5.11.1. Some unacceptable methods/agents for euthanasia include:

- a) Ether: unacceptable for killing any species of animals. Ether or diethyl ether is a combustible and explosive material and there are reports of exploded freezers or incinerators containing animal carcasses euthanized by ether. This material is also irritating for the mucous membranes of animals and causes excessive anxiety, pain, or suffering for the dying animal;
- b) Hypothermia, or snap freezing in liquid nitrogen: is an unacceptable method of killing any species of animals. Exceptionally, for euthanasia of reptiles and amphibians with total body weight under 4 grams, and for euthanizing premature rodent infants (less than 5 days old, without body coating, and weighing less than 4 grams) the snap freezing in liquid nitrogen could be used as an acceptable method of euthanasia under specific circumstances;
- c) Complete exsanguination or vascular incision in conscious and pain perceiving animals;

- d) Air embolism (injecting air);
- e) Using toxic substances that are not specifically made for euthanizing laboratory animals (such as pesticides and rodenticides); strychnine; injection of solvents, detergents, and household materials (such as acetone, cleaners, quarterly compound such as carbon tetrachloride, laxatives, dimethyl ketones, quarterly ammonium compounds, antacids); cyanides; chloral hydrate; chloroform; formalin/formaldehyde. Exceptionally, sponges -Porifera- could be euthanized by immersion in formalin/formaldehyde. However, one should consider the biological risks of using formalin/formaldehyde for [the health and safety of the] personnel performing the euthanasia.
- f) Neuromuscular blocking agents (such as nicotine, magnesium sulphate, potassium chloride, succinylcholine, pancuronium, vecuronium, atracurium, and other curariform agents) must not be solely used for euthanasia;
- g) Suffocating in bags or chambers, specifically for killing poultry and chickens; decompression (decreasing the ambient pressure around the animal's body); chest compression; drowning; decapitation; using nitrogen or argon (proper use of nitrogen and argon for euthanasia requires adherence to very specific criteria);
- h) Burning;
- i) Non penetrating captive bolt (unacceptable method of euthanasia for most animal species); a manual blow to the head (unacceptable method of euthanasia for most animal species; exceptionally for some small laboratory animals this method may be used according to

certain techniques and criteria described in specialized resources; Nonetheless, this is not a preferred method of euthanasia); stunning (may only render the animal unconscious; but it is not a euthanasia method);

- j) Other unacceptable methods of euthanasia for each species would be determined according to the valid and up-to-date resources of euthanasia.

2.5.11.2. Physical methods of euthanasia are not recommended [as a primary method of euthanasia] by this guideline. Physical methods of euthanasia can only be used if there is a scientific justification that using chemical methods of euthanasia would cause a substantial problem for a project, there are no other methods for acquiring valid results, and the project has significant importance in the advancement of human science and preserving the health of the other living beings. Before performing any physical method of euthanasia, one should use proper analgesics, or the animal should be rendered unconscious. If using proper analgesics or anesthetic method could cause a substantial problem for a specific project, then, performing the physical method of euthanasia is only permissible if there are no other methods for acquiring valid results and the project has significant importance in the advancement of the science and preservation of the health of the living beings. For proper performance of physical methods of euthanasia, the euthanizing person should have acquired the necessary skills and competence by enough practice on the carcasses of animals that are died or euthanized for other reasons, under the supervision of a competent person.

2.5.12. In the case of an unanticipated death of an animal, or requiring emergency euthanasia due to unanticipated reasons, the principal investigator should initiate an investigative

process to determine possible causes [of the issue] and should undertake necessary actions to prevent similar events from happening in other animals. If appropriate, a necropsy examination should be performed by a competent person to determine the cause of death or the underlying condition that led to the emergency euthanasia.

2.5.12.1. In many research projects, the mortality rate of animals is a valuable datum and should be reflected in the results. In cases where the mortality rate of animals is more than the predetermined threshold, and -according to the approved research proposal- it is necessary to replace the animals, the principal investigator must prepare a request for increasing the number of animals and submit it to the relevant ethics committee. In the submission, it is necessary to mention the reason(s) why the mortality rate exceeded the predetermined threshold, and what proposed method(s) are in place to avoid further mortality. The principal investigator is only allowed to increase the number of animals if this practice is approved by the ethics committee.

2.5.13. At the end of euthanasia, a competent person must confirm the death of the animal according to the species-specific death confirmation criteria. This issue applies to all developmental forms of animals, such as fertilized eggs, fetal, and embryonic stages of animals. Disposing of live animals, or disposing of animals that regain consciousness following euthanasia and disposal, could cause severe pain and suffering for them and this issue is considered a serious violation of the ethical rules of working with laboratory animals.

2.5.13.1. Considering different signs of death in various species of animals, the death confirmation criteria may vary between different species; therefore, the principal investigator is required to determine the death confirmation criteria according to the animal species of the study.

2.5.13.2. [Physical], behavioral, and emotional dependencies between animals, especially for those species with higher levels of neural system development, are substantially important matters. Therefore, when there is a tangible or intangible (behavioral or emotional) dependency between animals -such as the dependency between a mother animal and its infant(s)- it is necessary to determine the fate of the dependent animal(s) before euthanizing the depended animal. In this regard, a consultation can be sought from a veterinarian or a competent technician. Otherwise, it is very probable that the orphaned infant(s) or the dependent animals endure substantial suffering and distress following the euthanasia of the depended animal.

2.5.14. The carcass of a laboratory animal should be treated appropriately. This is important from ethical and health viewpoints. Animal carcasses and the remnants of their bodies are categorized under 'very hazardous hospital waste'. According to the Criteria and Methods for Practical Management of Medical Wastes and other Related Wastes developed by the Department of Environment, carcasses of animals are categorized as 'specialized medical wastes' and 'very contaminating wastes' and therefore should be treated as specialized waste until converted to normal waste.

2.5.14.1. Specifically, proper disposal of animal carcasses euthanized with barbiturates (such as sodium pentobarbital), xylazine, or certain other medications is of crucial importance, because the drug residue in these carcasses may be very dangerous or even fatal for other animals that may gain access to these carcasses.

2.5.14.2. Laboratory animals may have been intentionally infected by contagious agents during the course of an experiment. The remnant of these contagious agents in animal waste could be very dangerous for other animals and the environment.

- 2.5.14.3. The euthanized animals may have received various drugs (such as antimicrobial drugs) during the course of a project. [The remnants of] these drugs may have an unfavorable effect on the environment or pose danger to other living beings coming in contact with the remnants of the euthanized animal.
- 2.5.15. The temperature of the cold room for animal carcasses should preferentially be -20 degrees centigrade or colder. All regulations regarding the storage of infectious waste in this place should be observed.
- 2.5.15.1. Before placing animal carcasses in the cold room, the carcasses should be packed in sealed packages or bags, and there should be a label on the bag/package containing information about the date of death, and the name and contact number of the responsible person for the carcasses. A notice should be placed on the bag/package of the carcass, displaying the phrase 'Toxic and Dangerous' in the form of written text or special signs. For animal carcasses that are contaminated with contagious diseases, or containing radioactive materials, or any carcasses more dangerous than normal carcasses, it is necessary to take appropriate actions according to the valid and up-to-date resources.
- 2.5.16. Transportation of carcasses should be performed according to the special regulations detailed in valid and up-to-date scientific resources. Briefly, transportation should be performed with a vehicle having a cargo space that is non-penetrable with no leaks and is covered by suitable means. The cargo space should be washable and disinfectable. The flooring of the cargo space should not be covered with a carpet or moquette material, and if possible, should have an intact and seamless covering. The driver cabin should be separated from the cargo space and the carcasses should be placed in a cold container [at the cargo space]. All other aspects of transportation of carcasses should be performed according to pertinent local, institutional, and/or estate rules and regulations.

2.5.17. Discarding laboratory animal carcasses should be performed according to pertinent estate regulations, and the direction of the Department of Environment such as ‘Criteria and Methods for Practical Management of Medical Wastes and other Related Wastes’. Disposal of animal carcasses along with normal waste or regular biohazard waste is a threat to public health, is prohibited, and doing so has legal consequences.

2.5.17.1. For mass euthanasia and disposing of a large number of carcasses, one should perform a thorough investigation on the best method of their elimination before performing the euthanasia, and arrange for the required preparations. In this regard, pertinent estate regulations and directions, in particular directions of the Veterinary Organization, should be observed. In certain circumstances using international guidelines regarding disposal of a large number of animal carcasses may be useful.

2.5.17.2. Carcasses that are infected by rabies, or are suspected to be infected by rabies, should be treated according to the current directions of the Pasteur Institute of Iran.