ANNEX

to Recommendation of the
Eurasian Economic Commission's Board
No.___ dated _____, 20__

GUIDELINE
on Handling Laboratory Animals in Non-Clinical Trials/Studies

I. General provisions

1. There is a wealth of scientific evidence on the factors influencing on animal welfare, the ability of animals to feel and show signs of pain, stress, distress, suffering and injury with long-term negative health consequences. Therefore, the welfare of animals used for scientific purposes should be constantly improved through raising standards of their protection in accordance with up-to-date scientifically grounded requirements.

2. Despite the fact that there are more and more people supporting exclusion of animals from the scientific process around the world, the participation of animals in experiments is still necessary for protection of the human and animal health, as well as the environment. Herewith, a high level of the laboratory animals protection (hereinafter referred to as animals) should be ensured if their use cannot be avoided.

3. The general public is not indifferent to ethical aspects of the experiments using animals. For this reason, animals should always be regarded as sentients, and their use shall be rationally limited. Use of animals

for scientific or educational purposes is justified only in the cases where alternative methods are unavailable.

Receiving Animals for Study

4. It should be ensured that the use of animals in experiments does not endanger biodiversity.

5. Certain vertebrate species should be bred specifically for experimental procedures so that their genetic, biological and behavioral characteristics are well known to researchers. Such knowledge provides means of both improving the quality and reliability of scientific results and reducing the scatter of the data obtained; that decreases a number of research procedures and a number of animals used. Moreover, to improve animal welfare and species preservation, experiments on wild animals should be limited to cases where the intended objectives cannot be achieved using specially bred laboratory animals.

6. Since the origin of homeless and feral pets is unknown, and their capture and placement in laboratory environment is traumatic, their use in experiments is prohibited.

7. Research animals may be received from specialized laboratory animal husbandries or from breeders only.

II. Terms and Definitions

8. For the purposes of these Guideline, the terms below shall have the following meaning:

External Validity is the degree, to which the results of a specific study allow using or summarizing other studies, study conditions, animal lines and/or species, or people;

Internal Validity is the degree, to which the results of a specific study may be attributed to the effects of investigational intervention, and not to some other unknown factors (e.g. inconsistencies in the study design, conduct or analysis introducing biased error);

Distress is the suffering condition, in which an animal cannot fully adapt to stress factors and the stress caused by them;

Breeder is any juridical or natural person engaged in animal breeding;

Humane Endpoint is the earliest indicator in animal experiment evidencing severe pain, distress, suffering or impending death. When the humane endpoint is reached, measures should be taken to stop exposure, to minimize or reduce the effects on the trial subject through euthanasia of the animal, termination of the painful procedure or providing treatment to relieve pain and/or distress;

Power (in terms of a predetermined biologically significant effect) is the probability that a statistical test will detect an effect, if it exists (that is, the zero hypothesis has been correctly rejected);

Zero and Alternative Hypotheses: the Zero Hypothesis (H_0) is that there is no effect, such as a difference between groups or an association between variables. The Alternative Hypothesis (H_1) postulates that the effect exists;

Husbandry means any juridical person that breeds animals for their use for scientific purposes;

Outcome Index means any variable recorded during a study to evaluate the effect of a treatment or investigational intervention. It also known as Effect Variable, Response Variable;

User means any natural or juridical person using animals in procedures, whether this is done for profit or not;

Dealer means any natural or juridical person, other than the breeder, who delivers animals for scientific use;

Bias means overestimation or underestimation of the intervention true effect. Bias is caused by inconsistencies in an experiment design, conduct, or analysis leading to an error;

Procedure means any type of animal use (invasive or non-invasive) for investigational or other scientific purposes, with known or unknown outcome, or for educational purposes, which may cause pain, suffering, distress or injury to the animal with lasting consequences for health equivalent to or greater than those caused by the needle insertion, as well as any intervention resulting or likely to result in the birth or breeding of an animal, or creation and maintenance of genetically modified lines of animals. However, this term does not include euthanasia of animals solely for use of their organs and tissues;

Project means a work program having a specific scientific objective and including one or more procedures;

Sample Size means the number of experimental units in a group, also indicated as *n* value;

Effect Size means the quantitative measure of differences between groups or strength of correlations between variables;

Stress means the body state characterized by emotional and physical tension caused by the influence of various adverse factors.

Institution means a juridical person created by the owner to carry out scientific or educational activities, with any form of ownership, and represents any structure, building, group of buildings or other premises, and

which may include partially fenced or sheltered premises, as well as mobile facilities;

Euthanasia means the humane destruction of animals using methods ensuring rapid unconsciousness and death without pain or stress;

Experimental Unit means biologic unit exposed to intervention independently of all other units, so that any two experimental units may be assigned to different experimental groups. Sometimes it is called the randomization unit;

Experimental Animal means the animal intended for conducting an experiment/-s. The primary application of Experimental Animals in medicine and biology is the production, manufacturing, determination of safety, efficacy and quality of modern medicines, food products and some other substances in order to prevent their possible undesirable effect on the health and life of humans, animals or plants. Less commonly, this entails study of the animal natural habitat, maintaining the population, species diversity, etc. in the interests of ensuring a prosperous human habitat and preserving nature. In education, Experimental Animals are used to train subject specialists (veterinarians, physicians, biologists, livestock specialists, research analysts, etc.);

III. Planning, Conducting and Reporting in Animal Studies

9. To ensure the animal studies fulfill their role in evaluating safety and efficacy of a medicinal product for humans, the planning, conducting and reporting of each study must be completely transparent and credible, and that each method be reproducible.

10. The scientific background information for animal study shall demonstrate an explicit evidence gap and explain why an *in vivo* approach

would be justified. Systematic reviews of the animal literature provide the most robust evidence that the study issue has not been definitively resolved through demonstrating the amount of data available within the study area. They can also assist in selection of an experimental model by providing a comprehensive overview of the models used and their advantages and limitations.

11. The study rationale and context should be provided, as well as its relationship to other studies, including appropriate references to previous work. The evidence forming the basis of the hypothesis or objectives should be used and the selected experimental approach as the best suited one to answer the research questions should be clarified.

12. If any aspect of a human disease is being modeled in the study, the suitability of the model for the specific study objectives should be stated. This may include a description of how the disease, disorder, or injury onset in the animal is similar to the human condition (how the model responds to known clinically effective treatments; how similar the symptoms are to the clinical disease) and how animal characteristics have been selected to reflect the clinical population age, sex, and health state.

1. Study Purpose and Objectives

13. Explaining the study purpose through describing the issues that the study addresses allows to determine the study relevance.

14. Knowing whether a study is exploratory analysis or hypothesis testing is critical to its interpretation. A typical exploratory analysis may produce several results that can be used to generate hypotheses. Henceforth, it is expedient to plan a pilot study, the purpose of which is to clarify the design or the possibility for conducting larger subsequent experiments. Exploratory analyses provide aid in planning hypothesis testing experiments

through selecting the variables or outcome indices to focus on in subsequent studies.

15. A particular hypothesis testing is important for both study design and data analysis. For instance, an experiment designed to detect an anticipated effect would most likely need to be analyzed using statistical inference, and statistical estimate of the sample size should be done a priori. In a hypothesis testing study, there is also a predefined primary outcome measure that is used to assess the evidence in support of a particular study issue.

16. In contrast, preliminary, exploratory and pilot studies investigate a number of possible effects and are likely to generate more false positives as some would be positive by chance. Thus, results from well-designed hypothesis testing studies provide stronger evidence than results from exploratory or pilot studies. Independent study replication and meta-analysis can further increase confidence in the findings.

2. Study Planning

17. For each study, the design shall be developed on the basis of documents regulating such a study in accordance with the legislation of the Member State of the Eurasian Economic Union or at the interstate level (e.g. OECD protocols or the corresponding GOSTs on toxicology), taking into account all available data on the investigational medicinal product. The international 3R principles (Replacement, Refinement, Reduction) should be considered.

18. To ensure that the ways in which animals are bred, cared and used in experiments conducted in the Eurasian Economic Union are in line with

other international and national standards, they should be systematically reviewed. When selecting study methods, alternative research methods without using animals should always be considered.

19. The selection of study methods and biological model type has direct impact on the number and welfare of living test systems. Therefore, when selecting a method, the following principle should be followed: causing minimum of pain, suffering and distress to an animal along with obtaining reliable results. The selected methods shall exclude, as far as possible, the animal death due to the severe suffering experienced by the animal before death as the experiment endpoint. If possible, more humane experiment endpoints such as taking into account the clinical signs of impending death and giving animals the opportunity to die with the least suffering should be selected.

20. The choice of control or comparison group depends on the experiment purpose. Negative control is used to determine if a difference between groups is due to an intervention (e.g. wild animals versus genetically modified animals, placebo versus active treatment, sham surgery (sham-operated) versus surgical intervention). Positive controls can be used to support the interpretation of negative results or to determine whether an expected effect can be detected.

21. It may not be necessary to include a separate control without active treatment if, for instance, the experiment is aimed to compare treatments administered by different methods (e.g. intraperitoneal versus oral gavage) or animals used as their own controls in a long-term study. The pilot study (procedure practicability study) may also not require a control group.

22. When planning complex projects, a visual representation of the experiment design is easier to interpret than narrative description, therefore, a timing diagram or flowchart is recommended. Diagrams help to identify

which treatment types and procedures have been applied to specific animals or animal groups, and at what study stage they have been performed. They also help report complex design features such as factor intersection or nesting (hierarchical/layered designs), blocking (breakdown into blocks to reduce unwanted variations), or repeated measurements over time on the same experimental unit (repeated measurements design).

23. When considering any scientific project, the planning stage should take into account an independent assessment considering ethical aspects of animal use and ensuring implementation of the replacement, refinement and reduction principle in such projects, that is, the assessment of the project by persons not involved in its direct implementation and impartial to its results.

24. Each animal use case should also be justified, both ethically and scientifically; it should be carefully assessed for its scientific or educational significance and relevance of the expected experiment results. Possible harm to animals should be weighed against the expected benefits of the project.

25. In some cases, depending on the project type, the animal species used and the likelihood of achieving the project objectives, a final assessment may be required. Given that projects may significantly vary in complexity, duration, and timing of outcomes, the decision on conducting final evaluation should be based on these aspects.

3. Sample Size

26. At all stages of the study (from planning to reporting), the total number of animals used, number of experimental animals (directly involved in the experiment) allocated to each group, and total number in each experiment (e.g. in case of simulation on more animals) should be accurately indicated.

27. This information is critical for assessing validity of the statistical model and reliability of the experiment results.

28. The sample size in each group at the beginning of the study may differ from the number *n* in the analysis. This information helps to determine the reduction in the sample, whether there were exceptions and in which group they occurred. It is useful to include information on the total number of animals used in the study in the research report, as well as whether any animals have been reused between the experiments.

29. The sample power calculation should be carried out at the study design stage, and methods for determining the sample size should also be indicated, except in cases where the study design and the sample size are determined by regulatory documents. For hypothesis testing experiments, where statistical data are used to assess the effect size and to weight the evidence against the zero hypothesis, the sample size should be justified to ensure that the experiments are of the optimal size for the study verification. Too small sample sizes (underpowered studies) produce inconclusive results, while to large sample sizes (excessive studies) raise ethical concerns due to the unnecessary use of animals and may produce trivial results that are statistically significant but not biologically significant. With a small sample size, the following effects can be observed:

in the experiment, the probability of missing actual effects increases;

when an effect is discovered, this will often be an overestimate of the true effect size.

Therefore, low power studies contribute to low internal validity of studies and the risk of the used animals attrition in inconclusive studies.

4. Inclusion and Exclusion Criteria

30. The inclusion and exclusion criteria determine the suitability or disqualification of animals and data after the study initiation. To ensure scientific rigor, criteria are determined prior to the experiment start and data collection. Inclusion criteria should not be confused with the characteristics of the animals, they may be related to them (e.g. body weight must be in a certain range for a particular procedure) or to other study parameters. In studies in which selected data are re-analyzed for another purpose, inclusion and exclusion criteria should include information on how the data have been selected.

31. Exclusion criteria may be due to technical or animal health issues, such as complications expected during surgery, or circumstances in which testing procedures may be compromised (e.g. development of motor impairments that may affect behavioral measurements). Exclusion criteria for samples or data include failure to meet quality control standards (e.g. insufficient sample volumes, unacceptable contaminant levels, poor histological quality, etc.).

32. Exclusion criteria may also reflect the ethical principles of the study according to its humane endpoints. For instance, in tumor research, an animal may be excluded from the study and euthanized before a predetermined time point, if the subcutaneous tumor size exceeds the certain volume. If losses are expected, they should be taken into account when determining the number of animals to be included in the study. While exclusion criteria and humane endpoints are usually included in the ethical review application, communication of the criteria used to exclude animals from study or data points helps to interpret the data and provides important information to other investigators wishing to replicate the model.

5. Principles of Animal Grouping for a Study

33. The use of appropriate randomization methods when assigning animals to groups ensures that each experimental unit has equal likelihood of receiving a particular treatment and ensures balanced number in each group. The ad hoc animal selection from the cage (i.e. arbitrarily) is not statistically random, since this process involves a human. This may lead to offset affecting results since the investigator may (deliberately or innately) make judgments when assigning an animal to a particular group, or due to unknown and uncontrolled differences in experimental conditions or animals in different groups. Using a validated randomization method helps to minimize selection biased error and reduce systematic differences in the characteristics of animals assigned to different groups. Statistical data based on non-randomized group assignment are not valid. Thus, the use of randomization is prerequisite in any experiment intended for hypothesis testing.

34. Reporting the exact randomization method and type (simple, stratified, randomized complete blocks, etc.) used for randomization generation (e.g. computer-generated randomization with details of the algorithm or program used) allow to assess the results reliability and identify potential limitations. If randomization has not been used to assign the experimental units to groups, this shall be stated explicitly and explanation of how the compared groups were formed is required.

6. Ensuring Impartiality

35. Investigators often expect a particular outcome and may inadvertently influence an experiment or interpret data in a way that supports their preferred hypothesis. Blinding is the strategy used to minimize these subjective biases.

36. Unblinded evaluation of results leads to overestimation of treatment effects, and absence of measures to reduce bias, such as randomization and blinding, can increase the effect by 30% to 45%.

37. Ideally, investigators should not know what treatment the animals have received or will receive from the experiment start until data analysis. If this is not possible for every experiment stage, it should always be possible to run at least some of the stages blind. It is significant for the experiment arrangement and may require assistance from additional personnel (e.g. surgeon to perform interventions, technician to code investigational medicinal products).

38. For each study, it should be indicated whether investigator blinding has been used for each stage of the experimental process, and information on which procedures or conditions the investigators have been unaware of or knew about. If blinding was not used in any of the steps, this should be clearly stated, as well as the reason why blinding was not possible or not considered.

7. Outcome Indices

39. The outcome index is any variable recorded during a study (e.g. amount of tissue damaged, number of dead cells, specific molecular marker) to assess the effects of treatment or experimental intervention.

40. When planning, implementing, and describing a study, it is important to clearly state what has been measured, especially when these measurements can be implemented in different ways. For example, activity can be recorded as time spent for moving or distance travelled. Where possible, measurement results should be recorded in an unbiased manner (e.g. by blinding).

41. In hypothesis testing experiment, the primary outcome measure responses the primary biological issue. This is the most important result, which is determined during the experiment design stages and is used as the basis for calculating the sample size. For exploratory studies, it is not necessary to identify a single primary outcome, and multiple outcomes are often evaluated.

42. In a hypothesis testing study designed to identify effects on a primary outcome measure, data on secondary outcomes are used to estimate the additional effects of intervention, but subsequent statistical analysis of the secondary outcome measures may be underpowered, making the results and interpretation less reliable. Studies that claim to test a hypothesis but do not specify a predetermined primary outcome measure, or those that change the primary outcome measure after data is collected (also known as primary outcome switching), may randomly report only statistically significant outcomes in preference to more positive results.

8. Statistical Methods

43. The methods of statistical analysis used shall be consistent with the experiment objectives and design; they shall be determined in advance, before data collection begins. Exploratory and hypothesis testing studies may use descriptive statistics to summarize data (e.g. average and standard deviation, or median and quartile range). In exploratory studies that did not test a specific hypothesis, descriptive statistics are important for generating new hypotheses that can be tested in subsequent experiments, but do not allow drawing conclusions that go beyond the data obtained. In addition to descriptive statistics, hypothesis testing studies may use statistical inference to test a particular hypothesis.

44. A detailed description of analytical methods is required to ensure that the appropriateness of the methods selected can be evaluated and the results validity can be assessed. The description of the statistical analysis should be detailed enough to allow another investigator to reanalyze the raw data using the same method and obtain the same results.

9. Experimental Animals

45. Species, lineage, sublineage, sex, weight and age of animals are critical factors that can affect most experimental results. Reporting the characteristics of all animals used is equivalent to standardized patient demographics. These data confirm both the internal and external validity of the study results. This allows other investigators to repeat the experiment and summarize the results and assess whether the characteristics of the animals chosen for the experiment are consistent with the study objectives.

46. When reporting age and weight, summary statistics for each treatment group (e.g. average and standard deviation) should be included and, if possible, baseline values for individual animals (e.g. as additional information or as a link to a public data repository). Since body weight may change during the study, the time and date when the measurements were taken should be indicated. For most species, accurate age information is more informative than description of developmental status (e.g. a mouse that is referred to as an adult may be between 6 and 20 weeks old). However, in some cases information on the developmental stage is more informative than life age (e.g. in juvenile *Xenopus* (clawed frog), whose development rate can be controlled by incubation temperature).

47. Indication of the weight or sex of the animals used may not be appropriate for some studies. For example, sex may not be known for

embryos or juveniles, or weight measurement may be particularly stressful for some aquatic species.

48. The animals origin, their health or immune status, and history of previous tests or procedures on animals may influence their physiology and behavior, as well as their response to manipulations in the current study, and thus influence the study results. For instance, animals from the same strain but from different sources, or animals from the same source but at different times, may be genetically different. The immune or microbiological status of animals can also influence welfare, experimental variability and scientific results.

49. The health status of all animals participating in the study and any previous procedures to which the animals have been subjected should be reported. For instance, if animals are specific pathogen free (SPF), the pathogens from which they are free should be listed. If the health state is unknown or has not been tested, state it directly.

50. For genetically modified animals, the status of the genetic modification (e.g. knockout, overexpression), genotype (e.g. homozygous, heterozygous), altered gene/-s, genetic methods and technologies used to create the animals, how the genetic modification has been confirmed, and detailed information on animals used as controls (e.g. control littermates). Proper presentation of animal nomenclature is critical to understanding the data and enabling the detection and reproducibility of the study results.

10. Experimental Procedures

51. Important description information includes the procedures used to develop the model (e.g. pathology induction), the procedures used to measure outcomes, and pre- and post-experiment procedures, including animal handling, welfare monitoring, and euthanasia. Animal handling can be a

source of stress, and the specific method used (e.g. grabbing mice by the tail or folded hands) may affect the study results. Details of animal care and observations during the study and any quality assurance and control measures used should be documented so that other experimenters can replicate these methods. A diagram of experimental procedures with a timeline can give clear idea of how the study has been conducted.

52. The frequency and timing of experimental procedures and measurements should be neatly specified, including the cycle of light and dark time (e.g. 12 hours dark and 12 hours light), daily timestamps (e.g. turning on the lights at 8:00 am) and the experimental time sequence (e.g. interval between baseline measurements or interval between procedures and measurements). Along with natural circadian rhythms, they can influence study outcomes such as behavioral, physiological, and immunological parameters. The timing and frequency of the welfare assessment should also be specified, taking into account natural behavior patterns (e.g. nocturnal animals may not show behavioral signs of discomfort during the day).

53. Physiological acclimatization after a stressful event, such as transportation (e.g. between the dealer, animal facility, operating room and laboratory), prior to the start of the experiment, allows the animal's physiological responses to stabilize. Protocols differ depending on the species, lineage (e.g. physiological acclimatization after transportation of different species of animals can take from 24 hours to 1 week). Procedural acclimatization (immediately prior to the procedure) allows the animals to stabilize their response to unaccustomed manipulation, new environments, and previous procedures that may otherwise cause behavioral and physiological changes. Standard acclimatization periods may vary depending on research laboratories and the Member State legislation requirements.

54. It is important to indicate where the study has been conducted (e.g. dedicated laboratory or animal room, housing cage, open field arena, water maze) and whether physiological or procedural acclimatization periods were included in the study protocol, including their type and duration. If the study involved several locations, the location of each experiment and analysis of the samples should be indicated.

55. There are many approaches to evaluating any research problem; therefore, it is important to explain why a particular procedure or technique has been selected. This is especially relevant when the procedures are new or specific to the research laboratory, or are limited to animal models or experimental equipment (e.g. the route of administration is determined by the animal size).

11. Ethical Statement

56. Investigators are responsible for following the rules and instructions concerning animal use for scientific purposes. This includes ensuring that they have obtained appropriate approval for their study from the appropriate ethics committee and/or regulatory authority prior to commencement of work. The ethical statement provides assurance that the study has been ethically assessed. It also promotes transparency and understanding of the animal use in research and enhances public confidence.

57. An example of the scientific projects evaluation organization could be a bioethical commission set up as a working group in an institution conducting study using animals. The bioethical commission carries out expert assessment of research projects using laboratory animals.

58. When using animals in specialized educational institutions of higher education, students must study the technology of experimental work and the ethical standards that the experimenter must follow.

59. The organization of the educational process shall be based on this Guideline. In the structure of an educational institution, a bioethical commission can also be set up, which shall carry out expert assessment of educational modules using laboratory animals.

IV. Requirements for Animal Care and Housing in an Institution

60. Laboratory animal welfare shall be of primary concern in their housing, breeding and using. Organizations conducting scientific research should have a working group, whose activity includes issues related to animal welfare and advice to employees on the related issues. This group shall also monitor the progress and results of projects at the institution level, promote necessary working environment for effective animal care, and provide the personnel with the tools to apply and timely implement the latest technical and scientific innovations in terms of the replacement, refinement and reduction principle for improving the animal life. Recommendations given by such a group shall be properly recorded and available for analysis during inspections.

61. An example of such a group would be the Animal Welfare Committee, an internal initiative group within an institution responsible for the day-to-day evaluation and monitoring of the purposes and objectives for using laboratory animals.

62. The Animal Welfare Committee performs:

veterinary and sanitary assessment of all premises and areas for manipulations with animals, inventory in the laboratory vivarium and husbandry;

assessment of animal welfare (quality of care, housing, feeding, watering, etc.);

consideration of animal welfare issues of concern to employees of the organization;

policy of development of the vivarium and husbandry material base;

advising employees of the organization on issues of bioethics, humane treatment of animals.

63. In order to enable the competent authorities to monitor the implementation of the provisions hereof, each breeder, dealer and user should keep clear records of the number of animals used, their origin and fate.

64. For non-human primates, dogs and cats, a personal dossier shall be compiled, which is to be maintained throughout life, starting from birth, and contains the information necessary to provide them with care, housing conditions and treatment in accordance with their individual needs and features.

65. The conditions for their housing and care shall correspond to the species needs and features.

66. The implementation of control and supervisory measures in relation to breeders, dealers and users is carried out in accordance with the acts constituting the law of the Eurasian Economic Union.

1. Animal Housing and Care

67. The environment determines the animal health and welfare, and every aspect of it can potentially influence their behavioral and physiological responses, thereby influencing research results. Different studies may be sensitive to different environmental factors, and the specific environmental aspects that should be reported may depend on the study type.

68. The environment, both devoid of and enriched with additional elements, can influence a wide range of physiological and behavioral responses. Structural enrichment such as elevated surfaces, partitions,

resources for typical activities (e.g. nest material, rodent shelters; plants or gravel for aquatic species), as well as toys or other tools designed to provide a sense of novelty, used to encourage exploration, exercise (e.g. running wheel) are distinguished.

69. If no environmental enrichment has been foreseen, this should be clearly stated and justified. The scientific justification for food and water deprivation and for solitary confinement should also be provided.

2. Premises

Functions and Planning

70. All premises should be designed in such a way as to provide the life environment appropriate to the physiological and ethologic needs of the animal species they contain. The premises layout and operation should exclude access by unauthorized persons, as well as the escape of animals and their penetration from the outside.

71. It is recommended that the institution has an ongoing maintenance program in place to prevent and correct any malfunctions in the premise and equipment, and to ensure the continuity of the institution's operations, especially in animal life support. As part of a risk-based approach to the main processes of the institution, all short-term and long-term emergencies that may affect the animal life support (e.g. power, water supply, ventilation systems, etc. outages).

72. For risk assessment, it is recommended to use methods that allow identifying the problem before it appears and has an impact, and which are aimed at establishing the severity of the harm of the consequences, the likelihood of the hazard occurrence and its identification.

Animal Housing Premises

73. It is recommended that the premises have an effective schedule for regular cleaning of the premises and to maintain satisfactory hygiene standards. To assess the sanitary and hygienic indicators of the premise cleanliness, it is expedient to use those provided for medical institutions. At that, the risk-based approach should be used to determine the sampling and washing periodicity depending on the purpose of each premise and the sanitary and epidemiological situation in each specific region.

74. Walls and floors should be covered with material resistant to aggressive detergents, which cannot be damaged by animals and which does not adversely affect the health of animals and injure them. Instruments and equipment should be provided with additional protection against damage by animals and from injury to the animals themselves.

75. Incompatible animal species, such as a predator and its potential prey, or animals requiring different conditions, should not be kept in the same premise. In the case of a predator and a potential prey, they are not allowed to be in the area of visual, olfactory or sound contact.

General and Dedicated (Treatment) Premises

76. It is recommended that the institution has laboratory rooms for performing simple diagnostic tests, performing autopsies and/or for collecting samples that are sent for more detailed analyses to other laboratories. General and dedicated premises should be used when procedures or observations in the animal housing are undesirable.

77. Separate premises should be provided for the quarantine of newly introduced animals until their health status and potential health risks to animals already in the institution have been identified.

78. Separate premises should be allocated for keeping sick or injured animals.

Service Premises

79. Storage premises should be designed, used and maintained in such a way as to maintain the quality of feed and bedding material. Such premises should be free of pests and insects. Contaminated materials, including all types of waste that pose hazard to animals or personnel, shall be stored separately.

80. Cleaning and washing premises shall be large enough to accommodate the installations required for cleaning and washing equipment. The washing process should be organized so as to separate clean and dirty equipment in order to avoid contamination of freshly washed equipment.

81. The institution should provide conditions for hygienic storage and safe disposal of animal waste and their carcasses.

82. If sterile operations are required, one or more appropriately equipped premises should be provided for, as well as premises for postoperative recovery of animals.

Life Environment Control

Ventilation and Temperature

83. Thermal insulation, heating and ventilation of animal housings should provide conditions in which air circulation, dust content and gas concentration are maintained within limits that are not dangerous to animals and personnel.

84. The maximum allowable concentration (MAC) of harmful substances in indoor air shall be:

a) in the animal housing premises:

ammonia: 10 mg/m3,

carbon dioxide: 0.15 vol.%.

b) in production premises:

ammonia: 20 mg/m3,

formaldehyde: 0.5 mg/m3.

85. The temperature and relative humidity in the premises should be selected according to the type and age of the animals kept there. The temperature shall be measured and recorded daily.

Table 1

Reference range of
temperature and humidity parameters in the premises

Premise name	Temperature	Humidity
Lavatories, bathrooms, showers	20 ºC to 29 ºC	–
Disinfection and washing units	18 ºC to 26 ºC	< 75%
Biological waste decontamination and temporary storage premises	14 ºC to 24 ºC	< 75%
Laboratory animal housing premises (mice, rats, hamsters, degus)	18 ºC to 26 ºC	45% to 65%
Laboratory animal housing premises (gerbils)	20 ºC to 26 ºC	35% to 55%
Laboratory animal housing premises (guinea pigs)	15 ºC to 26 ºC	45% to 65%
Laboratory animal housing premises (rabbits)	15 ºC to 22 ºC	> 45%
Laboratory animal housing premises (ferrets)	15 ºC to 24 ºC	–
Primates:	23 ºC to 28 ºC	40% to 70% (higher humidity is acceptable)
Marmosets		
Monkeys, tamarins	23 ºC to 28 ºC (higher temperatures are acceptable)	
Cynomolgus monkeys, green monkeys	21 ºC to 28 ºC	
Rhesus macaques, short-tailed macaques, vervets	16 ºC to 25 ºC	
Long-tailed macaque	21 ºC to 28 ºC	
Mini-pigs housing premises	15 ºC to 27 ºC	40% to 75%

Premise name	Temperature	Humidity
	for newborn and prenursery pigs 27 ºC to 35 ºC	
Dog housing premises	15 ºC to 21 ºC	–
Prosectorium, procedure rooms for working with laboratory animals	18 ºC to 26 ºC	30% to 70%
Feed kitchens	18 ºC to 26 ºC	–
Warehouse for temporary storage of consumables	16 ºC to 25 ºC	60% to 70%
Warehouse of chemical reagents	8 ºC to 20 ºC	60% to 70%
Storage facilities (feed, bedding, hay)	8 ºC to 25 ºC	10% to 75%
Temporary clean inventory storage	5 ºC to 25 ºC	30% to 70%
Sample preparation room	20 ºC to 26 ºC	55% to 65%
Pharmaceutical storage	15 ºC to 25 ºC	55% to 65% (unless special storage conditions are specified in regulatory documentation)
Chemical-analytical, histological, biochemical, cellular laboratories	18 ºC to 26 ºC	40% to 60%
Histological archive (storage of raw material in formalin)	18 ºC to 25 ºC	40% to 75%
Microbiological laboratory	20 ºC to 26 ºC	30% to 60%
Archive	17 ºC to 19 ºC	50% to 55%
Office premises, passages	20 ºC to 25 ºC	15% to 75%

86. Animals should not be kept outdoors in uncontrolled climatic conditions that may cause them distress and introduce uncertainty into the study.

Lighting

87. Where natural light is not sufficient to provide a proper day/night cycle, artificial lighting should be provided to meet the biological needs of the animals and ensure satisfactory working environment.

88. The level of illumination should be sufficient for animal care and observation procedures.

89. Regular daylight hours and illumination intensity should be provided in accordance with the species-specific needs of the animals.

90. When keeping albino animals, the illumination level should be adjusted to take into account their increased sensitivity to light.

Noise Level

91. Noise levels, including ultrasound, shall not adversely affect the animal welfare.

92. If necessary, rooms for animals should be cladded with soundproofing and sound-absorbing materials.

Alarm Systems

93. Institutions using electrical and mechanical equipment for monitoring and emergency maintenance of the proper life environment should have a back-up service and a lighting system, and regularly check the operation of alarm systems.

94. Air heating and ventilation systems should be equipped with control and alarm devices.

95. Clear instructions for action in emergency situations shall be posted in a conspicuous place.

3. Animal Care

Health

96. Institutions should have an in-house program ensuring the animal health maintenance for their welfare and compliance scientific requirements. Such program shall include regular monitoring of animal health, a microbiological surveillance program and action plans in case of disease. This program shall also establish health status criteria and procedures for accepting new animals.

97. The risk-based approach should also be used for animal health monitoring, as there cannot be a single list of pathogens for every animal species, regardless of the region in which the institution is located. These indicators depend on the sanitary and epidemiological situation and the specificity of each region. The list of pathogens, frequency of sampling, sample size, etc. shall be determined based on the risk-based approach in each individual institution.

98. Animal inspection shall be carried out by a competent person at least once a day. In case of detection of sick or injured animals, the necessary measures should be taken.

Wild Animals

99. Transportation containers and vehicles should be suitable for the animal species and located at the trapping sites so that the animals can be sent for examination or treatment if necessary.

100. Particular attention should be paid to the acclimatization, quarantine, housing and maintenance of wild animals, as well as their care and, if necessary, stipulate the conditions for their release into the wild on completion of the procedures.

Animal housing and life environment enrichment (creation of multi-stimulus conditions)

Housing

101. Animals, except for those that naturally lead a solitary life, should be kept in permanent social groups of individuals compatible with each other. Selection and analysis of socially compatible animals shall be carried out and documented. When proper individual housing is permitted, its duration should be limited to the minimum necessary period, while visual, auditory, olfactory and/or tactile contact with relatives must be ensured. The introduction of new individuals into a group or relocation of individuals from one group to another should be carefully monitored to avoid problems of incompatibility and disruption of social bonds.

Environment Enrichment (Creation of Multi-Stimulus Conditions)

102. All animals should be provided with the space rich enough for a wide range of their natural behavioral responses. They shall have some degree of control and choice of conditions in order to reduce stress-induced behavior. It is recommended to create multi-stimulus conditions to expand the range of animal activity, including exercise, food search, play and a cognitive activity in accordance with their species. Means for enriching the habitat are selected in accordance with the species and individual features of animals. Institutional enrichment strategies shall be documented, regularly reviewed and updated.

Cages

103. Cages should be made of animal-safe materials. Their design and construction shall exclude the possibility of any injury to animals. Reusable

cages shall be made of materials capable to withstand washing and disinfection. The cage floor should be made taking into account the animal species and age features and convenient for removing their waste products.

Feeding

104. The feed form, composition and distribution shall correspond to the nutritional and behavioral needs of the animals.

105. The feed should be palatable and free from harmful substances. When selecting raw materials, manufacturing, preparation and distribution, measures should be taken to minimize chemical, physical and microbiological contamination.

106. The feed packaging, transportation and storage should exclude the possibility of its contamination, deterioration or destruction. All containers, waterers or other utensils used for feeding should be cleaned regularly and sterilized as necessary.

107. Each animal shall have access to feed and sufficient space for feeding to minimize competition.

Watering

108. All animals shall have constant access to clean drinking water.

109. The automatic watering system in use should be regularly checked, serviced and flushed to avoid watering problems. If solid bottom cages are used, measures should be taken to minimize the risk of flooding.

110. The supply of water to aquariums and boxes shall be in accordance with the species needs and tolerances for individual fish, amphibians and reptiles.

Rest and Sleep Space

111. Species-specific bedding or sleeping shelters should always be available, including nesting material or special designs for institution-bred animals.

112. Cages shall have reliable and comfortable places to rest in accordance with the species features of the animals. The place to sleep should be clean and dry.

Animal Handling

113. It is recommended that the institution have adaptation and training programs appropriate for the different animal species, procedures and duration of the project.

4. Species Specificity

Mice, Rats, Gerbils, Hamsters and Guinea Pigs

114. In this and the following Tables providing data for mice, rats, gerbils, hamsters and guinea pigs, the "cage height" means the distance between the cage floor and the top; at that, over 50% of the minimum cage area shall be at this height before placing aids to create multi-stimulus conditions (environmental enrichment). When planning procedures, it is necessary to take into account the likely growth of animals to ensure sufficient living space (according to Tables 2–6) for the entire study period.

Table 2

Mice

	Weight, g	Minimum cage dimensions, cm^2	Cage area/animal, cm^2	Minimum cage height, cm

	Weight, g	Minimum cage dimensions, cm^2	Cage area/animal, cm^2	Minimum cage height, cm
In colony and during experiments	< 20	330	60	12
	20–25	330	70	12
	25–30	330	80	12
	> 30	330	100	12
Breeding		330 For monogamous couples (outbred or inbred animals) or triads (inbred animals). For each additional female with litter, 180 cm^2 shall be added	–	12
In the breeder's colony*	< 20	950	40	12
	< 20	1500	30	12

* For a short period after weaning, mice may be housed in higher density groups provided they are housed in large cages with a sufficiently enriched environment, as long as there are no signs of deterioration in their welfare: increased aggression, morbidity and mortality, as well as stereotypy and other behavioral disturbances, weight loss, or other physiological or behavioral responses caused by stress.

Table 3

Rats

	Weight, g	Minimum cage dimensions, cm^2	Cage area/animal, cm^2	Minimum cage height, cm

	Weight, g	Minimum cage dimensions, cm^2	Cage area/animal, cm^2	Minimum cage height, cm
In colony and during experiments[1]	< 200 200–300 300–400 400–600 > 600	800 800 800 800 1500	200 250 350 450 600	18 18 18 18 18
Breeding		800 Female with litter; for each additional adult rat, 400 cm^2 shall be added	–	18
In the breeder's colony[2] Cage, 1500 cm^2	< 50 50–100 100–150 150–200	1500 1500 1500 1500	100 125 150 175	18 18 18 18
In the breeder's colony[2] Cage, 2500 cm^2	< 100 100–150 50–200	2500 2500 2500	100 125 150	18 18 18

[1] In long-term studies, if the space area per animal by the end of the experiment becomes less than indicated in the Table, then the constancy of the social group should be the priority.

[2] For a short period after weaning, rats may be housed in higher density groups provided they are housed in large cages with a sufficiently enriched environment, as long as there are no signs of deterioration in their welfare: increased aggression, morbidity and mortality, stereotypy and other behavioral disturbances, weight loss, or other physiological or behavioral responses caused by stress.

Table 4

Gerbils

	Weight, g	Minimum cage dimensions, cm^2	Cage area/animal, cm^2	Minimum cage height, cm
In colony and during experiments	< 40 > 40	1200 1200	150 250	18 18
Breeding		1200 for monogamous couples or triads with litter	–	18

Table 5

Hamsters

	Weight, g	Minimum cage dimensions, cm^2	Cage area/animal, cm^2	Minimum cage height, cm
In colony and during experiments	< 60 60–100 >100	800 800 800	150 200 250	14 14 14
Breeding		800 females or monogamous couples with litter	–	14
In the breeder's colony[1]	< 60	1500	100	14

[1] For a short period after weaning, hamsters may be housed in higher density groups provided they are housed in large cages with a sufficiently enriched environment, as long as there are no signs of deterioration in their welfare: increased aggression, morbidity and mortality, stereotypy and other behavioral disturbances, weight loss, or other physiological or behavioral responses caused by stress.

Table 6

Guinea pigs

	Weight, g	Minimum cage dimensions, cm^2	Cage area/animal, cm^2	Minimum cage height, cm
In colony and during experiments	< 200 200–300 300–450 450–700 > 700	1800 1800 1800 2500 2500	200 350 500 700 900	23 23 23 23 23
Breeding		2500 couples with litter; for each additional female, 1000 cm^2 shall be added	–	23

Rabbits

115. Inside the cage, a special platform raised above the floor shall be provided. This platform shall allow the animal to sit and lie freely, as well as freely climb under it, while the size of the platform should not exceed 40% of the entire cage area. In the case where the use of such platform is impractical for veterinary or scientific reasons, then the size of the cage should be 33% larger for one rabbit and 60% for a pair of rabbits. When keeping rabbits aged less than 10 weeks, the size of the platform should be at least 55 cm × 25 cm, and the height of the cage should correspond to the size of the animal.

116. The data given in Table 7 apply to cages and pens. For each rabbit from the third to the sixth add at least 3000 cm^2, and for each additional rabbit over six — at least 2500 cm^2.

Table 7

Rabbits aged over 10 weeks

Weight, kg	Minimum area for 1 or 2 socially compatible animals, cm^2	Minimum cage height, cm
< 3	3500	45
3–5	4200	45
> 5	5400	60

Table 8

Rabbits: the optimal dimensions of the raised platform for cages having the dimensions indicated in Table 7

Age, weeks	Weight, kg	Optimum platform size, cm × cm	The optimal height of the platform from the cage floor, cm
> 10	< 3	55 × 25	25
	3–5	55 × 30	25
	> 5	60 × 35	30

Rabbits aged under 10 weeks

117. The data in Table 9 apply to cages and pens.

Table 9

Cage dimensions, for rabbits aged under 10 weeks

Age, weeks	Minimum cage dimensions, cm^2	Minimum cage area /animal, cm^2	Minimum cage height, cm
< 7	4000	800	40
7–10	4000	1200	40

Table 10

Dimensions of a cage for a female rabbit with litter

Female weight, kg	Minimum cage dimensions, cm^2	Additional space for nests, cm^2	Minimum cage height, cm

< 3	3500	1000	45
3–5	4200	1200	45
> 5	5400	1400	60

Cats

118. Cats should not be kept alone for more than 24 hours straight. Cats that are persistently aggressive towards other cats should be kept separately only if they cannot be matched with a compatible individual. The social compatibility of animals kept in pairs or groups is subject to control at least once a week.

119. Females in the last two weeks of pregnancy or with kittens aged less than four weeks can be kept separately.

120. The minimum space for keeping a female and litter is the space required for one adult animal. This space should be gradually increased so that by the age of 4 months the kittens are housed in accordance with the space needs of an adult animal.

121. Places for feeding and droppings trays shall be located at a distance of at least 0.5 m from each other. Do not change their places.

Table 11

Cat cage dimensions

	Area*, m^2	Shelves, m^2	Height, m
At least one adult animal	1.5	0.5	2

Increase for each additional animal	0.75	0.25	–

* Floor area not including shelves.

Dogs

122. If possible, dogs walking should be provided. Dogs should not be kept alone for more than 4 hours straight. The indoor portion of the enclosure shall be at least 50% of the minimum space required for a dog (see Table 12).

123. The space requirements detailed below are based on recommendations for Beagles, but large breeds such as St. Bernards or Irish Wolfhounds shall be provided with space significantly exceeding the dimensions shown in Table 12. For all breeds other than Lab Beagles, the required space shall be determined in consultation with veterinarians.

124. Adult individuals. When dogs are kept in pairs or groups, each adult individual can be isolated in a compartment equal to half the total area of the enclosure (2 m^2 for dogs weighing up to 20 kg, 4 m^2 for dogs weighing more than 20 kg), if such isolation is necessary to achieve scientific results. The period for which the dog may be subjected to such isolation should not exceed 4 hours straight.

125. Nursing female and puppies should be kept in the same area as a single female of the same weight. Puppy enclosures should be designed so that the female can move to an additional compartment or to an elevated area away from the puppies.

Table 12

Cage dimensions for keeping an adult dog

Weight, kg	Minimum cage dimensions, m^2	Minimum space for 1 or 2 animals, m^2	Space for each additional animal, m^2	Minimum height, m
< 20	4	4	2	2
> 20	8	8	4	2

Dog puppies after weaning

126. The size of the cage for keeping puppies with female is shown in Table 13.

Table 13

Cage size for puppies with females

Weight, kg	Minimum cage dimensions, m^2	Minimum space/animal, m^2	Minimum height, m
< 5	4	0.5	2
5–10	4	1	2
10–15	4	1.5	2
15–20	4	2	2
> 20	8	4	2

Ferrets

127. The cage dimensions for ferrets are shown in Table 14.

Table 14

Ferret housing cage dimensions

Weight, g	Minimum cage dimensions, cm^2	Minimum space /animal, cm^2	Minimum cage height, cm

< 600	4500	1500	50
> 600	4500	3000	50
adult males	6000	6000	50
female with litter	5400	5400	50

Non-human primates

128. Young non-human primates should not be weaned from their mothers until they are 6 to 12 months old (depending on species).

129. The environment must allow non-human primates to perform the complex daily activity program. The enclosure should enable implementation of behavioral reactions of the widest possible range and allow the animals to feel safe. The enclosure should be equipped so that the animals can run, walk, climb and jump. The cage size for keeping monkeys and tamarins is indicated in Table 15

Table 15

Cage dimensions for keeping monkeys and tamarins

	Minimum cage area for 1* or 2 animals and litter up to 5 months, m^2	Minimum capacity/additional animal older than 5 months, m^3	Minimum height**, m
Monkeys	0.5	0.2	1.5
Tamarins	1.5	0.2	1.5

*Animals may be kept separately in exceptional circumstances only.

** The upper part of the enclosure shall be at least 1.8 m from the floor.

Baby monkeys and tamarins shall not be weaned from their mother until 8 months of age.

Table 16

Cage dimensions for housing squirrel monkeys (saimiris)

Minimum cage area for 1* or 2 animals, m^2	Minimum capacity/additional animal older than 6 months, m^3	Minimum height, m
2.0	0.5	1.8

*Animals may be kept separately in exceptional circumstances only.

Baby saimiri shall not be weaned from their mother until 6 months of age.

Table 17

Cage dimensions for macaques and vervet monkeys (pygmy green monkeys)*

Age	Minimum cage dimensions, m^2	Minimum cage volume, m^3	Minimum volume/animal, m^3	Minimum height, m
< 3 years[1]	2.0	3.6	1.0	1.8
> 3 years[2]	2.0	3.6	1.8	1.8
Animals for breeding[3]			3.5	2.0

* Animals should be kept separately in exceptional circumstances only.
[1] A minimum size enclosure may house up to 3 animals.
[2] A minimum size enclosure may house up to 2 animals.
[3] In colonies intended for breeding, young animals aged under 2 years, kept with their mothers, do not require additional space and/or capacity.

Baby monkeys and vervets shall not be weaned from their mothers until 8 months of age.

Table 18

Cynocephalus housing cage dimensions*

Age	Minimum cage dimensions, m^2	Minimum cage volume, m^3	Minimum volume/animal, m^3	Minimum height, m
< 4 years[1]	4.0	7.2	3.0	1.8
> 4 years[1]	7.0	12.6	6.0	1.8
Animals for breeding[2]	–	–	12.0	2.0

* Animals may be kept separately in exceptional circumstances only.
[1] A minimum size enclosure may house up to 2 animals.
[2] In colonies intended for breeding, young animals aged under 2 years, kept with their mothers, do not require additional space or capacity.

Baby cynocephaluses shall not be weaned from their mother until 8 months of age.

Farm Animals

130. In agricultural research, when the purpose of the project requires animals to be kept in conditions similar to those used for commercial breeding, the conditions of housing shall comply with the standards established in the relevant regulatory documents. The pen dimensions for housing farm animals are indicated in Tables 19 through 21.

Table 19

Cattle housing pen dimensions

Weight, kg	Minimum pen size, m^2	Minimum pen area/animal, m^2	Feeder length/animal at group cattle housing, m	
			unlimited feed amount	limited feed amount
< 100	2.50	2.30	0.10	0.30
100–200	4.25	3.40	0.15	0.50
200–400	6.00	4.80	0.18	0.60
400–600	9.00	7.50	0.21	0.70
600–800	11.00	8.75	0.24	0.80
> 800	16.00	10.00	0.30	1.00

Table 20

Sheep and goat housing pen dimensions

Weight, kg	Minimum pen size, m^2	Minimum pen area/animal, m^2	Minimum fence height, m	Feeder length/animal at group housing, m	
				unlimited feed amount	limited feed amount
< 20	1.0	0.7	1.0	0.10	0.25
20–35	1.5	1.0	1.2	0.10	0.30
35–60	2.0	1.5	1.2	0.12	0.40
> 60	3.0	1.8	1.5	0.12	0.50

Table 21

Pig and minipig housing pen dimensions

Weight, kg	Minimum pen size, m^2	Minimum pen area/animal, m^2	Minimum area/animal for lying in the pen (in thermally neutral conditions), m^2
< 5	2.0	0.20	0.10
5–10	2.0	0.25	0.11
10–20	2.0	0.35	0.18
20–30	2.0	0.50	0.24
30–50	2.0	0.70	0.33
50–70	3.0	0.80	0.41
70–100	3.0	1.00	0.53
100–150	4.0	1.35	0.70
> 150	5.0	2.50	0.95
Adult boar	7.5		1.30

* Pigs may be housed in smaller pens for short periods (e.g. by dividing the main space with partitions) if justified by veterinary or experimental needs (e.g. when individual conditions for food intake are required).

Horses

131. The shortest side of the stall shall be at least 1.5 times the animal height at the withers. The height of the covered stall shall allow the animals to play up. The area of the stall for housing horses is indicated in Table 22.

Table 22

Horse stall area

Height at the withers, m	Minimum stall area/animal, m^2			Minimum stall height, m
	housing separately or in a group of up to 3 individuals	housing in a group of 4 or more individuals	for foaling, mare with a foal	
1–1.4	9.0	6.0	16	3.00

1.4–1.6	12.0	9.0	20	3.00
> 1.60	16.0	$(2 \times WH)^{2}$ *	20	3.00

*To make sure there is enough space, the space calculation for each animal is carried out taking into account the horse height at the withers.

Birds

Domestic Chicken

132. In cases where, for scientific reasons, the following minimum cage dimensions are impractical, the duration of the birds retaining in smaller cages should be determined by the experimenter in consultation with veterinary personnel. In such cases, birds can be housed in smaller cages (at least 0.75 m^2), but with adequate environment enrichment. Cage dimensions for housing domestic chickens are indicated in Table 23.

Table 23

Domestic chickens housing cage dimensions

Weight, g	Minimum cage dimensions, m^2	Minimum cage area/bird, m^2	Minimum cage height, cm	Minimum feeder length/bird, cm
< 200	1.0	0.025	30	3
200–300	1.0	0.03	30	3
300–600	1.0	0.05	40	7
600–1200	2.0	0.09	50	15
1200–1800	2.0	0.11	75	15
1800–2400	2.0	0.13	75	15
> 2400	2.0	0.21	75	15

Domestic Turkeys

133. All sides of the cage shall be at least 1.5 m long. Where, for scientific reasons, smaller sizes are required, duration of the birds retaining in

such cages should be determined by the experimenter in consultation with the veterinary personnel. In these cases, birds can be housed in smaller cages, but with adequate environment enrichment and minimum area of 0.75 m^2 and minimum height of 50 cm for birds weighing less than 0.6 kg, minimum area of 75 cm for birds weighing less than 4 kg and minimum area of 100 cm for birds over 4 kg. Small groups of birds may be kept under these conditions according to the cage dimensions indicated in Table 24.

Table 24

Domestic turkeys housing cage dimensions

Weight, kg	Minimum cage dimensions, m^2	Minimum cage area/bird, m2	Minimum cage height, cm	Minimum feeder length/bird, cm
< 0.3	2.0	0.13	50	3
0.3–0.6	2.0	0.17	50	7
0.6–1	2.0	0.30	100	15
1–4	2.0	0.35	100	15
4–8	2.0	0.40	100	15
8–12	2.0	0.50	150	20
12–16	2.0	0.55	150	20
16–20	2.0	0.60	150	20
> 20	3.0	1.0	150	20

Quails

134. Quails should be housed in cages, the dimensions of which are indicated in Table 25

Table 25

Quail housing cage dimensions

Weig ht, g	Minimum cell dimensions, m²	Minimum cage area for a couple of birds, m^2	Cage area for each additional bird of the group, m^2	Minimum cage height, cm	Minimum feeder length/bird, cm
	1.0	0.5	0.10	20	4
>150	1.0	0.6	0.15	30	4

Ducks and Geese

135. When, for scientifically sound reasons, cages smaller than those indicated in the Table below are required, the duration of the bird retaining there shall be determined by the experimenter in consultation with the veterinarian. In such cases, the birds can be housed in smaller cages (at least 0.75 m^2) providing adequate environment enrichment. Similar conditions can be used to house small groups of birds in cages, the dimensions of which are indicated in Table 26.

Table 26

Cage dimensions for housing ducks and geese

Weight, g	Minimum cage dimensions, m^2	Minimum cage area/bird, m^{2*}	Minimum cage height, cm	Minimum feeder length/bird, cm
Ducks				
< 300	2.0	0.10	50	10
300–1200**	2.0	0.20	200	10
1200–3500	2.0	0.25	200	15
> 3500	2.0	0.50	200	15
Geese				
< 500	2.0	0.20	200	10
500–2000	2.0	0.33	200	15
> 2000	2.0	0.50	200	15

* The cage shall include a pond with a minimum area of 0.5 m^2 per each 2 m^2 of the cage; minimum pond depth shall be 30 cm. The pond may occupy up to 50% of the minimum cage size.

** Poults may be housed in cages with minimum height of 75 cm.

136. The minimum dimensions of the pond for housing ducks and geese are indicated in Table 27.

Table 27

Minimum pond dimensions for ducks and geese*

	Pond area per each 2 m^2 of cage, m^2	Depth, cm
Ducks	0.5	30
Geese	0.5	10–30

* The pond may occupy up to 50% of the minimum cage size.

Pigeons

137. Cages for pigeons shall be long and narrow (e.g. 2 m × 1 m), and not square, so that the birds can make short flights.

Table 28

Pigeon housing cage dimensions

Group size, individuals	Minimum cage dimensions, m^2	Minimum cage height, cm	Minimum feeder length/bird, cm	Minimum roost length per a bird (cm)
< 6 individuals	2.0	200	5	30
7–12 individuals	3.0	200	5	30
for each additional bird in the group > 12 individuals	0.15		5	30

Chestnut-Eared Finches

138. Cages for housing chestnut-eared finches shall be long and narrow (e.g. 2 m x 1 m), which will allow the birds to make short flights. For bird breeding research, couples may be housed in smaller cages, providing adequate environment enrichment, with minimum area of 0.5 m^2 and minimum height of 40 cm. Duration of such retaining should be justified by the experimenter in consultation with the veterinarian.

Table 29

Cage size for housing chestnut-eared finches

Group size, individuals	Minimum cage dimensions, m^2	Minimum cage height, cm	Minimum number of feeders
< 6	1.0	100	2
7–12	1.5	200	2
13–20	2.0	200	3
For each additional bird in the group > 20	0.05		1 per 6 birds

Amphibians

Aquatic Tailed Amphibians

139. The water surface area for housing amphibians is indicated in Tables 30 through 34.

Table 30

Water surface area for
housing aquatic tailed amphibians

Body length*, cm	Minimum water surface area, cm^2	Minimum water surface area per each additional animal of the group, cm^2	Minimum water depth, cm
< 10	262.5	50	13
10–15	525	110	13
15–20	875	200	15
20–30	1837.5	440	15
> 30	3150	800	20

* To be measured from neb to anus.

Table 31

Minimum water surface area for housing aquatic batrachians*

Body length[1], cm	Minimum water surface area, cm^2	Minimum water surface area per each additional animal of the group, cm^2	Minimum water depth, cm
< 6	160	40	6
6–9	300	75	8
9–12	600	150	10
> 12	920	230	12.5

* These requirements apply to amphibians housing reservoirs, but not to reservoirs for natural breeding and superovulation, procedures requiring smaller reservoirs for efficiency reasons. Space requirements for adult specimens are determined according to their sizes. The size of juveniles and tadpoles should either be ignored, or the dimensions of the vessel should be changed according to the scaling principle.

[1] To be measured from neb to anus.

Table 32

Terrarium dimensions for housing semiaquatic batrachians

Body length[1], cm	Minimum terrarium size[2], cm^2	Minimum terrarium area per each additional animal of the group, cm^2	Minimum terrarium height[3], cm	Minimum water depth, cm
< 5.0	1500	200	20	10
5.0–7.5	3500	500	30	10
> 7.5	4000	700	30	15

[1] To be measured from neb to anus.
[2] 1/3 of the terrarium shall be firm ground, 2/3 shall be water, which shall be enough for complete animal submergence.
[3] To be measured from the firm ground surface to the terrarium top. The terrarium height shall correspond to its internal layout.

Table 33

Terrarium dimensions for housing semi-amphibian batrachians

Body length[1], cm	Minimum terrarium size[1], cm^2	Minimum terrarium area per each additional animal of the group, cm^2	Minimum terrarium height[3], cm	Minimum water depth, cm
< 5.0	1500	200	20	10
5.0–7.5	3500	500	30	10
> 7.5	4000	700	30	15

[1] To be measured from neb to anus.
[2] 2/3 of the terrarium shall be firm ground, 1/3 shall be water in an amount sufficient for complete animal submergence.
[3] To be measured from the firm ground surface to the terrarium top. The terrarium height shall correspond to its internal layout.

Table 34

Terrarium dimensions for housing tree tailless amphibians

Body length[1], cm	Minimum terrarium size[2], cm^2	Minimum terrarium area per each additional animal of the group, cm^2	Minimum terrarium height[3], cm
< 3	900	100	30
> 3	1500	200	30

[1] To be measured from neb to anus.
[2] 2/3 of the terrarium shall be firm ground, 1/3 shall be water in an amount sufficient for complete animal submergence.
[3] To be measured from the firm ground surface to the terrarium top. The terrarium height shall correspond to its internal layout.

Reptiles

Table 35

Minimum water surface area for housing aquatic turtles

Body length[1], cm	Minimum water surface area, cm^2	Minimum water surface area per each additional animal of the group, cm^2	Minimum water depth, cm
< 5	600	100	10
5–10	1600	300	15
10–15	3500	600	20
15–20	6000	1200	30
20–30	10000	2000	35
> 30	20000	5000	40

[1] To be measured in a straight line from the carapace anterior to the posterior edge.

Table 36

Minimum terrarium size for housing land snakes

Body length[1], cm	Minimum terrarium dimensions, cm^2	Minimum terrarium area per each additional animal of the group, cm^2	Minimum terrarium height[2], cm
< 30	300	150	10
30–40	400	200	12
40–50	600	300	15
50–75	1200	600	20
> 75	2500	1200	28

[1] To be measured from neb to tail.
[2] To be measured from the firm ground surface to the terrarium top. The terrarium height shall correspond to its internal layout.

Fish

Water Supply and Quality

140. Water of adequate quality shall be available at all times. The water flow rate in the recirculation or filtration system in aquariums shall be sufficient to ensure the required water quality standards. If necessary, the water should be filtered or treated to remove substances harmful to the fish. The water quality shall meet the requirements that ensure the normal activity and physiological reactions of the specific fish species at the specific development stage. The water flow rate shall allow the fish to swim freely and not affect their normal behavior. The fish shall have enough time to acclimatize and adapt to changes in water quality.

Oxygen, Nitrogen Compounds, pH and Salinity

141. The oxygen concentration shall be appropriate for the specific species and environment in which these fish are kept. If necessary, additional

enrichment of water with oxygen should be carried out. The concentration of nitrogen compounds must be kept low.

142. The pH level is determined for each specific species and maintained at the most stable level. The salinity shall be adapted to the needs of certain fish species and their development stage. The change in salinity shall be carried out gradually.

Temperature, Lighting, Noise Level

143. The temperature should be kept as stable as possible and within the limits optimal for the fish species. The change in temperature shall be carried out gradually. Fish should be provided with adequate daylight hours. Noise levels should be kept to a minimum and, where possible, aquariums should be kept away from equipment that produces noise or vibration (such as generators or filtration systems).

Colony Density and Creating Suitable Environment Conditions

144. The colonies density depends on the fish needs in certain environmental conditions, as well as their health and welfare. Fish should be provided with sufficient water for normal swimming with consideration of their size, age, health and diet. The fish environment should be enriched in an appropriate way: with shelters or bottom substrate, unless this is not required due to their behavioral characteristics.

Fish Feeding and Handling

145. The fish shall be provided with food that suits their needs and at a frequency that suits them. Particular attention should be paid to feeding fry during their transfer from a natural diet to an artificial one; touching the fish with hands is allowed only in case of special need.

3.3. Animal Euthanasia Methods

146. Using an inappropriate euthanasia method can cause significant pain, distress and suffering to animals. Equally important is the professionalism of the specialist performing this operation. Euthanasia is carried out only by competent people using a method suitable for the animal species.

147. For euthanasia of animals, the methods listed in Table 37 are used. Other methods of euthanasia (other than those listed in Table 37) may be used on animals:

a) in unconscious state, and only after obtaining confidence that the animal will not regain consciousness before death;

b) used in agricultural research when the project purpose requires that the animals be kept under conditions similar to those in which they are kept on commercial farms.

148. Animal euthanasia is completed by one of the following methods:

a) confirmation of the permanent blood circulation stop;

b) brain destruction;

c) cervical dislocation;

d) bleeding;

e) confirmation of the rigor mortis onset.

Table 37

Animal Euthanasia Methods

Animals/Euthanasia Methods	Fish	Amphibians	Reptiles	Birds	Rodents	Rabbits	Cats, dogs, ferrets and foxes	Large mammals	Non-human primates
Anesthetic overdose	1	1	1	1	1	1	1	1	1
Pneumatic gun			2						
Carbon dioxide					3				
Cervical displacement				4	5	6			
Cranial concussion (blow on the head)				7	8	9	10		
Decapitation				11	12				
Electrocution	13	13		13		13	13	13	
Inert gases (Ar, N2)								14	
Gunshot proper guns, other weapons and ammunition			15				16	15	

the euthanasia type is prohibited for this animal species

Euthanasia Conditions:

1) If necessary, sedatives should be used first.
2) To be used for large reptiles only.
3) To be used only in case of gradual filling of a chamber with carbon dioxide. This method is not applicable to fetuses and newborn rodents.
4) To be used for birds weighing up to 1 kg only. Birds weighing more than 250 g should be given a sedative first.
5) Use only for rodents weighing up to 1 kg. Rodents weighing more than 150 g should be given a sedative first.
6) Use only for rabbits weighing up to 1 kg. Rabbits weighing more than 150 g should be given a sedative first.
7) Use only for birds weighing up to 5 kg.
8) Use only for rodents weighing up to 1 kg.
9) Use only for rabbits weighing up to 5 kg.
10) Use only for newborns.
11) Use only for birds weighing up to 250 g.
12) Use only if other methods are impractical.
13) Requires special equipment.
14) Use for pigs only.
15) Use only in the field by experienced shooters.
16) Use only in the field by experienced shooters, when other methods are impractical.

149. Euthanasia can be Planned or Forced. Planned euthanasia is carried out in accordance with the study plan and the need for its implementation should be scientifically justified.

150. Forced euthanasia is performed on laboratory animals experiencing moderate to severe pain, stress, and suffering during research operations, at standard housing, or disease, as a humane endpoint.

151. The process of euthanasia for each type of animal consists of 2 stages: initial and final.

152. Euthanasia methods used for different types of animals are indicated in tables 38 through 41.

Table 38

Euthanasia methods used for rats, mice, gerbils, degus, hamsters

Animal age	Initial euthanasia stage	Final euthanasia stage
Unborn embryos and fetuses: mice: up to 10 days of gestation[1] rats: up to 10 days of gestation[1] gerbils: up to 12 days of gestation[1] degus: up to 43 days of gestation[1] hamsters: up to 8 days of gestation[1]	Dam's euthanasia	Remove the uterus with litter in intact amniotic sac from the abdominal cavity, then perform the following: leave the uterus with fetuses for 1 hour or longer, for the fetuses death; embryos or fetuses up to 4 grams of body weight may be immersed in liquid nitrogen; if chemical fixation of the entire embryo or fetus is required, they should be euthanized prior to the chemical fixation procedure.
Unborn embryos and fetuses: mice: after 11 days of gestation[1] rats: up to 11 days of gestation[1] gerbils: up to 13	Dam's euthanasia (further, remove the uterus with litter from the abdominal cavity and expose the litter to	Decapitation; cervical dislocation; bleeding from the heart cavities or cutting main blood vessels; anesthetic overdose; embryos or fetuses up to 4 grams of body weight may be immersed in liquid nitrogen, bypassing the initial euthanasia stage; embryos or fetuses larger than 4 grams of body weight may be immersed

Animal age	Initial euthanasia stage	Final euthanasia stage
days of gestation[1] degus: up to 44 days of gestation[1] hamsters: up to 9 days of gestation[1]	anesthesia. Decapitation. Cervical dislocation.	in liquid nitrogen, they should be anesthetized beforehand; if chemical fixation of the entire embryo or fetus is required, it should be anesthetized or euthanized prior to the chemical fixation procedure. Do not use gases for this category[2]!
Newborn animals, up to 10 days	Anesthetic overdose (injection or inhalation); Cervical dislocation (up to body weight of 200 grams); Animal narcotizing. Do not use gases for this category[2]!	Decapitation; cervical dislocation; hypothermia is acceptable in newborn animals aged up to 7 days; at that, direct contact with ice and/or cold surfaces should be avoided; youngs may be immersed in liquid nitrogen, preliminary anesthesia should be performed; bleeding from the heart cavities or cutting main blood vessels (preliminary anesthesia should be performed); if chemical fixation of the entire animal body is required, preliminarily euthanasia or anesthesia should be performed before the chemical fixation procedure; removal of internal organs (heart, lungs, brain); cardiac perfusion.
Adults and newborn animals aged over 10 days	CO_2 use Cervical dislocation (not used for animals weighing over 200 grams and	Cervical dislocation (not used for animals weighing over 200 grams and hamsters); bleeding from the heart cavities; removal of internal organs (heart, lungs, brain); cutting main blood vessels; cardiac perfusion;

Animal age	Initial euthanasia stage	Final euthanasia stage
	hamsters) Anesthetic overdose (injection or inhalation); Animal narcotizing.	decapitation.

[1] Scientific evidence indicates that mammalian embryos and fetuses are unconscious throughout pregnancy period and childbirth. Embryos and fetuses cannot consciously experience feelings such as shortness of breath or pain. Therefore, they also cannot suffer dying in the dam's uterus after her death, regardless of the cause. The neural tube development into functional brain occurs after 50% of the gestation period. It is recommended that fetuses be humanely euthanized after 50% gestation by the specified methods.

[2] The development of excitatory and inhibitory receptor systems occurs throughout the entire gestation period and up to the second week of postnatal life (10-14 days). In this regard, newborn animals aged up to 14 days are resistant to hypoxia when using CO_2 and other inhalation anesthetics.

Table 39

Euthanasia methods used for guinea pigs

Animal age	Initial euthanasia stage	Final euthanasia stage
Unborn embryos and fetuses up to 34 days of gestation[1]	Dam's euthanasia	Remove the uterus with litter in intact amniotic sac from the abdominal cavity, then perform the following: for the fetuses death, leave uterus with the fetuses for 1 hour or longer; embryos or fetuses up to 4 grams of weight may be immersed in liquid nitrogen; if chemical fixation of the entire embryo or fetus is required, they should be euthanized prior to the chemical fixation procedure.
Unborn embryos and fetuses after 34 days of gestation[1]	Dam's euthanasia (further, remove the uterus with litter from the abdominal cavity and expose the litter to anesthesia.	Decapitation; cervical dislocation; bleeding from the heart cavities or cutting main blood vessels; embryos or fetuses over 4 grams of body weight may be immersed in liquid nitrogen; anesthetic overdose; chemical fixation.

Animal age	Initial euthanasia stage	Final euthanasia stage
Newborn and adult animals	Anesthetic overdose (injection or inhalation); Animal narcotizing. Do not use gases for this category	Decapitation; cervical dislocation; bleeding from the heart cavities or cutting main blood vessels (anesthesia should be performed); if chemical fixation of the entire animal body is required, preliminary euthanasia should be performed; removal of internal organs (heart, lungs, brain); cardiac perfusion.

[1] Scientific evidence indicates that mammalian embryos and fetuses are unconscious throughout pregnancy period and childbirth. Embryos and fetuses cannot consciously experience feelings such as shortness of breath or pain. Therefore, they also cannot suffer dying in the dam's uterus after her death, regardless of the cause. The neural tube development into functional brain occurs after 50% of the gestation period. It is recommended that fetuses be humanely euthanized after 50% gestation by the specified methods.

Table 40

Euthanasia methods used for rabbits, ferrets and pygmy pigs

Animal age	Initial euthanasia stage	Final euthanasia stage
Unborn embryos and fetuses: up to: rabbits: up to 10 days of gestation[1] ferrets: up to 12 days of gestation pygmy pigs: up to 30 days of gestation	Dam's euthanasia	Remove the uterus with litter in intact amniotic sac from the abdominal cavity, then perform the following: for the fetuses death, leave uterus with the fetuses for 1 hour or longer; embryos or fetuses up to 4 grams of body weight may be immersed in liquid nitrogen; if chemical fixation of the entire embryo or fetus is required, they should be euthanized prior to the fixation procedure.
Unborn embryos and fetuses: rabbits: after 11 days of gestation[1] ferrets: after 13 days of gestation[1] pygmy pigs: after 31 days of gestation[1]	Dam's euthanasia. Remove the uterus with litter from the abdominal cavity and expose the litter to narcotizing.	Decapitation; cervical dislocation; bleeding from the heart cavities or cutting main blood vessels (anesthesia should be performed for this procedure); anesthetic overdose; if chemical fixation of the entire embryo or fetus is required, preliminary euthanasia or anesthesia should be performed prior to the chemical fixation procedure; cardiac perfusion.
Newborn and adult animals	Anesthetic overdose (injection or inhalation); Animal narcotizing. Do not use gases for this category[2]!	Bleeding from the heart cavities or cutting main blood vessels; if chemical fixation is required, preliminary euthanasia should be performed prior to the chemical fixation procedure; removal of internal organs (heart, lungs, brain); blunt force head trauma[3]; cardiac perfusion.

[1] Scientific evidence indicates that mammalian embryos and fetuses are unconscious throughout pregnancy period and childbirth. Embryos and fetuses cannot consciously experience feelings such as shortness of breath or pain. Therefore, they also cannot suffer dying in the dam's uterus after her death, regardless of the cause. The neural tube development into functional brain occurs after 50% of the gestation period. It is

Animal age	Initial euthanasia stage	Final euthanasia stage
recommended that fetuses be humanely euthanized after 50% gestation by the specified methods. [2] The development of excitatory and inhibitory receptor systems occurs throughout the entire gestation period and up to the second week of postnatal life (10–14 days). In this regard, newborn animals aged up to 14 days are resistant to hypoxia when using CO_2 and other inhalation anesthetics. [3] Applicable to rabbits weighing up to 5 kg; they should be narcotized before.		

Table 41

Euthanasia methods used for dogs

Animal age	Initial euthanasia stage	Final euthanasia stage
Unborn embryos and fetuses up to 30 days of gestation[1]	Dam's euthanasia	Remove the uterus with litter in intact amniotic sac from the abdominal cavity, then perform the following: leave the uterus with fetuses for 1 hour or longer, for the fetuses death; embryos or fetuses up to 4 grams of body weight may be immersed in liquid nitrogen; if chemical fixation of the entire embryo or fetus is required, they should be euthanized prior to the chemical fixation procedure.
Unborn embryos and fetuses after 31 days of gestation[1]	Dam's euthanasia Further, remove the uterus with litter from the abdominal cavity and expose the litter to narcotizing.	decapitation; cervical dislocation; bleeding from the heart cavities or cutting main blood vessels (anesthesia is required); anesthetic overdose; if chemical fixation of the entire embryo or fetus, chemical fixation of the entire animal body is required, preliminary euthanasia or anesthesia should be performed prior to the chemical fixation procedure;

Animal age	Initial euthanasia stage	Final euthanasia stage
		cardiac perfusion.
Newborn and adult animals	Animal narcotizing. Do not use gases for this category[2]!	Anesthetic overdose (injection or inhalation);

[1] Scientific evidence indicates that mammalian embryos and fetuses are unconscious throughout pregnancy period and childbirth. Embryos and fetuses cannot consciously experience feelings such as shortness of breath or pain. Therefore, they also cannot suffer dying in the dam's uterus after her death, regardless of the cause. The neural tube development into functional brain occurs after 50% of the gestation period. It is recommended that fetuses be humanely euthanized after 50% gestation by the specified methods.

[2] The development of excitatory and inhibitory receptor systems occurs throughout the entire gestation period and up to the second week of postnatal life (10–14 days). In this regard, newborn animals aged up to 14 days are resistant to hypoxia when using CO_2 and other inhalation anesthetics.

5. Procedure Severity Classification

Procedure Severity Assessment and Further Animal Fate

153. To increase the study transparency and ensure the monitoring of its implementation, the procedures should be classified by the severity of pain, suffering, distress caused to an animal, as well as injuries that have long-term negative consequences for the animal health.

154. From the ethical point of view, it is necessary to define the upper pain, suffering, distress limit (humane endpoint), after which animals may not be used longer in the procedure. Procedures accompanied by severe pain, suffering or distress, which may be long-term and cannot be alleviated, should be avoided. In the course of study, the severity of pain, suffering and distress should be recorded and assessed. When drawing-up a report, not the degree of pain, suffering or distress experienced by animals anticipated at the experiment planning stage should be indicated, but the actual one. In the institution conducting preclinical studies, all procedures shall be monitored in terms of severity, and a comparative analysis of the anticipated and actual episodes shall be carried out.

155. The number of animals used in procedures may be reduced through their reuse, if this does not contradict the scientific objectives of the study and does not lead to a deterioration in the animal welfare. However, the benefits of animal reuse shall be weighed against the adverse effects of experiments on animal welfare considering features of each animal's life. In this regard, the issue of animal reuse in experiments should be considered individually depending on the specific situation.

156. The decision on the animal fate on completion of the experiment shall be made taking into account the prospects of this animal and the likely

risks to the environment. Animals whose welfare is at risk should be euthanized. In some cases, animals may be returned to their natural habitat. Animals such as dogs and cats can be adopted as pets in families. In this case, the breeder, dealer, or user should have an in-house socialization plan to ensure successful animal relocation to domestic conditions without additional distress or endangering the public safety.

157. Welfare of animals used in procedures depends to a significant degree on experience and professional competence of the persons responsible for performing the procedures, as well as persons performing the procedures or supervising the employees caring for the animals on a daily basis. Institutions conducting scientific research shall ensure that employees have the necessary education, skills and competencies. In addition, employees should be constantly supervised until they have gained sufficient experience and demonstrated the required level of competence.

158. Institutions conducting scientific research should have the equipment meeting the requirements for the housing conditions of the relevant animal species, and allowing effective procedures to be carried out with minimal distress to the animals.

159. To ensure ongoing monitoring of animal welfare, each institution shall have veterinary care available at all times and a designated person responsible for animal care and welfare.

160. The procedure severity should be classified according to the level of pain and suffering that is expected to be inflicted on the animal during the procedure, as well as the degree of damage that is expected to be caused during the procedure and have long-term negative consequences for the animal's health.

Procedure Severity Degrees

Without recovery from anesthesia

161. Procedures performed entirely under general anesthesia that do not require the animal to regain consciousness are classified as "without recovery from anesthesia".

Mild

162. Procedures on animals that may cause them to experience short-term mild pain, suffering or distress, as well as procedures that do not significantly affect the welfare or general condition of animals, are classified as "mild".

Moderate

163. Procedures on animals that may cause them to experience short-term moderate pain, suffering or distress; or prolonged mild pain, suffering or distress, and procedures that have a moderate negative impact on the welfare or general condition of animals, are classified as “moderate”.

Severe

164. Procedures on animals that may result in severe pain, suffering or distress, prolonged moderate pain, suffering or distress, and procedures that seriously affect the welfare or general condition of the animals are classified as "severe".

Procedure Severity Determination

165. When determining the procedure severity, any interventions or operations that occur as part of this procedure shall be taken into account. At that, the most severe consequences for each animal after applying all possible improvements in experimental methods should be considered.

166. When determining the severity of a particular procedure, the procedure type and other factors should be taken into account. All these factors shall be reconsidered on a case-by-case basis. These factors include:

manipulation type, animal conditioning to them;

nature of the pain, suffering, distress or injury with long-term negative effects on the animal health, which are caused by all elements of the procedure, their intensity, duration, frequency and recurrence of the methods used;

cumulative level of suffering experienced by the animal during the procedure;

barriers to natural behavior, including changes in housing and care standards.

167. Paragraphs 167 through 169 provide examples of procedures classified by severity based on factors related to the procedure type. They shall serve as a primary indicator in determining the severity degree most appropriate for a particular procedure type.

168. However, for the final procedure classification according to its severity, additional factors should be taken into account, evaluated on a case-by-case basis:

animal species and genotype;

animal maturity, age and sex;

level of the animal conditioning for the specific procedure;

in case of the animal reuse, actual severity of previous procedures shall be considered;

methods used to reduce or eliminate pain, suffering or distress, including improved living conditions and care;

humane endpoints.

Examples of different procedure types classified by severity based on the factors related to the procedure type

169. Mild procedure:

a) use of anesthesia apart of that used for euthanasia;

b) pharmacokinetic studies, where the medicinal product dose is administered once and blood is taken a limited number of times (in total less than 10% of the circulating blood volume), and the substance does not have a noticeable adverse effect;

c) obtaining images of animal organs using non-penetrating registration methods (e.g. magnetic resonance imaging) using necessary sedatives or anesthetics;

d) superficial operations (e.g. ear and tail biopsy, non-surgical subcutaneous implantation of mini-pumps and transmitters);

e) use of external telemetry devices that cause only minor inconvenience to the animals or insignificantly affect their normal activity and behavior;

f) administration of substances subcutaneously, intramuscularly, intraperitoneally, through a tube and intravenously into superficial blood vessels, if the substance has only a mild effect on the animals and the volumes administered are appropriate for the animal size and type;

g) induction of tumor growth or spontaneous tumors that do not cause pronounced clinical adverse effects (for example, small subcutaneous non-invasive nodes);

h) breeding of genetically modified animals, which is expected to produce animals with slight changes in phenotype;

i) modified diet that does not meet all the nutritional requirements of the animals, which is expected to cause mild clinical abnormalities for the study duration;

j) short-term (< 24 hours) keeping animals in metabolic cages;

k) studies requiring short-term removal of social partners, short-term individual maintenance of adult rats or mice of sociable lines;

l) models in which animals are exposed to painful stimuli that cause little and short-term pain, suffering and distress, and which animals can easily avoid;

m) the procedure can be qualified as "mild" in the case of complex or combination of the following operations and/or manipulations:

study of the body structure by non-invasive methods that require minimal restriction of mobility;

measurement of the electrocardiogram by non-invasive methods with minimal or no mobility restrictions on conditioned animals;

use of external telemetry devices that do not presumably cause harm to socially adapted animals and do not affect their normal activity and behavior;

breeding genetically modified animals that are not expected to have clinically detectable adverse phenotype;

adding inert labels to feed to track the digestion process;

fasting < 24 hours for adult rats;

open field test.

170. Moderate procedure:

a) pharmacokinetic studies with multiple administrations of a substance that causes moderate clinical effects and blood sampling (> 10% of circulating volume) in animal that is conscious for several days without blood replacement;

b) acute tests to determine dose-dependence, chronic toxicity and/or carcinogenicity, in which the endpoint of the experiment is not the animal death;

c) operations under general anesthesia with appropriate anesthesia leading to postoperative pain, suffering or deterioration of general condition, for example, thoracotomy, trepanation, laparotomy, lymphadenectomy, thyroidectomy, orthopedic surgery with effective stabilization and wound treatment, organ transplantation with effective prevention of their rejection, surgical implantation of catheters or biomedical devices (telemetry transmitters, mini-pumps, etc.);

d) patterns of induced or spontaneous tumor growth that are expected to cause moderate pain or suffering, or moderately affect normal behavior;

e) irradiation or chemotherapy in sublethal doses or in the case of lethal doses with the restoration of the immune system. Expected side effects are mild or moderate and short-lived (up to 5 days);

f) breeding genetically modified animals that are expected to have moderate change in phenotype;

g) creation of genetically modified animals during surgical procedures;

h) use of metabolic cells with moderate mobility restriction for a long period (up to 5 days);

i) studies using modified diet that does not meet all the nutritional requirements of the animals, which is expected to cause moderate clinical deviations for the study period;

j) fasting for 48 hours in adult rats;

k) creating conditions in which the animal cannot run away from or avoid painful stimuli and which ultimately lead to moderate distress.

171. Severe procedure:

a) toxicity studies in which the experiment endpoint is the animal death or the animal death is expected, or the occurrence of severe pathophysiological conditions (e.g. the determination of the substance acute toxicity);

b) studies in which equipment failure could cause severe pain, suffering or death of the animal (e.g. devices supporting heart function);

c) testing the vaccine effectiveness characterized by persistent impairment of the animal's condition, progressive disease leading to death or accompanied by prolonged moderate pain, suffering or distress;

d) irradiation or chemotherapy in lethal doses without restoration of the immune system or with the restoration of the immune system, which causes transplant rejection reaction.

e) patterns of induced or spontaneous tumor growth that are expected to cause progressive disease with fatal outcome, accompanied by long-term moderate pain, suffering or distress. For instance, cachexia-causing tumors, invasive bone tumors, metastatic tumors, and tumors with necrosis;

f) operations and other procedures on animals performed under general anesthesia that may result in severe or persistent moderate post-operative pain, suffering or distress, or cause serious and persistent impairment of the general condition of animals. For instance, in the case of nonunion fractures, thoracotomy without adequate pain relief or injury to cause multiple organ failure;

g) organ transplantation, in cases where the possible organ rejection will lead to serious distress or violation of the animal general condition (e.g. xenotransplantation);

h) breeding animals with genetic disorders that are expected to cause serious and permanent changes in their general condition, for example, Huntington's disease, muscular dystrophy, models of chronic recurrent neuritis;

i) use of metabolic cells with severe limitation of mobility for a long period;

j) unavoidable electric shock (e.g. for the learned helplessness test);

k) full isolation of social animal species (e.g. dogs and non-human primates, for an extended period);

l) stress due to immobilization to cause stomach ulcers or heart failure in rats;

m) tests involving forced swimming or exercise, where the endpoint is animal overexertion.

6. Animal Care and Follow-Up in an Experiment

172. Safe and effective anesthesia (pain relief) plan is critical to relieving pain, suffering, and distress. Without treatment, pain may affect animal biology and add variability to the experiment. However, specific pain management procedures can also make changes influencing experimental data. Incomplete communication about anesthetic procedures contributes to the persistence of inappropriate methodologies and the underutilization or inappropriate use of analgesia. Detailed description of the procedures used to relieve pain, suffering and distress provides investigators with practical information to reproduce this method.

173. The investigator should clearly describe the pain management strategy, including:

specific analgesic (composition, route, dose, concentration);

route of administration (path, volume, frequency, time and equipment used);

rationale for selection (e.g. animal model, disease and/or pathology, procedure, action mechanism, pharmacokinetics, personnel safety);

protocol modifications to reduce pain, suffering, and distress (e.g. changes in anesthesia protocol, increased monitoring frequency, procedure modifications, habituation, etc.)

174. If analgesics or other welfare measures reasonably expected for the procedure performed are not carried out for experimental reasons, scientific justification should be provided.

175. Reporting adverse events allows other investigators to plan appropriate welfare assessments and minimize the risk of these events occurring in their own studies. If an experiment tests the treatment efficacy, the occurrence of side effects can change the balance between the treatment benefit and risk.

176. Any adverse events that adversely affect the animal welfare in the study (e.g. cardiovascular and respiratory depression, central nervous system disturbance, hypothermia, reduced food intake) should be reported and whether they were expected or unexpected. If no adverse events were observed or reported during the study, state this clearly.

177. Humane endpoints are the predetermined morphological, physiological and/or behavioral signs that determine the circumstances under which an animal will be excluded from an experimental study. The use of humane endpoints can help to minimize harm while achieving scientific objectives. The humane endpoints that have been established for the particular study, species and strain should be reported. Include clear criteria for monitored clinical signs and clinical signs that led to euthanasia or other specific actions. Include such details as overall welfare measures (e.g. weight

loss, reduced food intake, poor posture) and welfare measures for specific procedures (e.g. tumor size in cancer studies, sensorimotor deficiency in stroke studies).

178. Report the monitoring timing and frequency, taking into account the animal's normal circadian rhythm and the timing of scientific procedures, as well as any increase in monitoring frequency (e.g. post-operative recovery, critical moments during disease investigations or after observing an adverse event). The availability of scorecards for monitored clinical signs may help other investigators develop clinically relevant assessments of welfare, especially for studies describing novel procedures.

179. This information should be reported even if no animal reaches any humane endpoint. If no humane endpoints have been established for the study, state this explicitly.

7. Recommendations on Administration Volumes for Laboratory Animals Depending on the Administration Route

Table 42

Recommended and maximum volumes for oral and intragastric administration of test objects to laboratory animals

Animal species	Recommended volume, ml/kg	Maximum volume, ml/kg
Mice	10	40[1]
Rats	10	20[2]
Degus	10	20[2]
Gerbils	10	20[2]
Hamsters	10	20[2]
Guinea pigs	10	30
Rabbits	1	20[3]
Ferrets	10	15

Animal species	Recommended volume, ml/kg	Maximum volume, ml/kg
Pygmy pigs	10	15
Dogs	5	20
Cats	10	15
Non-human primates	5	10

[1] To administer a larger volume, the dose may be divided (e.g. 20 ml/kg are to be administered 4 times daily to reach a total of 80 ml/kg).
[2] To accommodate a larger volume, the dose may be divided (e.g. 10 ml/kg are administered 4 times daily to reach a total of 40 ml/kg over a 24-hour period).
[3] Administer the test object to rabbits before the morning feeding.

Table 43

Intragastric tube dimensions depending on the body weight

Animal species	Animal body weight, g	Gauge	Dimensions	
			Diameter, mm	Tube length, mm
Mice	below 15	20	2.0	25
Gerbils	above 15	20	2.0	38
Rats	below 100	18	2.0	51
Degus	100–200			
Guinea pigs	200–300	18	2.0	76
Hamsters	above 300	16	2.8	76

Table 44

Gastric tube dimensions depending on the animal species

Animal species	Catheter diameter	Length, mm
Rabbits	2.7–4.3	to be measured before insertion
Ferrets	5.0	
Pygmy pigs	4.0–6.0	400–600

Table 45

Options of administration in conjunctival sac

Administration objective	Administration method
Experimental studies	
Study of the solutions local irritating effect on eye tissues	10–200 µl dosing device or Hamilton syringe. The administration volume and solution concentration depends on the animal species and the investigational medicinal product characteristics.
The drug efficacy study on models of allergic conjunctivitis, corneal injury, retina and other models of eye pathology	
Ophthalmic test	
Health-promoting administration	
Local ophthalmic anesthesia for laboratory animals in case of injury or before surgery	From a dropper dispenser, 1 drop to be administered into the conjunctival sac (mice, hamsters, rats, guinea pigs) and 2 drops (rabbits, predatory laboratory animals and pygmy pigs). After 15 minutes interval, the procedure is repeated.
Disinfection and healing of the eye wound in case of injury or after surgery	

Table 46

Administration volumes into conjunctival sac in different animal species

Animal species	Administration volume, µl
Mice	5
Gebrils	5
Rats	30
Degus	30
Hamsters	5
Guinea pigs	30
Rabbits	50
Ferrets	30
Pygmy pigs	50
Dogs	50
Cats	50

Table 47

Recommended volumes for intravitreal administration

Animal species	Recommended administration volume (µl/eye)
Mice	2
Rats	5
Rabbits	100
Dogs	100
Non-human primates	50

Table 48

Recommended volumes for intranasal administration of test objects to laboratory animals

Animal species	Administration volume per nostril (µl)
Mice, gerbils, hamsters	25
Rats	50
Degus	75
Guinea pigs	100
Rabbits	200
Ferrets	150
Pygmy pigs	1,000
Dogs	500
Cats	200
Non-human primates	200

Table 49

Permitted volumes for intramuscular administration of medicinal products to laboratory animals

Animal species	Recommended volume, ml/kg	Maximum single administration volume per injection site	Needle gauge, G
Mice	2–4	0,05 ml/site[1]	≤ 23
Rats	0.5–10[2]	0.2 ml/site[3]	≤ 23

Gerbils	2–4	0.2 ml/site[3]	≤ 23
Hamsters	0.5–1	0.2 ml/site[3]	≤ 23
Degus	2–4	0.2 ml/site[3]	≤ 23
Guinea pigs	0.5–2	0.25 ml/site[3]	≤ 22
Rabbits	0.25–0.5	0.5 ml/site[4]	≤ 20
Ferrets	0.25–1	0.5 ml/site[4]	≤ 21
Pygmy pigs	0.25–0.5	5.0 ml/site[5]	≤ 20
Dogs	0.1–1	3 ml/site[6]	≤ 21
Cats	0.25–1	0.5 ml/site[4]	≤ 21

Notes:
[1] Maximum five sites per paw are allowed when dividing a dose to several sites and alternating paws. The final volume shall not exceed 0.25 ml per paw.
[2] Administration of up to 20 ml/kg is allowed at dividing a dose to several sites.
[3] Maximum two sites per paw are allowed when dividing a dose to several sites and alternating paws. The final volume shall not exceed 0.5 ml per paw.
[4] Maximum two sites per paw are allowed when dividing a dose to several sites and alternating paws. The final volume shall not exceed 1.0 ml per paw.
[5] One site per each hindpaw and two sites per cervix are allowed. Total administration volume shall not exceed 20 ml.
[6] Two sites per each hindpaw and one site per each forepaw are allowed.

Table 50

Recommended volumes for intraperitoneal administration of test objects to laboratory animals

Animal species	Recommended volume, ml/kg	Needle gauge, G
Mice	20–80	≤ 23
Rats	10–20	≤ 23
Gerbils	20–80	≤ 23
Hamsters	10–20	≤ 23
Degus	10–20	≤ 23
Guinea pigs	10–20	≤ 22
Rabbits	5–20	≤ 20
Ferrets	5–20	≤ 21
Pygmy pigs	1–10	≤ 20
Dogs	1–20	≤ 21
Cats	5–20	≤ 21

Table 51

Permitted volumes for intravenous administration to laboratory animals[1]

Animal species	Recommended volume, ml/kg					Needle gauge, G
	bolus		infusion			
	rapid	slow[2]	volume, ml/kg	recommended rate, ml/min	maximum rate, ml/min	
Mice	5	25	50	1	2–4	≤ 25
Rats	1–5	20	50	1		≤ 23
Gerbils	5	20	50	1		≤ 23
Hamsters	5	20	50	1		≤ 25
Guinea pigs	1	5	10	1	2–4	≤ 23
Rabbits	2	10	20	1	2–4	≤ 21
Ferrets	2–5	10	20	1	2–4	≤ 23
Pygmy pigs	2.5	10	10	1	5	≤ 20
Dogs	1–5	10	20	5	5	≤ 23
Cats	5	10	20	1	5	≤ 23

[1] These volumes may be used also for endarterial injection.
[2] As slow injection.

Table 52

Permitted volumes for subcutaneous administration of medicinal products to laboratory animals

Animal species	Recommended volume, ml/kg	Needle gauge, G
Mice	10–20 (40[1])	≤ 23
Rats	5–10 (20[1])	≤ 23
Gerbils	10–20 (40[1])	≤ 23
Hamsters	5–10 (20[1])	≤ 23
Degus	5–10 (20[1])	≤ 23
Guinea pigs	5–10 (20[1])	≤ 22

Animal species	Recommended volume, ml/kg	Needle gauge, G
Rabbits	1–2.5 (15[1])	≤ 20
Ferrets[1]	10–20	≤ 21
Pygmy pigs	1 (3[1])	≤ 20
Dogs	1 (2[1])	≤ 21
Cats	1 (3[1])	≤ 21

[1] Maximum volume. Should be divided between 2–3 injection sites.

Table 53

Recommended volumes of intra-articular administration depending on the animal species and the selected joint

Animal species	Injection site	Administration volume, ml/joint
Rats	stifle joint	0.1
	tarsal joint	0.05
Rabbits	stifle joint	0.5
Dogs	stifle joint	1.0

Table 54

Recommended volumes for intravaginal administration to sexually mature individuals

Animal species	Volume, ml
Mice	0.02
Rats	0.35
Gerbils	n/a
Hamsters	n/a
Degus	n/a
Guinea pigs	n/a
Rabbits	2.0
Ferrets	n/a
Pygmy pigs	1–5
Dogs	2.0

Cats	n/a

3.7. Circulating blood volume (CBV) in laboratory animals and blood sampling

180. When taking blood samples from laboratory animals, this manipulation should be planned so that not to create prerequisites for the hypovolaemic shock or anemia.

181. The blood volume in animals recovers within 24 hours. However, the full blood composition restores after 2 weeks according to the formula 1 ml/kg/day.

182. References to tables.

Table 55

Circulating blood volume in various animal species

Animal species	Circulating blood volume, ml/kg
Mice	75
Rats	65
Gerbils	75
Degus	70
Hamsters	80
Guinea pigs	70
Rabbits	55
Ferrets	60

Table 56

Blood sampling frequency and blood amount permitted to be taken from laboratory animals

Single blood sampling (toxicity and specific studies)			Multiple blood sampling (pharmacokinetics and bioequivalence)		
% of circulating blood volume	recovery period	repletion demand	% of circulating blood volume per 24 hours	recovery period	repletion demand
7.5%	1 week	–	7.5%	1 week	–
10%	2 weeks	–	10–15%	2 weeks	up to 15% – /over 15% +
15%	4 weeks	+	20%	3 weeks	+

Table 57

Information on most common blood sampling sites
in various species of laboratory animals

Blood sampling site	Animal species	Need for anesthesia	Possibility of an inflammatory reaction and tissue destruction	Volume	Notes
Jugular vein /vena cava cranialis	all	+	low	+++	multiple blood sampling is possible
Retrobulbar plexus	small rodents	+/-	moderate/ high	++	sampling frequency NMT 4 times, 2 samples per eye within 15 days
Sublingual vein	small rodents	+/-	low	+++	sampling frequency 7–8 times
Gingival vein	small rodents	+/-	low	++	multiple blood sampling is possible
Mandibular vein	small rodents	+/-	low	++	
Ear marginal vein/ ear veins	all	-/+ local only	low	++	
Central auricular artery	rabbits	-/+ local only	low	++	
Forepaw lateral saphenous vein	all	+/-	low	++	

Blood sampling site	Animal species	Need for anesthesia	Possibility of an inflammatory reaction and tissue destruction	Volume	Notes
Saphena	all	+/-	low	++	
Low limb lateral metatarsal vein	Guinea pigs	+/-	low	++	
Femoral vein	all	+	low	+++	sampling frequency is limited NMT 2–3 times
Tail lateral vein	small rodents (except for hamsters; gebrils and degus with caution)	–	low	+++	multiple blood sampling is possible
Tail ablating (1–3 mm)	small rodents (except for hamsters)	+	moderate/ high	++	blood sampling is limited
Cardiac puncture	all	+	moderate	+++	terminal procedure

183. When sampling other biological materials, up-to-date scientific data on the possibility of such manipulations and humane volumes shall be used.

184. The animal participation in experiments remains essential for protection of human and animal health and the environment preservation. These Rules require a high protection level for laboratory animals if their use cannot be avoided.