Scientific Research Publishing

Open Journal of Veterinary Medicine, 2024, 14, 91-109
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Clinical/Behavioral Monitoring of Rodents and Rabbits Undergoing Scientific Experiments

Luiz Cesar Cavalcanti Pereira[1], Jhônata Willy Rocha Coelho[2], Hyago da Silva Medeiros Elidio[2], Rita de Cássia dos Passos Ferraz Da Silva[2], João Gabriel Regis Sobral[2], Bárbara Alves de Brito Soledade[2], Leandro Thomaz Vilela[2*], Isabele Barbieri Dos Santos[2]

[1]Institute of Science and Technology in Biomodels (ICTB), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil
[2]Laboratory Animal Facility of the Carlos Chagas Pavilion of the Animal Experimentation Center (CEA), Oswaldo Cruz Institute (IOC), Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil
Email: *leandro.vilela@fiocruz.br

How to cite this paper: Pereira, L.C.C., Coelho, J.W.R., Elidio, H.S.M., Da Silva, R.C.P.F., Sobral, J.G.R., Soledade, B.A.B., Vilela, L.T. and Dos Santos, I.B. (2024) Clinical/Behavioral Monitoring of Rodents and Rabbits Undergoing Scientific Experiments. *Open Journal of Veterinary Medicine*, **14**, 91-109.
https://doi.org/10.4236/ojvm.2024.145007

Received: April 10, 2024
Accepted: May 28, 2024
Published: May 31, 2024

Abstract

Background: We currently have international and national guidelines regarding the assessment and monitoring of clinical signs and humane endpoints in animals used in teaching and research, which make the performance of these activities mandatory for any experiment and professional working in this area. Assigning the severity of a research experiment is the result of an analysis of records of observations of the animal's behavior, and clinical signs. The aim of this study was to describe the importance of carrying out a severity assessment associated with clinical and behavioral monitoring of rodents and rabbits during experimentation to maintain the welfare of these animals undergoing scientific research. **Methods:** The literature search was carried out using the following terms: "Monitoring"; "Humane endpoints"; "Animal welfare", "Rodents"; "Rabbits", and as connectors "and"; "or", in the following databases: PubMed; LILACS/BIREME and SciELO. **Results:** A total of 987 articles were identified in the databases, and 20 of these studies were included in this review. **Conclusions:** Humane endpoint protocols and procedure severity tables are of the utmost importance, both from an ethical point and to refine the results of research conducted on laboratory animals. They should be drawn up jointly by the teams responsible for the project and the maintenance of the animals during the research period, and the data obtained should be published so that the scientific community can have access to it, helping to disseminate these practices, as well as helping to draw up new procedures. Monitoring and evaluating the welfare and clinical condition of animals undergoing scientific research procedures is the responsibility of the professors, researchers, veterinarians, and animal facility coordinators. The Ethics Committee on the Use of Animals must monitor all the activities conducted with

the animals, by inspecting the experimental procedures and the physical environment of the laboratory animal facility where the animals are housed.

Keywords

Monitoring, Humane Endpoints, Animal Welfare, Experimental Design, Laboratory Animals' Investigations

1. Introduction

Animal welfare is achieved when the animal can control internal and external variations, consequently remaining in homeostasis (the body's natural ability to maintain a stable and balanced internal environment), meaning how an animal is coping with the conditions in which it lives. An animal is in a good state of health if it is healthy and comfortable, well-nourished, safe, able to express innate behavior, and is not suffering unpleasant states of fear and distress [1].

In the area of animal experimentation science, the 3Rs principle was described in 1959 by scientists Willam Russell and Rex Burch, with a view to ethics and animal welfare in the use of animals in research [2]. The 3Rs principle encompasses the concepts of Replacement, Reduction and Refinement in the use of animals in experimentation. This concept brought critical thinking about the inhumane way in which animal experimentation took place. From that time to the present day, discussions on the 3Rs concepts have grown and gained more importance in science regarding the use of animals in experimentation. However, even with the development of alternative methods and the reduction in the use of animals for research aimed at education, science and health, the use of animals in research is still part of our current reality. Because of this, science and health professionals who deal with laboratory animals have an ethical responsibility to carry out this work with animals in the most refined way possible.

We currently have international and national guidelines on the assessment of the severity of the experiment and monitoring of humane end points in animals used in teaching and research, which make the performance of these activities mandatory for any experiment and any professional working in this area.

If the experiment's severity assessment process is effectively applied, animal welfare will be maintained, as well as the scientific validity and transparency of research results. Good internal and external communication about the severity assessment process and the application of the 3Rs bring even greater benefits. The aim of this study was to describe the importance of carrying out a severity assessment associated with clinical and behavioral monitoring of rodents and rabbits during experimentation to maintain the welfare of these animals undergoing scientific research.

2. Methodology

A literature review was carried out with the aim of providing an overview of the

evaluation of the severity of the experiment associated with the clinical and behavioral monitoring of rodents and rabbits during experimentation.

The literature search was carried out using the following terms: "Monitoring"; "Humane endpoints"; "Animal welfare", "Rodents"; "Rabbits", and as connectors (Boolean operators) "and"; "or", in the following databases: PubMed (National Center for Biotechnology Information, U.S. National Library of Medicine), in the PUB MED module (https://www.ncbi.nlm.nih.gov/pubmed/); LILACS/BIREME (Latin American and Caribbean Center on Health Sciences Information), in the All-Indexes module (https://lilacs.bvsalud.org/); SciELO (Scientific Electronic Library Online), in the Integrated method (http://www.scielo.br/) and the studies were selected based on predefined inclusion/exclusion criteria.

Inclusion/Exclusion Criteria

Manuscripts describing the importance of clinical/behavioral monitoring and assessment of study severity associated with humane endpoints for maintaining the well-being of rodents and rabbits used in scientific research were included.

Manuscripts that did not describe the association of clinical/behavioral monitoring and assessment of study severity with the humane endpoints of rodents and rabbits used in scientific research were excluded.

3. Results

A total of 987 articles were identified in the databases, and 20 of these studies were included in this review. After reading these 19 manuscripts included from the bibliographic survey, the following information was compiled.

3.1. The 3Rs Principle

In scientific research, there are still no alternative methods to replace all tests, and the use of animals is still essential for research. For this reason, even in research or investigative procedures with real scientific and social merit, researchers today are committed to developing procedures to replace the animals that are commonly used in procedures carried out using an in vitro approach, although this possibility still has major limitations, due to the existence of few validated technologies, all studies should strive to reduce the number of animals used, prioritizing techniques that will refine the management and intervention protocols, improving the results of trials, using as few animals as possible and adopting measures to reduce pain and suffering as much as possible [3].

In view of the above, and with the aim of improving the techniques used more and more, in an ethical and humane way, two English scientists, Willian Russell and Rex Burch, published a paper in 1950 [2] entitled: The Principles of Human Experimental Technique, which was summarized in three words: replacement, reduction and refinement, which in translation means, substitution, reduction and refinement, becoming known as the "principle of the 3 Rs", thus becoming a guideline for animal experimentation throughout the world, being a beacon used

by all who use animals in scientific experiments [4]. After Russell and Burch's publication in the late 1980s [2], laws and protocols were created and adopted by various countries, not only recognizing this concept but also creating legal and moral obligations used in the quest to replace, reduce, and refine, where possible, protocols and procedures involving the use of animals in experimental trials.

The main objectives of the 3Rs concept are to reduce the number of animals used, optimizing the number of animals in trials, from a quantitative point of view, replacing the use of animals whenever possible, and increasingly humanizing procedures, from a qualitative point of view, making refinement and reduction short-term objectives, with the main goal being the total replacement of the use of animals in experimental trials, through the development and validation of alternative methods to their use [5].

The scientific community knows that adopting the 3Rs can increase the quality of experimental trials, along with measures relating to refining experimental designs, reducing variance, standardizing procedures and conditions that optimize animal care, minimizing stress and unnecessary pain, thus producing better quality data [6].

When opting to use the severity assessment approach, the aim is to introduce a greater guarantee of the application of the 3 Rs to the experimental study, adopting it throughout the experimental trial, improving the results of the study in general, as well as better communication between all the characters involved, improving the consistency of the data [7].

3.2. Assessing the Severity of the Experiment

The severity of a procedure is determined by the degree and time of pain, suffering, stress, or lasting harm expected to be experienced by the animal during the procedure according to the following classification [8]:

- Mild (G1)—procedures that cause short-term pain, suffering or stress, and do not significantly impair the animal's general well-being.
- Moderate (G2)—procedures that cause moderate pain, suffering or stress in the short term, or mild pain, suffering or stress in the long term, as well as procedures that may moderately alter the general well-being of the animals.
- Severe (G3 and G4)—procedures that cause severe pain, suffering or stress to the animals, or long-term moderate pain, suffering or stress, as well as procedures that cause severe damage to the general welfare of the animals.
- Terminal procedures—procedures carried out entirely under general anesthesia, from which the animal will not regain consciousness and will be euthanized.

The consideration of severity in a procedure should be conducted on an ongoing basis, starting with the pre-study phase, through the study-specific daily monitoring of the animals during the project, to the actual severity assessment after the study has been completed, allowing further refinements to be identified for future studies [7]. Because of this, it is possible to ensure that the 3Rs are be-

ing applied throughout the study.

Severity assessment requires compliance with the following points: presence of people with expertise and experience, e.g. researchers, animal experimentation technicians, keepers and the responsible veterinarian; continuous and adequate education, training and education of all personnel involved; daily severity assessment systems adapted to the species and the project, including informed and structured observations of the animals at appropriate intervals (e.g. increased frequency during and after procedures); well-informed and effective protocols for assessing behavior and clinical signs; analysis of observations that allows analysis of the nature and level of suffering; knowledge of the severity of each procedure and what action to take if this is reached or exceeded; overall assessment of actual suffering (mild, moderate, severe) for statistical data; reflection on the degree of effectiveness of the application of the 3Rs and whether improvements could be made in future studies [7].

In the pre-study phase (drawing up the project), it is important to consider whether the use of live animals is necessary to fulfill the scientific objectives. When the use of live animals is necessary and justified, it is important to choose a suitable animal model for the study. All aspects of the study that could cause pain, suffering, anguish, or lasting harm should be identified, either through a literature search or by consulting animal experimentation technicians and the veterinarian responsible for animal welfare to describe ways of minimizing their effects. In addition, at this stage, it is necessary to develop an animal observation plan that is appropriate and adapted to the study and that can be understood by all those involved in the study to improve communication and the consistency of the information collected. It is important to emphasize the need to have enough trained staff to conduct the study and monitor the animals [7] [8].

There are behaviors and clinical signs that can be used to assess the severity of the procedures during the captivity period (in the cage, tank, etc.). The terminology used to describe these signs must be understandable by all those involved in using, monitoring, and caring for the animals. For any severity assessment system, the following points should be considered: the existence of a solid understanding of the health, behavior and normal welfare status of the species being observed; the aim of achieving the best possible quality of life for the animal; and ensuring that any suffering resulting from scientific procedures is detected and minimized in association with maintaining scientific objectives and results [7] [9]

The process for defining an assessment protocol during the captivity period should identify any adverse effects that may occur throughout the animal's life experience, including housing, handling, care provision, as well as adverse effects resulting from experimental procedures and their consequences. By analyzing all these adverse effects, we should identify indicators that can be used to effectively assess the animal's welfare during the captivity period. These indicators should be easy to understand, identify and record consistently and adapted to the species and experimental procedures used [7] [9] [10]

The clinical/behavioral signs are described in global categories, applicable to all species, as a starting point for producing a comprehensive list of specific indicators for each experimental procedure. In this way it is possible to produce a study-specific list of sufficient indicators, minimizing the risk of overlooking certain signs of suffering, without the need to create an overly complex system that is unnecessarily bureaucratic and time-consuming and makes evaluation extremely subjective. The categories are Appearance; Physiological functions; Environment; Behaviors; Procedure-specific indicators; Free observations (other relevant observations). The indicators in each of these categories can be adapted to any species. They should be used to produce a list of observable characteristics that can be assessed by a suitably trained individual to make an evaluation of the animal's general state of health and welfare. These indicators should be discussed and selected in conjunction with the people responsible for supervising the welfare of the animals and, if appropriate, as required by the Ethics Committee on the Use of Animals (CEUAs). They should be used to develop specific record-keeping systems for each study, during the captivity period, for observation, monitoring, and evaluation during the daily routine [7] [11]

Severity assessment should be conducted for each animal on a case-by-case basis, using observations made of the animals during daily monitoring. Additional parameters necessary for the purposes of the study may also be used, where appropriate and when available. Non-observable indicators (such as body temperature, body weight, biochemical parameters, or bio-metric data such as heart rate) may also be required for the study and should be considered when assessing severity if they can provide additional, relevant information [7] [11].

The severity of the experiment can be cumulative, in which case we must consider: the life experience of each animal, in which restrictions on the ability to refine housing, or the need for frequent capture, handling and restraint etc., can affect severity; procedures involving a series of steps/interventions; previous procedures, in the case of reuse; and elements such as provenance (e.g. early weaning) and transportation [7].

The effectiveness of refinements should also be taken into account when assessing the severity of the study, for example: appropriate analgesia, anesthesia and post-operative care protocols; enrichment, both environmental and group housing of social animals; housing characteristics and handling and care provision, which should be refined according to current best practice or may require restrictions such as confinement to smaller enclosures (e.g. metabolic cages or crates), grid flooring or exposure to environmental conditions likely to cause stress; and training the animal to cooperate or promoting habituation to procedures [7] [8].

The consistency of the severity assessment is based on the development of a specific assessment form for the experimental procedure. Evaluation forms should be developed and agreed upon before the project begins, and they should be adapted to the species and the study. All available and relevant information should be used effectively in the development of the study-specific evaluation

forms, e.g. previous experience, results of in vitro or in silico studies, literature searches, information from pilot studies and clinical signs observed in humans or other animals. Information should be available on which parameters need to be observed and how monitoring should be conducted during the animals' period of captivity. Separate assessment forms can also be drawn up for separate components, for example a standard surgical/post-operative care form used in combination with an assessment adapted to the study protocol [7] [9] [11]

3.3. Humane Endpoints

The humane death of laboratory animals involves ethical and legal issues and must be respected by all those involved in the process [12]. All animals used in experimental trials carry with them an ethical commitment of great significance, since they are bred exclusively for research and kept under restraint, with limited access to food, even determining their social grouping, as well as standardized environmental conditions, which reduces the possibility of the animals adapting, preventing them from adjusting their natural physiological conditions and behavioral manifestations that they would have in the wild. Therefore, welfare care becomes even more important in this context, and is directly dependent on the housing conditions in which the animal is kept, as well as the handling and submission of experimental protocols [9].

Any teaching or scientific research activity must establish the humane end point in the body of the proposal that will be submitted to the institution's Ethics Committee on the Use of Animals (CEUA), thus allowing immediate intervention to avoid unnecessary suffering of the animal, adopting criteria for the outcome and induction to death of these animals, such as the size of the wound ulceration and the physical and psychological suffering imposed on the animal [13]. Similarly, animals used in experimental studies of infectious diseases can present significant pain or suffering as part of the manifestation of the disease, and the earlier the search for a humane end point, the greater the chance that this animal will have its suffering and anguish reduced, without necessarily altering the outcome of the trial [14].

A humane endpoint is the moment at which the pain, discomfort or stress of an animal used in teaching or research is avoided, ended, minimized or reduced by actions such as activity is avoided, terminated, minimized or reduced by actions such as: 1) adoption of treatment to relieve pain, discomfort or stress; 2) interruption of a painful procedure; 3) exclusion of the animal from the study; or 4) humane death of the animal. A humane end point must allow the scientific objectives of the research protocol to be achieved while minimizing animal suffering. Every research project should contain descriptions of appropriate end points for the animal species and procedures being used.

Currently, in experimental trials where animals are used, the pain and suffering inflicted are already ethically unacceptable. Furthermore, they can generate significant errors in the test results, as they cause various physiological aspects to be altered, for example, serum or plasma concentrations of corticosterone,

growth hormones, glucose, prolactin, blood pressure and even heart rate [15].

The adoption of methods to assess pain in rodents and rabbits (**Figures 1-3**) is

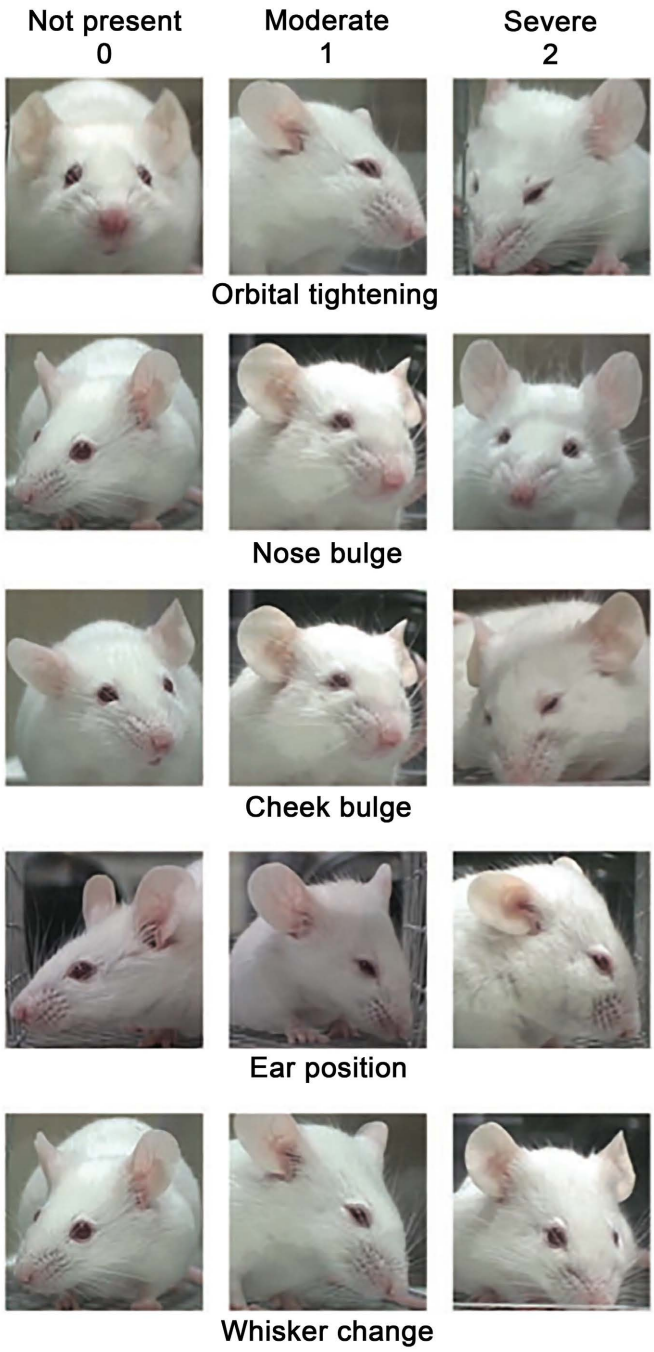

Figure 1. Facial scale of pain expression in mice. The Mouse Grimace Scale—Orbital tightening: Closing of the eyelid (narrowing of orbital area)./A wrinkle may be visible around the eye; Nose bulge: Bulging on the bridge of the nose./Vertical wrinkles on the side of the nose; Cheek bulge: Bulging of the cheeks; Ear position: Ears rotate outwards and/or backwards, away from the face./Ears may fold to form a "pointed" shape./Space between the ears increases. Whisker change: Whiskers are either pulled back against the cheek or pulled forward to "stand on end"./Whiskers may clump together./Whiskers lose their natural "downward" curve. Source: [16].

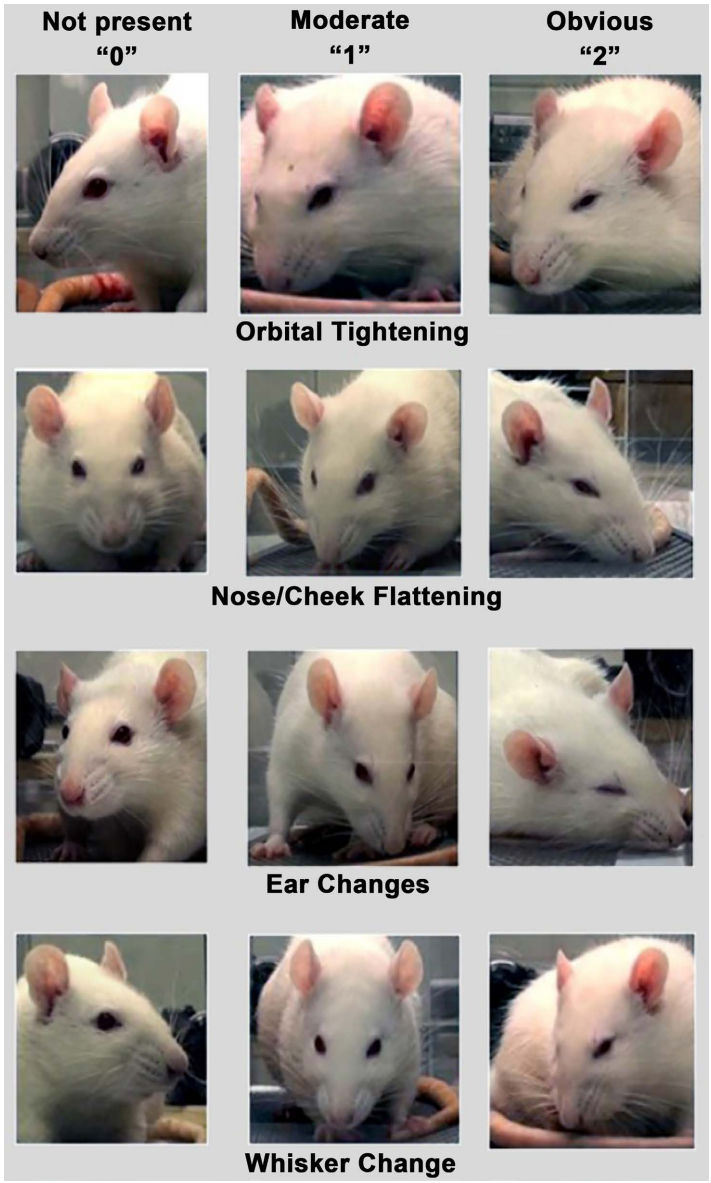

Figure 2. Facial scale of pain expression in mice. The Rat Grimace Scale—Orbital tightening: Closing of the eyelid (narrowing of orbital area)./A wrinkle may be visible around the eye. Nose/cheek flattening; Flattening and elongation of the bridge of the nose./Flattening of the cheeks (potentially sunken look). Ear changes: Ears curl inwards and are angled forward to form a "pointed" shape./Space between the ears increases. Whisker change: Whiskers stiffen and angle along the face./Whiskers may "clump" together./Whiskers lose their natural "downward" curve. Source: [17].

especially important, mainly because they are predated animals in the wild, and are therefore very resistant to expressing signs of pain, suffering or vulnerability. For this reason, there are behaviors and clinical signs that help to assess the degree of severity of the procedures imposed during the period of captivity, making it possible to create tables with these degrees, facilitating decision-making by those responsible for the study and for the welfare of the animals regarding the interruption of the study [9].

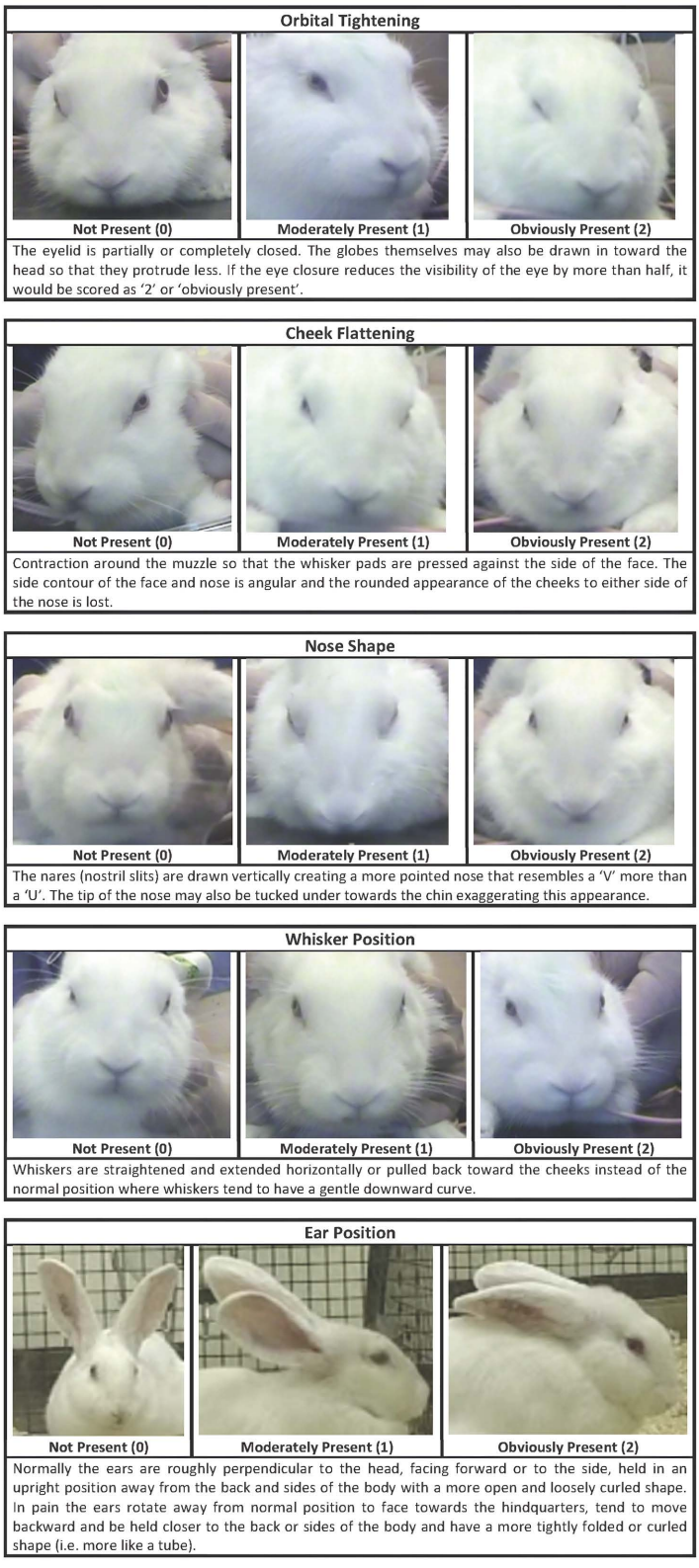

Figure 3. Facial scale of pain expression in mice. The Rabbit Grimace Scale [18].

This monitoring system is called the Humane End Point, which brings together a system for assessing the severity of each test, on an individual basis,

with a terminology and description that allows all those involved in the use, monitoring and care of the animals to easily understand, and it is essential for its use that the team has a solid knowledge of the behavior, health and normal state of well-being of the species used [7]

The End Point system aims to train all the professionals involved in a concrete system for physical and behavioral assessment of the animals used, as well as complete recording of all data and the frequency of monitoring [9].

It is important to note that the implementation of a monitoring system for the purpose of the Humane Ending of a scientific study requires the full training of a team of specialized professionals, with experience in multidisciplinary management, comprising the various areas of activity within the animal facility, from the keepers to the assistants, technicians and veterinary doctors, who will be involved in both the preparation and monitoring of interventions, which should take place on a daily basis, using well-defined protocols allowing for a reliable and complete assessment of all the data collected, allowing for continuous evolution and improvement [7].

All the data obtained during the observations made during the studies must be carried out in such a way as to allow the generation of reports capable of evaluating the animal's behavior and clinical signs, facilitating judgment, and avoiding or minimizing the pain and suffering of the animals used in the experimental studies [7].

All severity assessment protocols must have a simple approach, with a hierarchical definition of the responsibilities of each team member, and which allow them to be adapted to the species, strains, individuals, and procedures used. This process is used to define a form of assessment during the trial period, making it possible to identify any adverse effects that may occur to the animal under study, from housing to handling, also considering complications arising from the experimental procedures and their respective consequences [19].

From this set of information, daily analyses are conducted, identifying indicators that can be used to effectively assess the welfare of these animals, always adapting to each species, strain, procedures conducted and their consequences [9].

With the diversity of existing experimental protocols, respecting their specificities and distinct needs, the development of protocols for assessing the degree of severity (as described above) and the Humane End Point must be adjusted to each experimental protocol, and it is important to emphasize that the aim of adopting these End Point procedures is precisely to prevent animals from reaching a state of suffering, determining a point before their welfare is compromised for decision-making purposes [7]. In addition to the above, the Humane End Point Assessment also includes actions that are taken after the end of the experimental trials, when the project's objectives have been achieved, dealing with what will be done with the animals, also addressing what will be done if situations occur that were not foreseen in the trial protocol, such as unexpected

side effects, accidents, unforeseen illnesses with the animals, injury due to fights, escape, among other variables [9] [20].

The implementation of the Humane End Point in practice should be carried out in three stages: recognition of the signs of health, well-being, pain, distress and suffering of the species in question; a practical clinical approach, where the animal's natural behavior is observed from a distance and the animal's interaction with the observer during handling and carrying out a clinical examination of the animal (weighing, temperature and observation of clinical signs); recording clinical alterations in the humane end point table [9] [20].

The clinical score table is a spreadsheet for recording clinical signs, where a score is determined for the different abnormalities identified, based on defined criteria, allowing the health and welfare levels of the animal to be tracked and quantified [10] [20]. There are already end point table models in the literature for mice submitted to tumor inoculation experiments (**Figure 4**) and with infectious agents and surgical procedures (**Figure 5**) and rabbits subjected to polyclonal serum production (**Figure 6**) [9] [10] [21].

Parameter	Score			
	1	2	3	4
Coat	Normal	Lack of cleanliness	Dirty	Deplorable
Skin elasticity	Normal	Slightly dehydrated	Moderate dehydration	Severe dehydration
Spontaneous behavior	Normal	Lethargic	Aggressive, apathetic	Extremely aggressive, irresponsive
Tumor size	<3mm	</=5mm	</=8mm	>/=12mm
Jaundice	None	Minimal	Moderate	Severe
Weight	Normal <5%	<10%	<15%	<20%

Figure 4. Representation of the clinical score table for tumor inoculation procedures in mice. Source: CEUA/UNIFESP Humanitarian End Point Implementation Guide.

Parameter	Score				
	1	2	3		4
Coat	Normal	Lack of cleanliness	Dirty		Deplorable
Skin elasticity	Normal	Slightly dehydrated	Moderate dehydration		Severe dehydration
Spontaneous behavior	Normal	Lethargic	Aggressive, apathetic		Extremely aggressive, irresponsive
Surgical wound	Clean and dry	Red and moist	Red with purulent secretions		Purulent
Body score	Normal	Slightly emaciated	Moderately emaciated		Cachectic
Weight		Normal <5%	<10%	<15%	<20%

Figure 5. Representation of the clinical score table for procedures with surgical experiments in mice. Source: CEUA/UNIFESP Humanitarian End Point Implementation Guide.

CLINICAL-BEHAVIORAL SCORE	
ASPECT	**SCORE**
BODY WEIGHT	
5-10% weight loss	1
11-15% weight loss	2
16-20% weight loss	3
20% + weight loss	EUTHANASIA
POSTURE	
Moves slowly	1
Lying down with abdomen in contact with the floor most of the time	2
Does not move most of the time	EUTHANASIA
CHANGE IN BODY TEMPERATURE Normal range: 36,5ºC (35ºC -37,9ºC)	
Up to 2ºC above normal range	1
Up to 3ºC above normal range	2
> 3º of normal range	EUTHANASIA
Hypothermia < 35ºC	EUTHANASIA
ACTIVITY	
Little movement, changes posture, head and neck movements and no normal activity	2
Is immobile and does not exercise normal activity	EUTHANASIA
HUNGER	
Moderate decrease in intake	1
Decreased defecation	1
No swallowing	EUTHANASIA
No defecation	EUTHANASIA
INTERACTION	
No interaction with environmental enrichment objects	1
No curiosity	1
No sniffing	1
Absence of self-cleaning behavior	1
FACIAL EXPRESSION	
Half-closed eyes	1
Eyes closed	EUTHANASIA
Has floppy ears at some point	3
Ears flexed most of the time	EUTHANASIA
BEHAVIOR	
Mentions getting up, but remains lying down	2
Retracts and closes eyes	2
Shows tremors more frequently in the head and ears	2
ATTENTION TO THE AREA OF INOCULATION	
Lambe a área afetada Licks the affected area	1
Presses the inoculation site against the floor or cage	2
Keeps the limb suspended (if the inoculation is on the limb)	3
LESIONS IN THE AREA OF INOCULATION	
Ulcer	3
Abscess	EUTHANASIA
Take the following actions according to the total score obtained in the table above	**PONTUAÇÃO**
Increase the frequency of animal monitoring to twice a day	4
Implement, if possible, clinical veterinary care intervention	5 – 7
Euthanasia	8
Euthanasia - Any of these parameters alone indicate major physiological changes indicating pain and stress to the animal, leading to intense suffering.	EUTHANASIA

Figure 6. Representation of the clinical score table for polyclonal serum production in rabbit [21].

Animals should be monitored daily after the first day of infection and/or surgery and if the animal's total score after analyzing all the clinical score parame-

ters described in the table is equal to or greater than ten, the animal should be euthanized. Furthermore, even if the score of 10 is not reached, but the animal shows symptoms of extreme suffering such as convulsions, coma, paralysis of both limbs, hypothermia, more than 20% weight loss, 100% loss of strength when gripping, or some other specific sign that causes extreme suffering, it should be euthanized [9].

3.4. Responsibility for Monitoring the Animals

In Brazil, the National Council for the Control of Animal Experimentation (CONCEA) assigns responsibility for monitoring the welfare and clinical condition of animals to professors, researchers, veterinarians in charge and animal facility coordinators. If the animals are not assigned to a specific activity, responsibility for the daily monitoring of their welfare is shared by the coordinator and the veterinarian in charge of the animal facility where these animals are housed. Once an animal has been allocated to a teaching or research proposal, the teacher or researcher is responsible for the daily monitoring of its welfare. This responsibility is shared by the coordinator and the veterinarian in charge of the facility where the animal is housed [8] [22].

The records kept by the researchers, professors and veterinarians in charge should allow the Animal Ethics Committee to check that the quality and welfare of the animals is in accordance with the legislation. In addition, the records should also enable a critical assessment of the causes of unforeseen adverse events and contribute to the development of prevention strategies. The veterinarians technically responsible for the animal facility must immediately notify the professor or researcher of any unforeseen adverse event that could negatively impact animal welfare [8] [22].

The Ethics Committee on the Use of Animals (CEUA) should monitor all activities conducted with animals by establishing an inspection program and should also keep a record of the individual monitoring of animal activities in progress at the institution. The frequency and date of inspections will be determined by factors such as the number and accessibility of sites, the quantity, type and variety of teaching or scientific research activities, and the CEUA's meeting schedule. The facilities (laboratory animal facility) where the animals are housed must be inspected at least once a year. When inspections detect procedures that are not compatible with what is authorized, the CEUA must ensure that such activities are stopped immediately and that remedial action is initiated [8] [22].

In emergency cases, animals may be subjected to treatment or euthanasia. All appropriate measures must be approved by the veterinary technician responsible for the animal facility. Any treatment or euthanasia that deviates from the authorized proposal must be justified and reported in the form of a deviation (any unplanned change that occurs during a proposal after its initiation) and sent to CEUA immediately [8] [22].

3.5. Euthanasia

The word euthanasia comes from the Greek "euthanatos", or "good death", and is conceptualized as the humane way of bringing an animal to its death, without pain and with as little stress as possible, or the way of causing the animal's death in an assisted, controlled manner, relieving pain or suffering, with euthanasia, in these cases, being beneficial to the individual themselves, in cases of pain or suffering, at an irreversible level, without the possibility of pain control, treatment or rescue. This definition of the term euthanasia is used in all cases, both when inducing death is done for the good of the individual and for educational or scientific purposes, since the techniques used are similar [13].

The concept of euthanasia is part of CONCEA's Normative Resolution number 37 [13], which deals with euthanasia procedures carried out in animal facilities, which also states that all euthanasia procedures must be supervised by the technical manager of the facility, even if not in person, who must have the title of Veterinary Doctor, and active registration with the Regional Council of Veterinary Medicine, of the Federative Unit where the establishment is located, in addition to the Technical Manager training course [23].

In general, some criteria are adopted for euthanasia to be indicated, such as the severity of injuries, the impossibility of treatment, animals with terminal illnesses and intense suffering, and elderly animals who have difficulty performing their basic life support requirements individually. However, there are other situations in which euthanasia can also occur, such as in humane slaughter for consumption, and in teaching and scientific research activities. In these cases, the same methods are adopted for inducing death, which are painless, without mental suffering and quick [13].

There are different methods that can be chosen for the practice of euthanasia, and they should always be conducted by trained and qualified professionals and technicians, always under supervision, ensuring that the entire procedure takes place with respect and consideration for the animals and the principles proposed. Animal facilities should have a separate area, away from the rooms or accommodations of other animals, where euthanasia can be conducted [13].

Euthanasia is not just limited to the moment of death, but includes everything from removing the animals from their accommodation to physical restraint, which must be carried out in such a way as to minimize the stress, anxiety, apprehension and suffering of the animals in question, and these concerns must be taken into account when choosing the method to be used, These concerns must be taken into account when choosing the method to be used, ensuring that an appropriate method is chosen and that the animal loses consciousness quickly, without any unpleasant emotional or physical experience that is irreversible, so that the animal does not experience pain, stress, anxiety or apprehension, leading to immediate loss of consciousness, cardiorespiratory arrest and then loss of brain function [19].

The method of euthanasia used must be selected according to the species of

animal used, its age, the availability of means of restraint, the skill of the operator, the aim of the study and the number of animals to be euthanized, and can be divided into physical or chemical, where among the chemical methods, the most commonly used are injectable or inhalation agents, always remembering that the objective of the study must be taken into consideration when choosing the method, which can hinder the use of chemical methods, and should always prioritize the choice of the most humane method possible, taking into account the objectives of the trial and the animal species [13].

An extremely important factor in assessing the level of stress imposed on the animal is knowledge of the animal's behavior, and the faster the loss of consciousness followed by death, the less stress and consequent suffering the animal will be subjected to during the euthanasia procedure, and the brain depression caused by the methods must always precede cardiorespiratory arrest [13].

During the restraint process, all the principles of animal welfare should be respected, and the process should be completed as quickly as possible [13].

4. Conclusions

Below we highlight the essential recommendations that should be carried out during the clinical/behavioral monitoring of rodents and rabbits under experimentation to maintain the well-being of these animals undergoing experimental procedures during scientific research.

Any system for assessing the severity of an experiment must effectively detect deviations from a normal state of health and well-being, allowing the observer to record and transmit a clear and consistent assessment of each animal.

The final assignment of the severity of the experiment is the result of an analysis of records of observations of the animal's behavior, clinical signs and other relevant parameters during the period of captivity. Input from relevant researchers, animal experimentation technicians, veterinarians and animal care staff is required in the development phase of the study to ensure that appropriate data is available to enable the correct assignment of the final severity of the experiment.

Humane End Point protocols and procedure severity tables are of the utmost importance, both from an ethical point of view and to refine the results of research conducted on laboratory animals. They should be drawn up jointly by the teams responsible for the project and the maintenance of the animals during the research period, and the data obtained should be published so that it can be accessed by the scientific community, helping to disseminate these practices, as well as helping to draw up new procedures.

Monitoring and evaluating the welfare and clinical state of the animals is the responsibility of the professors, researchers, veterinarians in charge and animal facility coordinators. The CEUA must monitor all activities conducted with the animals, through inspections of experimental and teaching procedures and the facilities (laboratory animal facility) where the animals are housed.

The euthanasia procedure begins when the animals are removed from their

housing, including physical restraint, which must be conducted in such a way as to minimize stress, anxiety, apprehension, and suffering. Management based on habituation and human-animal interaction should be used to keep this moment as stressful as possible. The method of choice for euthanasia must guarantee a rapid loss of consciousness, devoid of any unpleasant emotional or physical experience, which is irreversible, so that the animal does not experience pain, stress, anxiety, or apprehension, leading to immediate loss of consciousness, cardiorespiratory arrest, and subsequent loss of brain function.

Conflicts of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of Data and Materials

The datasets used and/or analyzed during the current study can also be requested from the corresponding author on reasonable request.

Authors' Contributions

Jhônata Willy Rocha Coelho, Hyago da Silva Medeiros Elidio, Rita de Cássia dos Passos Ferraz da Silva, João Gabriel Regis Sobral and Bárbara Alves de Brito Soledade: bibliographic review, selection of the articles used in this study according to the inclusion criteria and data analysis.

Luiz Cesar Cavalcanti Pereira, Leandro Thomaz Vilela and Isabele Barberi dos Santos: experimental design of the study, data analysis, preparation and writing of the manuscript.

References

[1] Riviera, E.A.B. (2017) Welfare of Laboratory Animals. In: Lapchik, V.B.V., Mattaraia, V.G.M. and Ko, G.M., Eds., *Care and Management of Laboratory Animals*, 2nd Edition, Ateneu, Rio de Janeiro, 35-45. https://link.springer.com/book/10.1007/978-1-4020-2271-5

[2] Russell, W.M.S. and Burch, R.L. (1959) The Principles of Humane Experimental Technique. Methuen & Company, London.

[3] Fischer, M.L., Farias, M.K. and Carrano, E. (2020) The Inclusion of Field Research in the Brazilian Guidelines for the Care and Use of Animals for Scientific and Teaching Purposes. *Revista Conexão Ciência*, **15**, 7-20.

[4] Braga, L.M.G.M. and Pitrez, P.M.C. (2010) Animals in Research and Teaching: Ethical and Technical Aspects. *XII Salão de Iniciação Científica-PUCRS*, 3-7 de outubro de 2011, 173. https://editora.pucrs.br/anais/seminarioic/20112/3/1/1.pdf

[5] Tenter, A.M., Heckeroth, A.R. and Weiss, L.M. (2000) *Toxoplasma gondii*: From Animals to Humans. *International Journal for Parasitology*, **30**, 1217-1258. https://doi.org/10.1016/S0020-7519(00)00124-7

[6] Flecknell, P. (2002) Replacement, Reduction, and Refinement. *Altex*, **19**, 73-78. https://altex.org/index.php/altex/issue/view/66?iid=65&aid=5

[7] Directive 2010/63/EU. European Union. On the Protection of Animals Used for

Scientific Purposes. Working Document on a Severity Assessment Framework. Brussels, July 11-12, 2012. https://ec.europa.eu/environment/chemicals/lab_animals/pdf/guidance/severity/en.pdf

[8] National Council for the Control of Animal Experimentation (2023) Brazilian Guide to the Production, Maintenance or Use of Animals for Teaching Activities or Scientific Research. CONCEA, Brasília. https://www.gov.br/mcti/pt-br/composicao/conselhos/concea/arquivos/arquivo/publicacoes-do-concea/guia_concea_1ed_animais-_ensino_ou_pesquisa_2023.pdf

[9] Cavalcanti, P.V. (2024) Guide for the Implementation of the CEUA/UNIFESP. Humanitarian End Point. 2nd Edition, UNIFESP, São Paulo. https://ceua.unifesp.br/images/documentos/CEUA/Guia_EndPoint_CEUA_UNIFESP_2024.pdf

[10] Leach, M.C., Thornton, P.D. and Main, D.C.J. (2008) Identification of Appropriate Measures for the Assessment of Laboratory Mouse Welfare. *Animal Welfare*, **17**, 161-170. https://doi.org/10.1017/S0962728600027676 https://www.researchgate.net/publication/233521726_Identification_of_appropriate_measures_for_the_assessment_of_laboratory_mouse_welfare

[11] Vlissingen, J.M.F., Borrens, M., Girod, A., Lelovas, P., Morrison, F. and Torres, Y.S. (2015) The Reporting of Clinical Signs in Laboratory Animals: Relatório do Grupo de Trabalho da FELASA. *Laboratory Animals*, **49**, 267-283. https://doi.org/10.1177/0023677215584249

[12] Hawkins, P., Playle, L., Golledge, H., Leach, M., Banzett, R., Coenen, A., Cooper, J., Danneman, P., Flecknell, P., Kirckden, R., Niel, L. and Raj, M. (2006) Consensus Meeting on Carbon Dioxide Euthanasia of Laboratory Animals. https://www.nc3rs.org.uk/sites/default/files/documents/Events/First%20Newcastle%20consensus%20meeting%20report.pdf

[13] National Council for the Control of Animal Experimentation (CONCEA) (2018) Guidelines for the Practice of Euthanasia. Normative Resolution No. 37 of February 15. https://www.gov.br/mcti/pt-br/composicao/conselhos/concea/arquivos/arquivo/legislacao/anexo-da-resolucao-normativa-no-37-de-15-de-fevereiro-de-2018.pdf

[14] Olfert, E.D. and Godson, D.L. (2000) Humane Endpoints for Infectious Disease Animal Models. *IIAR Journal*, **41**, 99-104. https://academic.oup.com/ilarjournal/article/41/2/99/749074?login=false https://doi.org/10.1093/ilar.41.2.99

[15] Morton, D. (2000) A Systematic Approach for Establishing Humane Endpoints. *ILAR Journal*, **41**, 80-86. https://pubmed.ncbi.nlm.nih.gov/11406701/ https://doi.org/10.1093/ilar.41.2.80

[16] Langford, D., Bailey, A., Chanda, M., *et al.* (2010) Coding of Facial Expressions of Pain in the Laboratory Mouse. *Nature Methods*, **7**, 447-449. https://doi.org/10.1038/nmeth.1455

[17] Sotocina, S.G., Sorge, R.E., Zaloum, A., *et al.* (2011) The Rat Grimace Scale: A Partially Automated Method for Quantifying Pain in the Laboratory Rat via Facial Expressions. *Molecular Pain*, **7**. https://doi.org/10.1186/1744-8069-7-55

[18] Keating, S.C.J., Thomas, A.A., Flecknell, P.A. and Leach, M.C. (2012) Evaluation of EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physio-Logical, Behavioural and Facial Expression Responses. *PLOS ONE*, **7**, e44437. https://doi.org/10.1371/journal.pone.0044437

[19] Cardoso, C.V.P. (2002) Laboratory Animals; Breeding and Experimentation. Fiocruz, Rio de Janeiro, 57 p. https://www.arca.fiocruz.br/handle/icict/37618

[20] Silva, K.S.M. and Lima, M.R. (2023) Guide to Humane Termination Applied to Rodents Used in Research/Organizers Klena Sarges Marrvaz da Silva, Monique Ribeiro de Lima. Atena, Ponta Grossa.
https://doi.org/10.22533/at.ed.824231008

[21] Dos Santos, I.B. (2023) Polyclonal Serum Production. In: *Guide to Humane Termination Applied to Rodents Used in Research/Organizers Klena Sarges Marrvaz da Silva*, *Monique Ribeiro de Lima*, Atena, Ponta Grossa, 104-115.

[22] National Council for the Control of Animal Experimentation (CONCEA) (2022) Updates the Text of the Brazilian Guidelines for the Care and Use of Animals in Teaching or Scientific Research Activities-DBCA. Normative Resolution No. 55, of October 5.
https://www.in.gov.br/web/dou/-/resolucao-n-55-de-5-de-outubro-de-2022-434869177

[23] Marques, R.G., Marcelo, M.M. and Petroianu, A. (2014) Brazilian Law for Scientific Use of Animals. *Acta Cirúrgica Brasileira*, **24**, 2009-2069.
https://pubmed.ncbi.nlm.nih.gov/19169547/
https://doi.org/10.1590/S0102-86502009000100015