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HUMANITARIAN FINALIZATION IN ANIMALS SUBJECTED TO SCIENTIFIC EXPERIMENTS

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Abstract: Humane termination aims to minimize the distress, pain or suffering of animals used in research, ending the procedure or experiment in order to avoid unnecessary suffering of the animal, without the loss of data for the research. Every research protocol must contain descriptions of appropriate *End point*s for the animal species in use. The Humane Endpoint protocols and the tables of degrees of severity of the procedures are of paramount importance, both for the ethical aspect and for refining the results of research carried out on laboratory animals, and must be prepared jointly by the teams responsible for the design and maintenance of the animals during the research period, with the data obtained being published for access by the scientific community, helping to disseminate such practices, as well as helping to develop new procedures.
Keywords: Degree of Severity; Humanitarian endpoints; Animals, Scientific research.

PRINCIPLE OF THE 3RS

In scientific research, there are still no alternative methods that can replace all tests, and the use of animals is still essential for the progress of research. Therefore, even in research or investigation procedures of effective scientific and social merit, researchers are today committed to developing conduct in favor of replacing animals that are commonly used for procedures carried out with an *in vitro* approach, however, due to this possibility still has major limitations, due to the existence of few validated technologies, all studies must focus on reducing the number of animals used, prioritizing techniques that will refine the management and intervention protocols carried out, improving the results of the tests, using the minimum animals as possible and adopting measures to reduce pain and suffering as much as possible (FISCHER et al, 2020).

In view of what was said, and with the aim of improving the techniques used every day, in an ethical and humanitarian way, two English scientists, Willian Russel and Rex Burch, in 1950 published the work entitled: *The Principles of Human Experimental Technique,* which came to be summarized in three words: *replacement, reduction and refinement,* which in translation means replacement, reduction and refinement, becoming known as the "principle of the 3 Rs", thus becoming a guide for animal experimentation around the world, being a guide used by all who use animals in scientific experiments (BRAGA, PIETRIZ, 2010).

After the publication of Russel and Burch, at the end of the 1980s, new laws and protocols were created, adopted by several countries, not only recognizing this conception but also creating legal and moral obligations used in the search to replace, reduce and refine, when possible, protocols and procedures that involve the use of animals in experimental tests.

The 3Rs concept has as its main objectives the reduction of the number of animals used, optimizing the number of animals in the tests, from a quantitative point of view, replacing, whenever possible, the use of animals, increasingly humanizing the procedures, from the point of view of from a qualitative point of view, making refinement and reduction become short-term objectives, with the main goal being the total replacement of the use of animals in experimental tests, through the development and validation of alternative methods to their use (TENTER, 2000).

It is known to the scientific community that the adoption of the 3Rs can increase the quality of experimental trials, along with measures related to refining experimental designs, reducing variance, standardizing procedures and conditions that optimize animal care, minimizing their stress and pain. unnecessary, thus producing better quality data (FLECKNEL, 2002).

When choosing 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 test, improving the results of the study in general, as well as a better communication between all the characters involved improving data consistency (EU DIRECTIVE, 2010).

EUTHANASIA

The word euthanasia comes from the Greek "euthanatos", or "good death", being conceptualized as the humane form or way of taking the animal to death, without pain and with as little stress as possible, or the way of causing the death of the animal. animal in an assisted manner, being controlled, relieving pain or suffering, with euthanasia, in these cases, being beneficial to the individual himself, in cases of pain or suffering, at an irreversible level, without the possibility of pain control, treatment or assistance (CONCEA, 2018).

This definition of the term euthanasia is used in all cases, both when the induction of death is done for the good of the individual, and for didactic or scientific purposes, since the techniques used are similar (CONCEA, 2018).

The concept of euthanasia is part of CONCEA's Normative Resolution number 37, which deals with euthanasia procedures carried out in animal facilities, which also states that all euthanasia procedures must be supervised by the facility's technical manager, 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 Responsible training course (MARQUES et al, 2014).

Some criteria are adopted so that euthanasia, in general, is indicated, such as: severity of injuries, impossibility of treatment, animals with terminal illnesses, in intense suffering, elderly animals with difficulty in carrying out their basic life support requirements. individual way. 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 which cases the same methods for inducing death are adopted, which are painless, without mental suffering and rapid (CONCEA, 2018).

There are different methods that can be chosen to practice euthanasia, and they must always be carried out 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 proposed principles. Animal facilities must have a separate place, away from the rooms or housing of other animals, to carry out euthanasia (CONCEA, 2018).

Euthanasia is not limited to the moment of death, but ranges from the removal of animals from their housing to physical containment, which must be carried out in order to minimize stress, anxiety, apprehension and suffering of the animals in question, these being concerns considered when choosing the method to be used, ensuring the choice of an appropriate method, and the loss of consciousness quickly, devoid of unpleasant emotional or physical experience, which is irreversible, meaning that the animal does not present pain, stress, anxiety or apprehension, leading the animal to immediate loss of consciousness, cardiorespiratory arrest and then loss of brain functions (CARDOSO, 2006).

The euthanasia method used must be selected according to the animal species used, age, availability of means of containment, the skill of the operator, the objective of the study and the number of animals to be euthanized, which can be divided into physical or chemical, where, among chemicals, the most commonly used are injectable or inhalational agents, giving preference to chemical methods when compared to physical ones, such as cervical dislocation or decapitation, always remembering that the objective of the study must be taken into consideration at the time of choice of method, which may impede the use of chemical methods, and you must always choose the most humane method possible, taking into account the objectives of the test and the animal species (CONCEA, 2018).

An extremely important factor for evaluating the level of stress imposed on the animal is knowledge of its behavior, and the faster the loss of consciousness followed by death, the less stress and consequent suffering the animal is subjected to during the procedure. of euthanasia, and the cerebral depression caused by the methods must always precede cardiorespiratory arrest (CONCEA, 2018).

During the containment process, all applied animal welfare principles must be respected, and the process must be completed as quickly as possible (CONCEA, 2018).

ASSESSMENT OF THE DEGREE OF SEVERITY OF THE EXPERIMENT

Consideration of severity in a procedure must be carried out on an ongoing basis, starting with the pre-study phase, through study-specific daily monitoring of animals during the project, until assessment of actual severity after completion of the study, allowing identification of new refinements for future studies (EU DIRECTIVE, 2010). Due to this, it is possible to ensure that the 3Rs are being applied throughout the study.

The assessment of effective severity requires the following points: presence of

people with specialties and experience, for example researchers, animal experimentation technicians, handlers and the responsible veterinarian; continuous and adequate education, training and training of all personnel involved; daily severity assessment systems adapted to the species, strain and project, including informed and structured observations of 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; global assessment of actual suffering (mild, moderate, severe) to generate statistical data; reflection on the degree of effectiveness of the application of the 3Rs and whether improvements can be made in future studies (EU DIRECTIVE, 2010);

In the pre-study phase (project preparation), it is important to consider whether the use of live animals is necessary to meet scientific objectives. When the use of live animals is necessary and justified, it is important to choose an appropriate animal model for the study. All aspects of the study that may cause pain, suffering, distress or lasting harm must be identified, through literature research or by consulting animal experimentation technicians and the veterinarian responsible for animal welfare with the aim of describing ways to minimize their effects.

Furthermore, at this stage, it is necessary to develop an animal observation plan that is appropriate and adapted to the study that can be understood by everyone involved in the study to improve communication and consistency of the information collected. It is important to highlight the need to have a team in sufficient numbers and with adequate training to carry out the study and monitoring of animals (DIRECTIVE UE, 2010; CONCEA,2023).

There are behaviors and clinical signs that can be used to assess the severity of procedures during the captivity period (in the cage, tank, cage, etc.). The terminology used to describe these signs must be understandable by everyone involved in the use, monitoring, and care of animals. For any severity assessment system, the following points must be considered: existence of a solid understanding of the health, behavior and normal state of well-being of the observed species; objective of achieving the best possible quality of life for the animal; and ensure that any suffering resulting from scientific procedures is detected and minimized associated with maintaining scientific objectives and results (DIRECTIVE UE, 2010; CAVALCANTE, 2024).

The process for defining an evaluation protocol during the captivity period must identify any adverse effects that may occur throughout the animal's life experience, including housing, handling, care, as well as adverse effects resulting from experimental procedures and their consequences. By analyzing all these adverse effects, we must identify indicators that can be used to effectively assess the animal's well-being during the period of captivity. These indicators must be easy to understand, identify and record consistently and adapted to the species and experimental procedures used (Leach MC et al. (2008), DIRECTIVE UE, 2010; CAVALCANTE, 2024).

Clinical/behavioral signs are described in global categories, applicable to all species, as a starting point for producing a comprehensive list of indicators specific to each experimental procedure. This way, it is possible to produce a list, specific to the study, of sufficient indicators, minimizing the risk of ignoring certain signs of suffering, without the need to create an overly complex system that is unnecessarily

bureaucratic and time-consuming and makes the assessment extremely subjective. The categories are Appearance; Physiological functions; Environment; Behaviors; Procedure-specific indicators; Free observations (other relevant observations). The indicators for each of these categories can be adapted to any species. They must be used to produce a list of observable characteristics that can be evaluated by an individual with appropriate training to assess the animal's general health and welfare. These indicators must be discussed and selected together with the people responsible for supervising animal welfare and, if appropriate, as required by the Animal Use Ethics Committee. They must be used to develop specific record keeping systems for each study, during the period of captivity, for observation, monitoring and evaluation during the daily routine (DIRECTIVE EU, 2010; VLISSINGEN et al, 2015).

Assessment of effective severity must be carried out for each animal, case by case, using observations made of the animals during daily monitoring. Additional parameters necessary for the purposes of the study may also be used, whenever appropriate and when available. Non-observable indicators (such as body temperature, body weight, biochemical parameters or biotelemetric data, such as heart rate) may also be necessary for the study, which must be considered in the assessment of severity, if they can provide additional information and relevant (EU DIRECTIVE, 2010; VLISSINGEN et al, 2015).

The severity of the experiment can be cumulative, in this case we must consider: the life experience of each animal, in which restrictions on the ability to refine the housing, or the need for frequent capture, handling and containment, etc., can affect the severity; procedures involving a series of steps/interventions; previous procedures, in case of reuse; and elements such as provenance (e.g. early weaning) and transport (EU DIRECTIVE, 2010).

The effectiveness of refinements must also be taken into account when evaluating the severity of the study, such as: adequate analgesia, anesthesia and postoperative care protocols; enrichment, both environmental and group housing of social animals; housing characteristics and management and care provision – which must be refined according to current best practices or may require restrictions, such as confinement in smaller enclosures (e.g. cages or metabolic cages), grid flooring or exposure environmental conditions that may cause stress; and training the animal to cooperate or promoting habituation to the procedures (DIRECTIVE UE, 2010; CONCEA, 2023).

The consistency of the severity assessment is based on the development of an assessment form specific to the experimental procedure. Assessment sheets must be developed, agreed in advance before the start of the project, and adapted to the species and the study. All available and relevant information must be used effectively in the development of specific evaluation forms for the study, for example 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 must be available on which parameters need to be observed and on how monitoring must be carried out during the animals' captivity period. 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 (DIRECTIVE EU; 2010; VLISSINGEN et al., 2015; CAVALCANTE 2024).

HUMANITARIAN ENDPOINTS (*END POINTS*)

The humane death of laboratory animals involves ethical and legal issues and must be respected by everyone involved in the process (HAWLKINS et al., 2006).

All animals used in experimental trials carry with them an ethical commitment of great significance, since they are created exclusively for research and maintained in a containment regime, access to food is limited, being determined up to the social group of coexistence, in addition to standardized environmental conditions, which reduces the animals' ability to adapt, preventing the adjustment of their natural physiological conditions and behavioral manifestations that they would have in free life. Therefore, care for well-being becomes even more important in the context, and directly dependent on the housing conditions to which the animal is conditioned, in addition to the management and submission of experimental protocols (CAVALCANTE, 2024).

Any teaching or scientific research activity must establish the humanitarian *End point* in the body of the proposal that will be forwarded to the institution's Committee on Ethics in the Use of Animals (CEUA), thus allowing immediate intervention, avoiding unnecessary suffering to the animal, adopting criteria for the outcome and induction of death of these animals, such as the size of the wound ulceration and the physical and psychological suffering imposed on the animal (CONCEA, 2018). Likewise, animals used in experimental studies of infectious diseases may experience significant pain or suffering as part of the manifestation of the disease, and the sooner the search for a humane *End point* is achieved, the greater the chance that that animal will experience suffering and distress. decreased, without necessarily altering the test result (OLFERT, 2000).

Currently, in experimental tests where animals are used, the pain and suffering inflicted are already ethically unacceptable, in addition, they can generate significant errors in test results, as they cause several physiological aspects to be altered, for example, serum or plasma concentrations. corticosterone, growth hormones, glucose, prolactin, blood pressure and even heart rate (MORTON, 2000).

The adoption of pain assessment methods in rodents is very important mainly due to the fact that, in free life, they are preyed upon animals, thus presenting great resistance in expressing signs of pain, suffering or vulnerability, therefore, there are behaviors and clinical signs that they assist in evaluating the degree of severity of the procedures imposed during the period of captivity, allowing the creation of tables with these degrees, facilitating the decision-making of those responsible for the study and for the well-being of the animals regarding the interruption of the study (CAVALCANTE, 2024).

This monitoring system is called Humanitarian Final Point, or in English, *End point*, which brings together a severity assessment system relating to each test, individually, which has a filling terminology with description that allows easy understanding of everyone involved in the use, monitoring and care of animals, and it is essential for their use that the team has solid knowledge about the behavior, health and normal state of well-being of the species used (EU DIRECTIVE, 2010).

The *End point* system aims to train all professionals involved, in a concrete system of physical and behavioral evaluation of the animals used, in addition to complete recording of all data, as well as the frequency of monitoring (CAVALCANTE, 2024).

It is important to highlight that the

implementation of a monitoring system for the purpose of Humane Finalization of a scientific study requires full training of the team of specialized professionals, with experience in multidisciplinary management, who comprise the different areas of activity within the animal facility, from the handlers, to assistants, technicians and veterinarians, who will act in both the preparation and monitoring and interventions, which must occur on a daily basis, making use of well-defined protocols allowing a reliable and complete evaluation of all data collected, allowing its continuous evolution and improvement (EU DIRECTIVE, 2010).

All data obtained during observations carried out during the studies must be carried out in such a way as to allow the generation of reports capable of evaluating the behavior and clinical signs of the animal, facilitating judgment and avoiding or minimizing pain and suffering of animals used in experimental studies (EU DIRECTIVE, 2010).

All severity assessment protocols must have a simple approach, with a hierarchical definition of the responsibilities of each team member, and that enable their due adaptation to the species, lineages, individuals and procedures used, this process being used to define a form of evaluation during the period during which the test is carried out, enabling the identification of any adverse effects that may occur with the animal under study, from housing to handling, also considering possible complications arising from the experimental procedures and their respective consequences (CARDOSO, 2006).

From this set of information, daily analyzes are carried out, identifying indicators that can be used in the effective assessment of the well-being of these animals, always adapting to each species, lineage, procedures performed and their possible consequences (CAVALCANTE, 2024).

With the diversity of existing experimental protocols, respecting their specificities and distinct needs, the elaboration of severity degree assessment protocols (as described above) and Humanitarian *End point* must be adjusted to each experimental protocol, and it is important to highlight that the objective of adoption of these *End point* procedures, is precisely to prevent animals from reaching a state of suffering, determining a point prior to compromising their well-being for decision making (EU DIRECTIVE, 2010). In addition to the above, the Humanitarian *End point* Assessment also includes actions that are taken after the end of the experimental tests, having achieved the project objectives, dealing with what will be done with the animals, also addressing what will be done if situations not foreseen in the trial protocol, such as unexpected side effects, accidents, unforeseen illnesses involving the animals, injuries from fights, escape, among other variables (CAVALCANTE, 2024; SILVA & LIMA, 2023).

The implementation of the Humanitarian Endpoint in practice must be carried out in three stages: recognition of signs of health, well-being, pain, distress and suffering of the species in question; 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 clinical examination of the animal (weighing, temperature and observation of clinical signs); recording of clinical changes in the humanitarian *End point* table (CAVALCANTE, 2024; SILVA & LIMA, 2023).

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 tracking and quantification of the animal's health and well-being levels (EU Directive,

Parameter	Punctuation			
	1	2	3	4
Coat	Normal	Lack of cleaning	Dirty	Deplorable
Skin Elasticity	Normal	Little dehydrated	Moderate Dehydration	Severe Dehydration
Behavior	Normal	Lethargic	Aggressive, apathetic	Very aggressive, irresponsive
Tumor Size	<3mm	</=5mm	</=8mm	>/=12mm
Jaundice	None	Minimum	Moderate	Serious
Weight	Normal <5%	<10%	<15%	<20%

Figure 1: Representation of the clinical score table for tumor inoculation procedures
Source: Guide at CAVALCANTE (2024).

Parameter	Punctuation			
	1	2	3	4
Coat	Normal	Lack of cleaning	Dirty	Deplorable
Skin elasticity	Normal	Little dehydrated	Moderate Dehydration	Severe dehydration
Behavior	Normal	Lethargic	Aggressive, apathetic	Very aggressive, irresponsive
Surgical Wound	Clean and dry	Reddish and moistened	Red with secretions	Purulent
Body Score	Normal	Slightly slimmed	Moderately Slim	Cachectic
Weight	Normal <5%	<10%	15%	<20%

Figure 2: Representation of the clinical score table for procedures with surgical experiments.
Source: Guide at CAVALCANTE (2024).

2010; SILVA & LIMA, 2023). In the literature, there are already *End point* table models for mice subjected to tumor inoculation experiments (Figure 1) and with infectious agents and surgical procedures (Figure 2) (CAVALCANTE, 2024; DIRECTIVE UE, 2010).

Monitoring of animals must occur daily after the first day of infection and/or surgery and if the animal's sum of points after analysis of all clinical score parameters, described in the table, is equal to or greater than 6, the animal must be subjected to euthanasia. Furthermore, even if score 6 is not reached, but the animal presents symptoms of extreme suffering such as convulsions, coma, paralysis of both limbs, hypothermia, more than 20% weight loss, loss of 100% of grasping strength, or some other specific sign that causes extreme suffering, the person must be subjected to euthanasia (CAVALCANTE, 2024).

FINAL CONSIDERATIONS

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 a severity category is the result of an analysis of observation records during the period of captivity, behavior, clinical signs and other relevant parameters. Input in the study development phase from relevant scientists, animal technicians, veterinarians and animal care personnel is required to ensure that appropriate data are available to allow the correct assignment of the final severity of the experiment.

The Humane Endpoint protocols and the tables of degrees of severity of the procedures are of paramount importance, both for the ethical aspect and for refining the results of research carried out on laboratory animals, and must be prepared jointly by the teams

responsible for the design and maintenance of the animals during the research period, with the data obtained being published for access by the scientific community, helping to disseminate such practices, as well as helping to develop new procedures.

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