

Regulatory frameworks governing the use of animals in education and research: a comparison between Brazil and the European Union

Abstract

This study conducts a comparative qualitative analysis of the regulatory frameworks governing the use of animals in education and research in Brazil and the European Union, focusing on ethical, legal, and technical aspects related to animal welfare. Through a chronological and structural examination of key documents - Directive 2010/63/EU and its updates in the EU, and Law No. 11.794/2008 with its normative resolutions in Brazil - the research evaluates similarities, differences, and advances in both systems. Findings show that the European Directive establishes a comprehensive and harmonized framework, encompassing vertebrates and cephalopods, and promoting transparency through non-technical summaries and public data on animal use. Brazilian regulation demonstrates notable progress, supported by detailed operational standards and oversight by CONCEA and CEUAs, but remains limited to vertebrates and lacks comparable mechanisms for public transparency. Both regions exhibit strong adherence to the principles of Replacement, Reduction, and Refinement (3Rs), reflecting a shared ethical commitment to humane scientific practices. The study concludes that while Brazil and the EU differ in scope and implementation, both legal systems represent significant achievements in the governance of animal use. Continued harmonization, personnel training, and the development of alternative methods are essential to strengthen ethical standards in science and education.

Keywords: ethical use, animal welfare, regulation, legislation

Volume 14 Issue 1 - 2026

Julio C Q Penha

Center for Research in Laboratory Animals, Federal Fluminense University, Brazil

Correspondence: Julio C Q Penha, Center for Research in Laboratory Animals, Federal Fluminense University, Brazil, Tel +5521994760788

Received: April 1, 2026 | **Published:** April 29, 2026

Introduction

Animals have been used in educational and research activities for thousands of years. There are records in the literature dating back to 2000 before Common Era (BCE) describing practices in which peoples of the Babylonian and Assyrian empires performed surgeries involving animals. In Ancient Greece, studies have shown that Aristotle conducted dissections to observe anatomical differences among various animal species.^{1,2} Later, during the Common Era (CE), Galen studied pigs, monkeys, parrots, and many other animal species, contributing significantly to the advancement of experimental medical sciences. Like Aristotle, he believed that animals experienced less pain, which facilitated their use in experimental activities.¹

Over the centuries, the indiscriminate use of animals in education and research continued, intensifying even further during the Middle Ages due to the Catholic Church's prohibition of human cadaver use. Concerns regarding the use of animals began to gain momentum only around the 18th and 19th centuries, particularly in Europe. During this period, attempts to regulate such practices emerged, marked by the creation of one of the first laws - the Act to Prevent the Cruel and Improper Treatment of Cattle - and by the establishment of animal protection societies, such as the Society for the Prevention of Cruelty to Animals (SPCA) in England. Following these developments, the movement gained strength and spread across Europe and other parts of the world, eventually reaching the Americas.¹

In the 20th century, a major milestone for the ethical care and use of animals in education and research was the emergence of the 3Rs principle. In 1955, the concept was first proposed in an article by William Russell. Two years later, it was presented for the first time at the Symposium on Humane Technique in the Laboratory in London. Finally, in 1959, William Russell and Rex Burch published the book

The Principles of Humane Experimental Technique, in which they established the 3Rs of research involving animals: Replacement, Reduction, and Refinement.¹

From that point on, educational and research activities involving animals took new directions. New principles emerged as extensions of the 3Rs, such as the 3Ss, 3Vs, 6Ps, 10Fs, and 10Rs.³ The principle proposed by Russell and Burch served as a guiding framework for the development of regulations in several countries around the world, including those within the European Community and Brazil.⁴

Brazil is located in South America and occupies a prominent position in the regional landscape, exercising political leadership both within the Southern Common Market (Mercosur) and across the American continent. The country acts as a mediator of consensus and a promoter of regional integration, seeking to balance economic, social, and political interests among the member states of the bloc. As observed by Penha et al.,⁴ in their comparative study on the regulation of animal use in educational and research activities across American nations, Brazil stands out on the continent for possessing one of the most comprehensive legal frameworks in this area.

The European Union (EU), in turn, constitutes one of the most advanced and influential models of political and economic integration in the world, exercising significant leadership both within Europe and on the global stage. It has consolidated itself as a model of supranational governance, promoting political stability, development, and integration among its Member States.⁴ The bloc also stands out as a diplomatic and normative power, including in the regulation of animal use in education and research activities. This study aims to conduct a comparative analysis of the laws regulating educational and research activities involving animals in the European Union and Brazil, which are considered among the most comprehensive in the world.

Material and methods

This study employed a comparative qualitative analysis of the regulatory frameworks governing the use of animals in teaching and research within the European Union and Brazil. The analysis was conducted through a chronological evaluation and examination of key aspects with potential implications for animal welfare and research ethics. Official government websites were consulted in the official language of each respective territory.⁴

The assessment of comprehensiveness was based on predefined qualitative analytical criteria systematically applied to both regulatory systems. The evaluation considered the normative scope, structural depth, and functional mechanisms established within each framework. Specifically, the following dimensions were analyzed: the scope of application of the laws and the categories of animals covered; the possibility of animal reuse; the existing regulatory and oversight bodies; ethics committees on the use of animals; requirements related to personnel involved in activities involving animal use; the training of personnel engaged in such activities; the manner in which projects involving the use of animals are regulated and evaluated; reporting requirements on animal use; methods of euthanasia; the incorporation of the 3Rs principles; and the presence of provisions concerning facility standards, housing conditions, and animal care.⁴

The comparisons were structured through a direct analytical approach, whereby the relevant legal instruments were systematically examined side by side for each topic addressed in the study. This method enabled the identification of similarities and differences between the two regulatory systems, which were subsequently analyzed and discussed.⁴

A complementary literature search was also performed across major scientific databases, including Science Direct (Elsevier), Web of Science, and Google Scholar. The search strategy combined the keywords “animal,” “experimentation,” “teaching,” “regulation,” “law,” “legislation,” “Europe,” “European Union,” and “Brazil,” using the Boolean operators “AND” and “OR” in English, Spanish, and Portuguese. Only articles published within the past 20 years were included, considering that Brazil’s primary legislation on this matter was enacted in 2008 and the most important recent revision of the European Directive occurred in 2010.⁴ Data collection and analysis were carried out between January and August 2025.

Results and discussion

This study is based exclusively on documentary and normative analysis of the primary legal instruments and complementary regulatory documents governing the use of animals in teaching and research in Brazil and the European Union. As such, it does not encompass empirical assessment of how these regulations are implemented in practice at the institutional level, nor does it evaluate enforcement effectiveness, compliance rates, or practical challenges faced by oversight bodies and ethics committees. Additionally, although official documents and guidance materials were consulted in their respective official languages, variations in interpretation and national transposition of supranational provisions - particularly within European Union Member States - may result in differences not fully captured in this analysis. Therefore, the conclusions presented reflect the formal legal and regulatory structures in force during the study period and should be interpreted within the inherent limitations of a qualitative documentary approach.

Legal provisions

The guidelines concerning the use of animals in educational and research activities within the European Union are established in Directive 2010/63/EU of the European Parliament and of the Council, published on September 22, 2010, and updated by Directive 2019/1010/EU in 2019. This directive outlines the measures for the protection of animals used for scientific or educational purposes, establishing rules regarding the application of the 3Rs principle in the use and breeding of animals, as well as the evaluation and authorization of projects involving their use. The current regulation in the European Community replaced Directive 86/609/EEC, which was in force from 1986 to 2010. Directive 2010/63/EU comprises 66 articles and 8 annexes, and its preamble contains 56 significant considerations, some of which are also discussed in this study.⁵⁻⁸ This study also addresses the guidance documents published by the European Commission, which aim to provide detailed clarification and support for the implementation of the Directive’s provisions by the Member States.

Another noteworthy document in Europe, published in 1986 by the Council of Europe through the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, is the European Treaty 123, commonly known as ETS 123. Although it does not carry the force of law, this document provided guidance for Member States that voluntarily chose to adhere to its provisions. It was the first document produced in Europe that sought to establish parameters aimed at the care, welfare, and ethical use of laboratory animals, serving as a model for the Directives that would later be published.^{9,10}

It is important to note that, according to Article 2 of the European Directive, Member States may, at their discretion, transpose the provisions of the Directive into national regulations, including the option to adopt stricter measures, and tailor the information contained therein according to their local characteristics. Twenty-six Member States have published their own regulations, such as Royal Decree No. 53 of 2013 in Spain, Decree-Law No. 113 of 2013 in Portugal, and Legislative Decree No. 26 of 2014 in Italy.¹¹⁻¹³

In Brazil, the guidelines are established by Law No. 11.794 of October 8, 2008, also known as the Arouca Law, which set forth measures for the breeding and use of animals in educational and scientific research activities throughout the national territory. Additionally, there is Decree No. 6.899 of July 15, 2009, which regulates Arouca Law. The Law comprises 27 articles, while the Decree contains 61. Furthermore, Brazil also has Normative Resolutions and Technical Guidelines, which carry the force of law and provide detailed instructions on the provisions contained in the aforementioned legal instruments; these are also addressed in this study.^{4,14,15}

Scope of application of the Laws: According to Article 5 of the European Directive, procedures may only be carried out with the objective of fundamental and/or translational or applied research, aimed at the prevention, prophylaxis, diagnosis, or treatment of diseases, health problems, or other abnormal conditions, or their effects on humans, animals, or plants; the assessment, detection, regulation, or alteration of physiological conditions in humans, animals, or plants; or the welfare of animals and the improvement of production conditions for animals raised for agricultural purposes. Procedures may also be conducted for the development, quality control, efficacy, and safety of medicines, foodstuffs, animal feed, and other substances or products; the protection of the natural environment in the interest

of human or animal health or welfare; research aimed at species conservation; higher education or training for acquiring, maintaining, or improving professional qualifications; and investigations in the field of forensic medicine.⁵ Marinou and Dontas⁸ highlighted in their study the need for a clearer definition regarding the use of animals for educational purposes and skills training within the European Community, particularly taking into account the 3Rs.⁸

In Brazil, paragraph 1 of Article 1 of Law No. 11.794 stipulates that the use of animals for educational purposes is limited to institutions of higher education and technical-level vocational education establishments in the biomedical field. Paragraph 2 further defines scientific research activities involving animals as all those related to basic science, applied science, technological development, and the production and quality control of drugs, medicines, food, immunobiologicals, instruments, or any other items tested on animals. The regulation does not consider zootechnical practices related to agriculture as research activities, as specified in paragraph 3.¹⁴ When comparing the information contained in the two regulations, it becomes apparent that European legislation provides greater detail. However, when analyzed in general terms, both share similar scopes of application.

Animals included within the scope of the Laws: Directive 2010/63/EU applies to live non-human vertebrate animals, including independently feeding larval forms and fetal forms of mammals from the last third of their normal development, as well as live cephalopods. In contrast, Brazilian legislation is more general but restricted to animals belonging to the phylum Chordata, subphylum Vertebrata, excluding humans. Brazilian law makes no reference to invertebrate animals.^{5,14,15}

The issue of invertebrate sentience has long been a subject of debate within the global scientific community, acting as a key reference point for shaping ethical standards and enhancing animal welfare policies. Research suggests that some invertebrate groups - particularly cephalopods and certain arthropods - demonstrate sophisticated behaviors, learning capacities, and reactions linked to pain perception, which challenge traditional assumptions about their cognitive and sensory abilities. Acknowledging this sentience is an essential step toward fostering more humane treatment practices and supports the creation of regulations that guarantee the ethical execution of scientific research involving invertebrates.¹⁶⁻¹⁸ Current legislation in the Member States of the European Community includes cephalopods, reflecting recognition of their sentience in both educational and research contexts, as stated in Recital 8 of the Directive's preamble. Conversely, Brazilian law has yet to advance to the point of incorporating invertebrate species within its legal framework.

The European Directive also addresses, in Chapter II, certain provisions regarding the use of endangered species (Article 7), non-human primates (Article 8), animals captured from the wild (Article 9), and stray or feral animals of domestic species (Article 11).⁵ Some of these provisions are not addressed in Brazilian legislation.

Reuse of animals

The European Directive, in Article 16, provides for the reuse of animals and the conditions under which it may occur. This provision is also referenced in Recital 25 of its preamble. According to the regulation, an animal that has already been used in one or more procedures may only be reused in a new procedure if another animal that has not previously been subjected to any procedure could equally be used, and the following conditions are met: the actual severity of

the previous procedures was classified as mild or moderate; it has been demonstrated that the animal's overall health and welfare have fully recovered; the new procedure is classified as mild, moderate, or non-recovery; and the new procedure complies with veterinary advice, taking into account the animal's entire life. The regulation further allows that, under exceptional circumstances, the competent authority may authorize the reuse of an animal that has undergone a procedure classified as severe, provided it has not been used more than once in a procedure causing severe pain, distress, or equivalent suffering.⁵

The reuse of animals is prohibited under Brazilian legislation. According to paragraph 8 of Article 14 of the Arouca Law, "the reuse of the same animal after achieving the main objective of the research project is forbidden". However, in 2024, Normative Resolution No. 69 was published in Brazil, providing guidance on the relocation of animals in educational or scientific research activities. The resolution allows for sequential use of animals, defined as procedures involving the same animal carried out at different times within a teaching or research activity proposal, necessary to achieve its primary objective; and the relocation of animals, defined as the inclusion of an animal that has previously participated in a teaching or research activity into a new proposal. It is important to note that prior authorization from the Ethics Committee on the Use of Animals (CEUA) is required, along with compliance with specific conditions: the animal's overall health must have been fully restored and certified by a veterinarian; the animal's welfare must have been fully restored; the invasiveness level of the previous procedure must have been classified as "mild" or "moderate"; the invasiveness level of the procedure in the new activity must be classified as "mild," "moderate," or "terminal procedure"; and clinical records, medical files, other documents related to the animal's history, reports from previous studies in which the animal participated, and the protocol of the study in which the animal will be relocated must be included in the process evaluated by CEUA.^{14,19}

The reuse of animals provided for in European regulations, and the sequential use and relocation established in Brazilian legislation, are directly related to the application of the Reduction principle. It is important to note, however, that both regulations emphasize that the welfare of the animals involved must be ensured, avoiding stress, pain, and/or distress, thereby also relating to the Refinement principle.

The European Directive also provides for the rehoming or release of animals, where permitted by Member States. Article 29 stipulates that breeding and/or use facilities whose animals are intended for rehoming must maintain a regime that ensures the socialization of the rehomed animals. In the case of wild animals, whenever appropriate, a rehabilitation program must be in place before the animals are returned to their natural habitat.⁵ In Brazil, Law No. 11.794 establishes that, exceptionally, when animals used in experiments or demonstrations are not subjected to euthanasia, they may leave the animal facility after the procedure, following consultation with the relevant CEUA regarding applicable safety criteria, provided they are placed with responsible individuals or legally recognized animal protection organizations willing to assume responsibility for them.¹⁴

As the degree of harm experienced by animals is a key factor in evaluating their potential reuse, sequential use, or relocation, both the European Directive and Brazilian legislation establish severity (non-recovery, mild, moderate, and severe) and invasiveness (terminal procedures, mild, moderate, and severe) classifications that must be considered in decision-making, including project evaluations and other relevant circumstances. Table 1 presents the severity classifications outlined in Article 15 and Annex VIII of the European

Directive, alongside invasiveness levels according to Normative Resolution No. 55, currently in force in Brazil. It is noteworthy that Brazilian regulations not only provide descriptions for each level of invasiveness but also include examples of procedures corresponding to each classification.^{5,20}

Table 1 Levels of severity and invasiveness present in the legislation of the European Union and Brazil

Severity ⁵	Description	Invasiveness ²⁰	Description
Non-recovery	Procedures performed entirely under general anesthesia from which the animal does not regain consciousness.	Terminal Procedures	Procedures performed entirely under general anesthesia, from which the animal will not regain consciousness and will be subjected to euthanasia.
Mild	Procedures performed on animals that are likely to cause mild pain, suffering, or distress of short duration, as well as procedures that do not cause significant harm to the animals' welfare or overall condition.	Mild (G1)	Procedures that cause short-term pain, suffering, or distress and do not significantly impair the animals' overall welfare.
Moderate	Procedures performed on animals that are likely to cause moderate pain, suffering, or distress of short duration, or mild pain, suffering, or distress of long duration, as well as procedures likely to cause moderate harm to the animals' welfare or overall condition.	Moderate (G2)	Procedures that cause moderate pain, suffering, or distress of short duration, or mild pain, suffering, or distress of long duration, as well as procedures that may moderately affect the animals' overall welfare.
Severe	Procedures performed on animals that are likely to cause severe pain, suffering, or distress, or moderate pain, suffering, or distress of long duration, as well as procedures likely to cause severe harm to the animals' welfare or overall condition.	Severe (G3 and G4)	Procedures that cause severe pain, suffering, or distress to animals, or moderate pain, suffering, or distress of long duration, as well as procedures that cause serious harm to the animals' overall welfare.

Regulatory and oversight bodies

It is established in Article 26 of the European Union Directive that Member States must set up Animal Welfare Bodies (AWBs) in establishments where animals are bred and/or used. Among the functions of these bodies, as described in Article 27, are: providing advice to staff members involved in matters related to animal welfare concerning acquisition, housing, care, and use; advising on the application of the 3Rs principles and on technical and scientific developments related to them; establishing and reviewing internal operational processes concerning monitoring, information sharing, and follow-up in matters of animal welfare; monitoring project progress with a focus on the application of the 3Rs principle; and advising on rehoming programs.⁵ It is worth noting that, since the AWB is established within the animal facility, the diversity of its members will depend on their availability within the establishment, a fact also observed by Marinou and Dontas in their study.⁸ As an example, in Spain, the responsible bodies are referred to Órgano de Experimentación y Bienestar Animal (OEBA). In the country, at facilities where animals are used for educational and/or research activities, the OEBA serves as the ethics committee, similar to the system in Brazil.¹¹

Article 26 also outlines the minimum composition of AWBs, which must include the person(s) responsible for animal welfare and care and, in facilities where experimental procedures are conducted, a scientific member, with input from the designated veterinarian. Article 25 provides information regarding the designated veterinarian, who must be specialized in laboratory animal medicine.⁵ Drawing a parallel with Brazilian ethics committees, the legislation stipulates that CEUAs must be composed of veterinarians, biologists, faculty members, and representatives of legally established animal protection organizations within the country. In educational institutions, this includes faculty members, whereas in research institutions, faculty members are replaced by researchers. Institutions engaged in both teaching and research must include both a faculty member and a researcher.^{14,21}

In 2014, the European Commission published the guide A Working Document on Animal Welfare Bodies and National Committees to Fulfil the Requirements under the Directive to assist Member States in meeting the requirements set out in Articles 26 and 27 of the Directive regarding AWBs. The document emphasizes the importance of these bodies, highlighting benefits for the animals, for science, and for the staff involved in the activities. Furthermore, the document also provides information on National Committees, which will be discussed.²²

Article 49 of the Directive stipulates that each Member State must establish a National Committee for the protection of animals used for scientific purposes. These committees are responsible for advising the competent authorities and AWBs on matters related to the acquisition, breeding, housing, care, and use of animals in procedures, and for ensuring the sharing of best practices. In Brazil, this role is performed by the National Council for the Control of Animal Experimentation (CONCEA), and Brazilian legislation mandates the creation of institutional committees, which differs from the national committee structure established under European regulations.^{5,14}

In Brazil, Law No. 11.794, in its Article 4, established the CONCEA as the main body responsible for overseeing the use of animals in the country. CONCEA is tasked with formulating and ensuring compliance with regulations regarding the humane use of animals for educational and scientific research purposes, accrediting institutions for the breeding or use of animals in education and research, and monitoring and evaluating the introduction of alternative techniques that replace the use of animals in these activities. It is also responsible for establishing and periodically reviewing standards for the use and care of animals in education and research in accordance with international conventions to which Brazil is a signatory, as well as technical standards for the installation and operation of breeding centers, animal facilities, and laboratories for animal experimentation, including working conditions within such facilities. Additionally, CONCEA establishes and periodically reviews standards for accrediting institutions that breed or use animals for teaching and

research, maintains an up-to-date registry of procedures conducted or in progress and of researchers based on information provided by CEUAs, reviews and decides on appeals against CEUA decisions, and advises the Executive Branch regarding educational and research activities covered by the Law. CONCEA is linked to the Ministry of Science and Technology.¹⁴

Ethics committees on the use of animals

The European Union Directive does not explicitly require the establishment of ethics committees within the text of the law, as is observed in regulations from various countries, including Brazil. However, by referring to AWBs, it can be inferred, based on the functions described in the Directive, that these bodies perform activities analogous to those carried out by ethics committees.⁵ Indeed, the transposition of the Directive into national laws of Member States may provide for such committees, as occurs in Royal Decree No. 53, published in 2013 by Spain, which in Article 39 states: “In the case of users, the ethics committee on animal experimentation (...)”.¹¹ Pietrzykowski mentions in his study that the Directive encourages its Member States to establish bodies, such as Animal Ethics Committees (AECs), to conduct independent evaluations of research projects involving animals. The study also notes, as described by the author, that the majority of Member States have a local or regional committee responsible for assessing projects.²³

In Brazil, Law No. 11.794 establishes in Article 8 that the prior constitution of a CEUA is an indispensable condition for the accreditation of institutions conducting educational or research activities involving animals. The law also defines the minimum composition of these committees, as previously mentioned. Article 10 of this law outlines all the responsibilities of the committees, including: ensuring compliance with the provisions of the Law and other applicable regulations regarding the use of animals in education and research, particularly CONCEA resolutions; conducting prior review of educational and research procedures to be carried out at the institution to which the committee is linked, to determine their compliance with applicable legislation, including ethical use and animal welfare considerations; maintaining an up-to-date registry of procedures performed or in progress at the institution and submitting reports to CONCEA; maintaining a registry of researchers conducting educational and research procedures and sending this information to CONCEA; issuing certificates required by research funding agencies, scientific journals, or other entities; and immediately notifying CONCEA and health authorities of any accidents involving animals at accredited institutions, providing the necessary information to enable corrective action.¹⁴

There are also two specific regulations governing the activities of CEUAs. One of these is Normative Resolution No. 51, dated May 19, 2021, which establishes provisions for the establishment and operation of Ethics Committees on the Use of Animals and of animal facilities,²¹ and Normative Resolution No. 52, also dated May 19, 2021, which establishes the unified forms for requesting authorization to use animals in education or scientific research, as well as the procedures for authorization and certification by the Ethics Committees on the Use of Animals.²⁴ Normative Resolution No. 51, in addition to reiterating the provisions regarding CEUAs established in Law No. 11.794, outlines the responsibilities of institutions concerning the operation of the committees and provides greater detail on their functioning. Among the committee’s competencies, not specified in Law No. 11.794, are the evaluation of the qualifications and experience of personnel involved in the production, maintenance, or use of animals in educational or scientific re-search activities to ensure their proper

use; the promotion of the adoption of the principles of Refinement, Reduction, and Replacement in the use of animals in teaching and re-search, with a focus on ethical use and animal welfare; and the establishment of preventive programs and the conduct of inspections to ensure the proper operation and adequacy of facilities under their responsibility, in accordance with the standards and regulations defined by CONCEA.²¹

Regarding inspections, Brazilian legislation establishes that this activity is the responsibility of CEUAs. In contrast, the European Directive, in Section 2, Article 34, stipulates that competent authorities must carry out periodic inspections at all locations where laboratory animals are housed. The Directive recommends that the frequency of inspections be determined based on a risk assessment, considering factors such as the type of animal facility, the number and species housed, compliance with regulatory requirements, the number and type of projects conducted, and any information that may indicate non-compliance. The Directive mandates that at least one-third of experimental facilities be inspected annually, as well as breeding and experimental facilities involving non-human primates. Additionally, a suitable proportion of inspections must be conducted without prior notice. Brazilian legislation specifies that the frequency and timing of inspections should be determined by factors such as the number and accessibility of facilities, the quantity, type, and variety of educational or research activities, and the CEUA’s schedule. CEUAs are required to inspect animal housing areas at least once a year.^{5,20,21}

Requirements related to personnel involved in activities

The European Directive, in Article 24, stipulates that Member States must ensure that each animal facility has one or more on-site personnel responsible for supervising the welfare of the animals and the care provided to them; that staff handling the animals have access to species-specific information relevant to the facility; and that personnel possess the appropriate qualifications, are competent, receive continuous training, and are supervised until they have demonstrated the required competence. Regarding veterinarians, Article 25 of the Directive provides that each facility must have a designated veterinarian, as previously mentioned, or, if more appropriate, a suitably qualified expert, whose role is to provide advice on animal welfare and treatment.^{5,21}

Brazilian legislation, through Normative Resolution No. 51 of 2021, establishes the mandatory presence of a Coordinator and a Technical Responsible in animal facilities. The Coordinator must be a professional with proven experience in laboratory animal science, focusing on welfare, quality in production, and proper management of animals in facilities, capable of managing the facility to provide suitable conditions for the performance of scientific research and teaching activities. The Technical Responsible must be a veterinarian, in charge of medical care and animal welfare in the teaching or research activities conducted at the facility to which they are assigned by the institution. The regulation also allows other professionals with specific responsibilities to act within the limits of their legal competencies, when applicable.²¹

Training of personnel involved in activities

In Brazil, Normative Resolution No. 49, published on May 7, 2021, established the mandatory requirement for training of all individuals involved in the handling of animals used in production, maintenance, research, or educational activities. The resolution states that such training must correspond to each person’s specific responsibilities, regardless of the degree of invasiveness of the protocol, in order

In Brazil, such detailed guidance is not provided as it is in the European Union. The document produced by the European Commission allows for greater standardization of the skills that must be developed by those who handle animals throughout the territory. This is highly positive, as it supports the pursuit of ethical use and animal welfare. However, as mentioned by Marinou and Dontas,⁸ some variations can be observed among the Member States due to the flexibility allowed in the transposition of the Directive within their territories.⁸

Projects involving the use of animals

European legislation, in Section 3 of Directive 2010/63/EU, establishes the requirements related to projects involving the use of animals. According to Article 36, Member States must ensure that no project is carried out without prior authorization from the competent authority, based on a formal application submitted and duly evaluated. Article 37 specifies that each project must include, at a minimum: a detailed proposal and a non-technical summary (when necessary to protect confidential information and intellectual property); the scientific and ethical justification for the use of animals; the origin, estimated number, species, and life stages of the animals to be used; a description of the procedures to be performed; the application of the principles of the 3Rs (replacement, reduction, and refinement); the use of anesthesia, analgesics, and other measures to minimize pain; strategies to avoid, reduce, and alleviate any kind of suffering from birth to death, when applicable; the use of humane endpoints; the experimental design and statistical models employed to minimize the number of animals and the environmental impact; the reuse of animals and its cumulative effects; the proposed classification of procedure severity; measures to prevent unjustified duplication of experiments; the housing, breeding, and care conditions of the animals; the planned methods of euthanasia; and the qualifications and competence of the personnel involved in the execution of the project.⁵

The evaluation of projects, according to Article 38, involves assessing criteria related to the scientific or educational justification; whether the objectives warrant the use of animals; and whether the project allows procedures to be carried out in the most humane manner possible, while respecting the environment. During the evaluation, the objectives and expected scientific or educational benefits are considered, as well as compliance with the 3Rs, the assessment and assignment of procedure severity classification, and a harm-benefit analysis. This analysis aims to determine whether the harm caused to animals, in terms of suffering, pain, and distress, is justified by the expected outcomes, taking ethical considerations into account, and whether the results may ultimately benefit humans, animals, or the environment. Additionally, the necessity and timing of retrospective project evaluation are assessed. It should be noted that, according to the legislation, project evaluations are conducted impartially and may take into account the opinions of independent parties.⁵

It is important to mention that the guide produced by the European Commission, A Working Document on the Development of a Common Education and Training Framework to Fulfil the Requirements under the Directive, includes a module related to project evaluation (Module 25). Its purpose is to provide guidance and information for training individuals involved in project assessment, aiming to help them understand the context, principles, and evaluation criteria, develop a consistent approach to assessment, and formulate well-informed, impartial, and justified opinions.²⁶ Harmonizing the approach of evaluators is extremely important for project assessment. In Brazil, unlike in the European Union, there are no documents specifying in detail how such evaluations should be conducted, only general

information, as will be described later in this section. Jankoski and Fischer,²⁷ in their study, identified challenges in the Brazilian context, such as the lack of regular training for members of ethics committees responsible for evaluating teaching and research projects.²⁷

Continuing the analysis of the European Directive, project authorization, granted for a maximum period of five years, must specify, according to Article 40, the researcher or instructor responsible for carrying out the activities; the individuals responsible for overall execution and compliance with the project authorization; the facilities where the activities will be conducted, if applicable; and any specific conditions following the project evaluation, including whether and when the project should undergo retrospective assessment. Article 41 establishes a timeframe of up to 40 working days for the decision on project evaluation, with the possibility of a 15-working-day extension if justified. Another important aspect, set forth in Article 42, is the possibility of submitting simplified projects, classified with severity levels of “non-recovery,” “mild,” or “moderate,” and that do not involve non-human primates. In cases requiring modification or renewal, according to Article 44, a favorable opinion from a new evaluation is necessary. Projects are subject to revocation of authorization if not conducted in accordance with the granted approval. All documentation related to the authorization process must be retained for at least three years from its conclusion.⁵

In Brazil, according to Law No. 11.794, teaching and research projects involving animals must be reviewed in advance by the institution’s CEUA to determine their compliance with the applicable legislation.¹⁴ The information provided in the proposals must be sufficient to demonstrate to the CEUA that the intended use of animals is consistently justified. To this end, the ethical, educational, and scientific merits must be clearly articulated and defined within the proposal. Essential elements in the CEUA’s evaluation include the measures to be implemented by the team regarding the 3Rs principles and the assurance of animal welfare.²¹

Normative Resolution No. 55, in item 5.3, specifies the information that must be included in proposals submitted for CEUA approval: the proposal title; the anticipated start and end dates of the activities; information regarding all collaborators involved in the activities, including their names, roles, and details of the training qualifying them to perform specific procedures involving animals; the origin of the animals, details of the facilities, and the locations where procedures will be conducted; justifications for the use of animals, assessing the ethical, educational, and scientific merits versus the potential impact on animal welfare, with specific justifications required for all procedures; the potential benefits of the proposal, including a clear description of its general context and the anticipated knowledge gains that may result in benefits for human health, animal health, or personnel training, as well as other advancements or improvements; a descriptive summary of how the proposal was planned in relation to its objectives and the use of animals; the experimental design, including the application of the 3Rs principles; how animal monitoring will be conducted, detailing how animal welfare will be assessed throughout the activities, what actions will be taken if a problem is identified, including criteria for intervention, treatment, or removal of animals from the proposal, and the names and contact details of those responsible for daily monitoring and emergency response; designation of a Veterinarian responsible for animal care and welfare actions; and specification of any risks to other animals or humans arising from the teaching or research activity.²⁰

According to item 5.4, the evaluation and approval of proposals must take place in CEUA meetings where a minimum quorum is

present at the time of the decision. Decisions regarding the approval, modification, or rejection of a proposal, or the cancellation of its approval, must be made by consensus or by a favorable vote of the relative majority of its members. Requests for extensions of deadlines or modifications to the number of animals in already authorized proposals must be submitted with scientific justification and a report on the work already carried out, without the need to resubmit all the proposal documents.²⁰

Unlike European legislation, Brazilian law does not specify a deadline for the evaluation of proposals or the issuance of decisions. Another notable difference between the regulations of the two territories is the retrospective evaluation, provided for in Article 39 of the European Directive. This evaluation assesses whether the project's objectives were achieved; the harm inflicted on the animals, including the number and species used and the severity of the procedures; and factors that may contribute to improving the application of the principles of replacement, reduction, and refinement. According to the Directive, all projects involving non-human primates and those including procedures classified as severe are subject to retrospective evaluation.⁵ In Brazil, there is no provision for such evaluations in the existing legal instruments.

Non-technical summaries of projects involving the use of animals: As previously mentioned, the European Directive, in its Article 43, requires the provision of Non-Technical Summaries (NTS) for projects involving the use of animals, subject to the protection of intellectual property and confidential information. These summaries must include information regarding the objectives of the project, the anticipated harms and benefits, and the number and types of animals to be used, as well as a demonstration of compliance with the principles of replacement, reduction, and refinement. It is important to note that the summaries are anonymized and must not include the name or address of the researcher or any other personnel involved.⁵ Brazilian legislation does not require the preparation of such summaries. It is important to highlight that this practice, as implemented in the European community, ensures greater transparency of the activities conducted within the territory, facilitates broader access to the scientific knowledge generated, and reduces the likelihood of unnecessary repetition of procedures already performed, as also noted by Marinou and Dontas.⁸

With the aim of standardizing the information contained in these summaries, in 2021 the European Commission published the guide Working Document on Non-Technical Project Summaries to assist Member States in complying with the requirements set out in Article 43 of the Directive. The document details all the information that must be included in NTSs and also provides illustrative examples.²⁸

Reports on the use of animals: Directive 2019/1010/EU updated the text of Article 54 of the Directive originally published in 2010, which addresses the reporting and presentation of statistical data regarding the use of animals within the European territory. According to this article, Member States are required to collect and make publicly available, on an annual basis, statistical data on the use of animals in procedures, including information on the actual severity of the procedures and the origin and species of non-human primates used in such procedures.⁶ The European Commission has been publishing this information since 1991, initially at five-year intervals. Over time, the interval between publications has been reduced, and currently the data are published annually, as stipulated by the Directive.⁸ Busquet et al.,²⁹ published a study in which they evaluated the quality of the data released by the European Union. The authors observed that, after the entire period that began in 1991 when the data first started being

published, the information could only be considered satisfactory from 2015 onwards, taking into account the requirements established by the Directive regarding the content of the published data.²⁹

In Brazilian legislation, there is no requirement to make such data publicly available. However, in 2024, CONCEA published a report on the use of animals in teaching and research from 2019 to 2023, based on the premise that the agency is responsible for monitoring the number of animals used in teaching and research across the various Brazilian institutions accredited for such purposes. The objective, as stated in the document, is to provide a transparent and understandable overview of animal use in scientific activities in the country. It is worth noting that CEUAs are required to annually submit reports to CONCEA with data on the projects conducted at teaching and research institutions in Brazil.³⁰

Methods of euthanasia

Euthanasia is a procedure aimed at minimizing suffering and ensuring a humane death when necessary. Its application must strictly adhere to current national and/or internationally recognized guidelines, which establish technical and ethical criteria for its execution. The chosen method should be as rapid, painless, and effective as possible, taking into account the species, age, and condition of the animal, and must be performed by qualified professionals. Brazilian legislation provides information regarding the practice of euthanasia in teaching and research activities involving animals.

Law No. 11.794, in its Article 14, Paragraph 1, stipulates that animals must be subjected to euthanasia, in strict compliance with the prescriptions relevant to each species, according to current guidelines, whenever, at the conclusion of the experiment or at any stage thereof, the procedure is technically recommended, or when the animal experiences intense suffering.¹⁴ Normative Resolution No. 37 of 2018, issued by CONCEA, establishes the guidelines for its execution, detailing the criteria to be adopted, the necessary conditions from the perspective of both the operator and the animal, and the confirmation of the animal's death. It addresses all methods and their characteristics, including those that are recommended, conditionally accepted, or unacceptable for each taxonomic group.³¹

Normative Resolution No. 55 of 2022 stipulates that procedures must be performed by trained personnel, following evaluation and authorization by the CEUA, in accordance with the relevant technical guidelines. Euthanasia should be carried out in a quiet environment, away from other animals, and death must be confirmed before the carcass is disposed of. Whenever possible, tissues and structures from the carcass should be shared among instructors and researchers, thereby aligning with the principle of Reduction in the use of animals.²⁰ This information is also included in the European Directive, which states that, in order to promote the principle of reduction, Member States shall, whenever appropriate, facilitate the development of programs for the sharing of organs and tissues from euthanized animals.⁵ Brazilian regulations also stipulate that neonates dependent on an animal that has died must be provided with appropriate care or subjected to euthanasia. The method chosen should always be the most suitable for the animal's developmental stage, and it is recommended that the cause of death be investigated whenever an animal dies unexpectedly or euthanasia is performed due to unforeseen complications.²⁰

The European Directive provides, in its Article 6 and Annex IV, information regarding the practice of euthanasia. According to the Directive, Member States must ensure that animals are euthanized with minimal pain, suffering, and distress by a competent person,

whether in animal facilities or in the field. Annex IV of the Directive outlines appropriate methods for each taxonomic group, although with less detail compared to the Brazilian normative resolution. Competent authorities may grant exceptions to the procedures described in the annex, provided that, based on scientific evidence, the method to be used is considered at least equally humane; or when, based on a scientific justification, the objective of the procedure cannot be achieved using the method indicated in the annex. The Directive also stipulates that, if animals need to be euthanized under emergency circumstances for reasons of animal welfare, public health, public safety, and animal health, or environmental protection, exceptions to the provisions of the Directive may occur.⁵

Presence of the 3Rs

The principle of the 3Rs, proposed by Russell and Burch in 1959, has guided the development of various legal instruments in several countries worldwide, including the European and Brazilian regulations.¹⁴ Replacement, reduction, and refinement can be explicitly observed in several sections of the legislation, as will be described below.

The European Directive makes reference to the 3Rs throughout almost its entirety. They can already be perceived in the preamble of the law, which, in its considerations, provides important information—particularly regarding the principle of Replacement. Item 10 states that the Directive represents an important step toward achieving the objective of fully replacing the use of live animals in scientific and educational procedures as soon as it is scientifically feasible to do so. Furthermore, Item 11 specifies that the principles must be applied while strictly observing the hierarchy of obligations concerning the use of alternative methods. When no alternative method has been recognized under Union legislation, the number of animals used may be reduced by employing other methods and applying testing strategies, such as *in vitro* assays or other approaches capable of reducing and refining the use of animals. These principles can also be observed in several of the 56 considerations contained in the preamble of the Directive.⁵

In Article 4, the Directive establishes that Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy not involving the use of live animals is employed in place of a procedure (Replacement); that the number of animals used is reduced to a minimum without compromising the objectives of the project (Reduction); and that animal breeding, housing, and care, as well as the methods used in procedures, are refined in order to eliminate or minimize any potential pain, suffering, distress, or lasting harm inflicted on the animals (Refinement). Article 13, which addresses the choice of methods to be used, states that Member States shall ensure that procedures are not carried out if another recognized method or testing strategy is available to achieve the desired results without the use of live animals (Replacement). Furthermore, the choice of method must take into account the use of the smallest possible number of animals (Reduction); the involvement of animals with the lowest capacity to experience pain, suffering, distress, or lasting harm (partial Replacement); and the causing of the least possible pain, suffering, distress, or lasting harm (Refinement), while offering the greatest likelihood of producing satisfactory results. The article also includes information on humane endpoints, referring to Refinement, which is likewise mentioned in Article 14, highlighting the use of anesthesia in procedures involving the use of animals.⁵

As previously mentioned, the 3Rs are also embedded in the operational guidelines of bodies responsible for animal welfare, in the requirements for teaching and research projects, and in Chapter

V of the Directive, which addresses the prevention of duplication of procedures and alternative approaches. The prevention of duplication of procedures, referred to in Article 46, relates to the principle of Reduction, while the discussion of alternative approaches in Article 47 refers to the 3Rs more broadly. This article states that Member States shall contribute to the development and validation of alternative approaches capable of providing the same or a higher level of information than that obtained through procedures involving animals, but which do not use animals, use fewer animals, or involve less painful procedures. Member States shall also take all appropriate measures to encourage research into such alternatives.⁵

Regarding Brazilian legislation, in addition to the presence of the 3Rs in Law No. 11.794, they can also be observed in several Normative Resolutions issued by CONCEA. In Chapter IV of the aforementioned law, which addresses the conditions for the breeding and use of animals for educational and scientific research purposes, Article 14, Paragraph 3, establishes that, whenever possible, teaching practices should be photographed, filmed, or recorded in order to allow their reproduction for future demonstrations, thereby avoiding the unnecessary repetition of didactic procedures involving animals - a reference to the principle of Replacement. Paragraph 4 specifies that the number of animals used in a project and the duration of each experiment must be the minimum necessary to produce conclusive results, sparing the animals from suffering as much as possible - a reference to the principle of Reduction. Finally, Paragraph 5 refers to the principle of Refinement, stating that experiments likely to cause pain or distress must be carried out under appropriate sedation, analgesia, or anesthesia.¹⁴

Normative Resolution No. 55, which establishes the Brazilian Guideline for the Care and Use of Animals in Teaching and Scientific Research Activities, refers to the 3Rs throughout nearly its entire content.³¹ At the outset, the guideline emphasizes the responsibilities of all animal users, including the promotion, development, and use of alternative methods that replace or reduce the use of animals in teaching and scientific research activities (Replacement); the minimization of the number of animals used in such activities without compromising the quality of the results to be obtained (Reduction); and the refinement of methods and procedures to prevent pain, suffering, and stress in animals used in teaching or research. In the section “General Guidelines”, item 3 includes specific sub items for each of the principles, providing detailed descriptions. In other sections, such as “Responsibilities of Animal Ethics Committees,” “Responsibilities of Instructors and Researchers,” “Conduct During Project Implementation,” “Acquisition and Care of Animals in Breeding or Maintenance Facilities,” and “Use of Animals in Teaching Activities,” the 3Rs can also be clearly observed.²⁰

In Brazil, there are normative resolutions that officially recognize alternative methods for use in the country. Normative Resolution No. 54 of January 10, 2022, establishes guidelines for the recognition of validated alternative methods aimed at the Replacement, Reduction, or Refinement of the use of animals in teaching and research activities. Table 2 presents the normative resolutions and the number of methods recognized under each.³²

In addition, the principle of the 3Rs can be identified in Normative Resolution No. 32 of September 6, 2016, which establishes the guidelines for integrity and good practices in the production, maintenance, and use of animals in teaching and scientific research activities;³⁷ in Normative Resolution No. 37 of 2018, which provides detailed guidance on the practice of euthanasia;³¹ in Normative Resolutions No. 51 and 52, both of 2021, which relate to the operation

of Animal Ethics Committees and the use of standardized forms for the submission of teaching and research project proposals;^{21,24} in Normative Resolution No. 53 of May 19, 2021, which establishes restrictions on the use of animals in teaching, complementing the Brazilian Guideline for the Care and Use of Animals in Teaching and Scientific Research Activities;³⁸ and Normative Resolution No. 58 of February 24, 2023, which prohibits the use of vertebrate animals, except humans, in scientific research, development, and quality control of personal hygiene products, cosmetics, and perfumes that contain ingredients or compounds whose safety and efficacy have already been scientifically established, among other provisions.³⁹

Table 2 Normative resolutions of the National council for the control of animal experimentation that recognize alternative methods in Brazil, and the number of methods recognized under each resolution

Normatives	Number of recognized alternative methods
Normative Resolution No. 56 of October 5, 2022 ³³	15
Normative Resolution No. 45 of October 22, 2019 ³⁴	1
Normative Resolution No. 31 of August 18, 2016 ³⁵	7
Normative Resolution No. 18 of September 24 2014 ³⁶	17

Information on requirements for facilities, housing and care of animals

The European Directive sets forth, in Article 22, requirements related to facilities and equipment, stating that Member States shall ensure that all breeding and experimental establishments are equipped with facilities and equipment appropriate for the species of animals housed therein and, where procedures are conducted, suitable for the performance of those procedures. Articles 23, 24, and 25, as previously mentioned, stipulate that each facility must have sufficient personnel to carry out its activities, and that such personnel must possess adequate training and qualifications for their tasks. Regarding animal care, Article 33 establishes that Member States shall ensure that all animals have appropriate housing, environment, food, water, and care necessary for their health and well-being. Any limitations on an animal's ability to satisfy its physiological and ethological needs must be minimized, and the environmental parameters under which animals are kept must be monitored daily. Furthermore, measures must be taken to ensure that any anomalies or avoidable pain, suffering, distress, or lasting harm are eliminated as quickly as possible, and that the transportation of animals is conducted under suitable conditions.⁵

Annex III of the Directive establishes requirements related to establishments and the provision of care and housing for animals, including facilities, the environment in which animals are kept and its monitoring, and animal care.⁴⁰ There are species-specific sections covering rodents (mice, rats, gerbils, hamsters, and guinea pigs); rabbits; dogs; cats; ferrets; non-human primates (marmosets and tamarins, squirrel monkeys, genus *Macaca*, vervet monkeys, and baboons); farm animals (cattle, sheep and goats, pigs and minipigs, and equines); birds (domestic chickens, domestic turkeys, quails, ducks and geese, pigeons, and zebra finches); amphibians (aquatic urodeles, aquatic anurans, semi-aquatic anurans, semi-terrestrial anurans, and arboreal anurans); reptiles (aquatic chelonians and terrestrial snakes); and fish.⁵

Brazilian legislation provides such information in various normative resolutions. Normative Resolution No. 55 of 2022 addresses conduct

during the implementation of teaching and research projects (item 7) and the acquisition and care of animals in breeding or maintenance facilities (item 8).²⁰ There are specific normative resolutions for each taxonomic group, which establish the conditions that must be observed for the breeding, maintenance, and experimentation of animals kept in teaching or research facilities. The resolutions include mandatory items regarding facility infrastructure and procedures to be conducted with the animals, as well as recommended items. Table 3 presents the normative resolutions and the corresponding taxonomic groups covered by each.

Table 3 Normative resolutions of the National council for the control of animal experimentation that establish the conditions to be observed for the breeding, maintenance, and experimentation of animals kept in teaching or research facilities, along with their respective taxonomic groups

Normative	Taxonomic group
Normative Resolution No. 57 of December 6, 2022 ⁴⁰	Rodents and lagomorphs
Normative Resolution No. 59 of May 2nd, 2023 ⁴¹	Dogs and cats
Normative Resolution No. 60 of May 2nd, 2023 ⁴²	Non-human primates
Normative Resolution No. 61 of May 2nd, 2023 ⁴³	Fishes
Normative Resolution No. 62 of May 2nd, 2023 ⁴⁴	Amphibians and snakes
Normative Resolution No. 63 of May 2nd, 2023 ⁴⁵	Small ruminants
Normative Resolution No. 64 of May 2nd, 2023 ⁴⁶	Large ruminants
Normative Resolution No. 65 of May 2nd, 2023 ⁴⁷	Equines
Normative Resolution No. 66 of May 2nd, 2023 ⁴⁸	Swines
Normative Resolution No. 67 of May 2nd, 2023 ⁴⁹	Birds

In Brazil, there is also the Brazilian Guide for the Production, Maintenance, or Use of Animals for Teaching or Scientific Research Activities, published in 2023, which aims to serve as guidance and reference for facilities using the species covered by Law No. 11.794. The guide generally addresses, in each chapter, topics such as animal care and management and the maintenance of their facilities, in accordance with the mandatory and recommended criteria for each taxonomic group as outlined in the previously mentioned normative resolutions.⁵⁰

Conclusion

A comparative analysis of Brazilian and European Union legislation demonstrates significant advances in both regions regarding the ethical and legal regulation of the use of animals in teaching and research. European Directive 2010/63/EU establishes a comprehensive and detailed framework that serves as a reference for numerous countries worldwide, while Brazilian legislation, primarily through Law No. 11.794/2008 and its complementary normative resolutions, represents a robust and continually evolving system, adapted to national scientific and institutional contexts. However, some discrepancies remain evident. The European Directive includes invertebrate species, such as cephalopods, recognizing their sentience, whereas the Brazilian framework is still limited to vertebrates. Moreover, European regulations require non-technical summaries and public access to data on animal use, promoting transparency and societal engagement-elements not yet fully incorporated into the Brazilian context. Both systems converge in the application of the 3Rs principles, which form the ethical foundation of animal experimentation. In the legal documents analyzed, these principles were observed almost in their entirety, demonstrating the commitment of Brazil and all European Union Member States to the pursuit of ethical science grounded in animal welfare.

Continuous improvement in the training of personnel involved in these activities, as well as in the evaluation and monitoring of projects

involving animal use, remains essential. Strengthening training programs and developing alternative methodologies are crucial steps to consolidate humane and scientifically robust practices in both regions. Collaborative initiatives between Brazil and the European Union could foster knowledge ex-change and greater alignment of ethical standards. Leveraging the experience of the European bloc in eliminating disparities among the regulatory, legislative, and administrative provisions of Member States concerning the protection of animals used for experimental and other scientific purposes - aimed at its application in American countries, particularly within MERCOSUR, of which Brazil is a signatory - could provide a pathway for consolidating ethical practices in animal use across the Americas. In conclusion, de spite contextual differences, Brazil and the European Union share the same ethical commitment to animal welfare in scientific and educational activities. Advancing legislation, expanding transparency, and promoting international cooperation are key strategies to ensure that research involving animals continues to evolve under the highest standards of responsibility, integrity, and respect for life.

Acknowledgments

None.

Conflicts of interest

The author declares that there is no conflicts of interest.

References

1. Penha JCQ, Cruz ACM, Castro HC. Animals used in research and its protection: from the beginnings to the XXI century. *Enciclop Biosfera*. 2023;20:261–281.
2. Franco NH. Animal experiments in biomedical research: a historical perspective. *Animals*. 2013;3:238–273.
3. Penha JCQ, Cruz ACM, Castro HC. The 3Rs and their outspread: important tools for animal experimentation. *Cienc Anim*. 2023;33:138–149.
4. Penha JCQ, Cruz ACM, Castro HC. Animals in teaching and research in the Americas: analysis of legislation. *J Am Assoc Lab Anim Sci*. 2025;64:1–12.
5. European Union. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. *Off J Eur Union*. 2010;L276:33–79.
6. European Union. Regulation (EU) 2019/1010 of the European Parliament and of the Council of 5 June 2019 on the alignment of reporting obligations in the field of environmental legislation. *Off J Eur Union*. 2019;L170:115–127.
7. European Union. Council Directive 86/609/EEC of 24 November 1986 on the protection of animals used for experimental and other scientific purposes. *Off J Eur Commun*. 1986;L358:1–29.
8. Marinou KA, Dontas IA. European Union legislation for the welfare of animals used for scientific purposes: areas identified for further discussion. *Animals*. 2023;13:2367.
9. Council of Europe. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. *Eur Treaty Ser*. 1986;123.
10. Guillén J, Prins J, Howard B, et al. The European framework on research animal welfare regulations and guidelines. In: Guillén J, ed. *Laboratory Animals*. 2nd ed. London, UK: Elsevier; 2018:117–202.
11. Spain. Royal Decree 53/2013, of February 1, 2013. Establishes basic standards for the protection of animals used for experimentation and other scientific purposes, including teaching. *Boletín Oficial del Estado*. 2013;(34).
12. Portugal. Decree-Law No. 113/2013, of August 7, 2013. Transposes Directive 2010/63/EU on the protection of animals used for scientific purposes. *Diário da República*. 2013;(151):4709–4739.
13. Italy. Legislative Decree No. 26/2014, of March 4, 2014. Implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. *Gazzetta Ufficiale Italiana*. 2014;(61).
14. Brazil. Law No. 11.794, of October 8, 2008. Regulates procedures for the scientific use of animals and establishes related provisions. *Diário Oficial da União*. 2008.
15. Brazil. Decree No. 6.899, of July 15, 2009. Provides for the organization and functioning of the National Council for the Control of Animal Experimentation (CONCEA) and related provisions. *Diário Oficial da União*. 2009.
16. Mather JA, Anderson RC. Ethics and invertebrates: a cephalopod perspective. *Dis Aquat Organ*. 2007;75:119–129.
17. Drinkwater E, Robinson EJH, Hart AG. Keeping invertebrate research ethical in a landscape of shifting public opinion. *Methods Ecol Evol*. 2019;10:1265–1273.
18. Valente CS. Rethinking sentience: invertebrates as worthy of moral consideration. *J Agric Environ Ethics*. 2025;38:1–19.
19. Brazil. Normative Resolution No. 69, of October 30, 2024. Provides for the relocation of animals used in teaching or scientific research activities. *Diário Oficial da União*. 2024.
20. Brazil. Normative Resolution No. 55, of October 5, 2022. Updates the Brazilian guideline for the care and use of animals in teaching or scientific research activities (DBCA). *Diário Oficial da União*. 2022.
21. Brazil. Normative Resolution No. 51, of May 19, 2021. Provides for the establishment and operation of Animal Use Ethics Committees (CEUAs) and animal facilities. *Diário Oficial da União*. 2021.
22. European Commission. National competent authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes: a working document on animal welfare bodies and national committees.
23. Pietrzykowski T. Ethical review of animal research and the standards of procedural justice: a European perspective. *Bioeth Inq*. 2021;18:525–534.
24. Brazil. Normative Resolution No. 52, of May 19, 2021. Provides for unified forms for authorization requests for the use of animals in teaching or scientific research and for authorization and certification by Animal Use Ethics Committees (CEUAs). *Diário Oficial da União*. 2021.
25. Brazil. Normative Resolution No. 49, of May 7, 2021. Establishes mandatory training requirements for personnel involved in teaching and scientific research activities using animals. *Diário Oficial da União*. 2021.
26. European Commission. National competent authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes: a working document on the development of a common education and training framework. 2018.
27. Jankoski LGQ, Fischer ML. The role of bioethics in animal ethics committees. *Rev Bioet*. 2019;27:549–565.
28. European Commission. National competent authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes: a working document on non-technical project summaries.
29. Busquet F, Kleensang A, Rovida C, et al. New European Union statistics on laboratory animal use-what really counts. *ALTEX*. 2020;37:167–186.
30. Brazil. Report on the use of animals in teaching and research from 2019 to 2023. 2024.
31. Brazil. Normative Resolution No. 37, of February 15, 2018. Guidelines for euthanasia practice of the National Council for the Control of Animal Experimentation (CONCEA). *Diário Oficial da União*. 2018.

32. Brazil. Normative Resolution No. 54, of January 10, 2022. Recognition of alternative methods to the use of animals in teaching and scientific research activities. *Diário Oficial da União*. 2022.
33. Brazil. Normative Resolution No. 56, of October 5, 2022. Recognition of alternative methods to the use of animals in research activities in Brazil. *Diário Oficial da União*. 2022.
34. Brazil. Normative Resolution No. 45, of October 22, 2019. Recognition of alternative methods to the use of animals in research activities in Brazil. *Diário Oficial da União*. 2019.
35. Brazil. Normative Resolution No. 31, of August 18, 2016. Recognition of alternative methods to the use of animals in research activities in Brazil. *Diário Oficial da União*. 2016.
36. Brazil. Normative Resolution No. 18, of September 24, 2014. Recognition of alternative methods to the use of animals in research activities in Brazil. *Diário Oficial da União*. 2014.
37. Brazil. Normative Resolution No. 32, of September 6, 2016. Guidelines on integrity and good practices for the production, maintenance, or use of animals in teaching or scientific research activities. *Diário Oficial da União*. 2016.
38. Brazil. Normative Resolution No. 53, of May 19, 2021. Restrictions on the use of animals in teaching complementing the Brazilian guideline for the care and use of animals (DBCA). *Diário Oficial da União*. 2021.
39. Brazil. Normative Resolution No. 58, of February 24, 2023. Prohibition of the use of vertebrate animals in research and development of personal hygiene products, cosmetics, and perfumes with proven ingredients. *Diário Oficial da União*. 2023.
40. Brazil. Normative Resolution No. 57, of December 6, 2022. Conditions for breeding, maintenance, and experimentation of rodents and lagomorphs. *Diário Oficial da União*. 2022.
41. Brazil. Normative Resolution No. 59, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of dogs and cats. *Diário Oficial da União*. 2023.
42. Brazil. Normative Resolution No. 60, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of nonhuman primates. *Diário Oficial da União*. 2023.
43. Brazil. Normative Resolution No. 61, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of fish. *Diário Oficial da União*. 2023.
44. Brazil. Normative Resolution No. 62, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of amphibians and reptiles. *Diário Oficial da União*. 2023.
45. Brazil. Normative Resolution No. 63, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of small ruminants. *Diário Oficial da União*. 2023.
46. Brazil. Normative Resolution No. 64, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of large ruminants. *Diário Oficial da União*. 2023.
47. Brazil. Normative Resolution No. 65, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of equids. *Diário Oficial da União*. 2023.
48. Brazil. Normative Resolution No. 66, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of swine. *Diário Oficial da União*. 2023.
49. Brazil. Normative Resolution No. 67, of May 2, 2023. Conditions for breeding, maintenance, and experimentation of birds. *Diário Oficial da União*. 2023.
50. Brazil. Brazilian guide for the production, maintenance, or use of animals in teaching or scientific research activities. 1st ed. Brasília, Brazil: Ministry of Science, Technology and Innovation; 2023.