

A Comprehensive Review of Laboratory Animals Use in Biomedical Research: Welfare Concerns and Alternative Approaches

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Abstract Laboratory animals have been central to biosciences research worldwide since decades. Animal welfare is increasingly being recognized as a critical component for both the ethical acceptability and scientific sustainability of such practices. Welfare entails preventing abuse, ensuring appropriate housing, feeding, disease prevention, treatment, and minimizing unnecessary discomfort or pain. The guiding principles of Replacement, Reduction, and Refinement (3Rs) not only improve welfare but also enhance translational value by increasing the reliability of animal models. Researchers bear ethical and legal obligations to safeguard animal well-being, as minimizing distress also improves reproducibility of results. The capacity of animals to adapt to their environment and exercise control over their lives is essential to welfare. Accordingly, animal experiments should be undertaken only when no alternative exists, and only when potential benefits outweigh the expected harm. Globally, millions of vertebrates are used each year in research, teaching, and testing. Approximately, 70% are employed in drug development, vaccine production, cancer research, and related biological studies, while the remaining 30% serve diagnostic and educational purposes. Among them, mice and rats dominate, accounting for about 61% and 14%, respectively, with rabbits also contributing significantly, particularly in atherosclerosis studies. Together, these species represent more than 80% of all research animals within the European Union. Harmonization of global laws and regulations remains essential to balance ethical responsibility with scientific progress. By promoting the 3Rs, reducing discomfort, and aligning practices with societal expectations, research can advance while maintaining respect for the animals on which it relies.

Keywords: Biomedical research, Experimental models, Laboratory animals, Mice, Rabbit, Rat, Welfare

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1. Introduction

The Animal Welfare Act (AWA), was originally enacted in 1966 as the Laboratory Animal Welfare Act. The U.S. Department of Agriculture was authorized to oversee the regulation of laboratory animals. Amendments under the Food Security Act of 1985, implemented in 1986, further strengthened welfare standards, mandated training for personnel, enhanced enforcement, and promoted reduction of experimental duplication through better information sharing.

A wide variety of animals are used in biomedical research worldwide, including mice, rats, rabbits, guinea pigs, hamsters, monkeys, chimpanzees, cats, dogs, pigs,

birds, and others [1,2,3,4]. Out of the 144 mammalian species employed in experimentation, approximately 42% are rodents [4]. Globally, 75 to 100 million vertebrate animals are estimated to be used annually in research, education and testing, with Europe alone accounting for about 10.7 million [5]. Mice (*Mus musculus*) are most commonly used in biomedical research [2, 6]. One experimental study was conducted at the Institute of Tropical Medicine, Antwerp, Belgium to confirm the pathogenicity of environmental strains of *Cryptococcus neoformans* var. *neoformans* in murine model [6]. In addition, rats (*Rattus norvegicus*) are the frequently employed as a laboratory animal in research with studies reporting that rats and mice accounted for 85% of Medline articles and 70.5% of Lilacs publications [7]. The development of chemotherapeutic agents and vaccines to

combat the pandemic of COVID-19 was possible only due to the use of laboratory animals [8].

Although laboratory animal welfare encompasses multiple dimensions, research has predominantly focused on health aspects, often neglecting issues such as handling practices and human–animal interactions [9]. A comparable trend is observed in farm animal welfare studies, where emphasis is placed on productivity outcomes and human–animal relationships [10]. Therefore, the objective of this review is to examine key welfare concerns associated with laboratory animals, and to evaluate alternative approaches to their use in scientific research.

2. Review on the Welfare of Laboratory Animals

One of the earliest definitions of animal welfare was introduced in 1965 by the Brambell Committee, which outlined the ‘Five Freedoms’ as minimum standards for farm animal welfare. These freedoms include:

- ✓ Freedom from thirst, hunger, and malnutrition
- ✓ Freedom from discomfort
- ✓ Freedom from pain, injury, and disease
- ✓ Freedom to express normal behavior
- ✓ Freedom from fear and distress

These principles have since become foundational in animal welfare science. Welfare is regarded as the state of the individual at a given time, reflecting how well the animal is coping with its environment. It encompasses both, the ease or difficulty of coping, as well as any failure to cope, and it exists on a continuum from very good to very poor. Assessment of animal welfare can be performed scientifically [11].

Animal welfare is influenced by multiple factors, and as a result, there is no universally accepted definition of an ideal welfare state. However, the concept of animal welfare can be understood through three key perspectives [12]:

- ✓ Biological state: Welfare is considered good when the animal is healthy, and able to grow and reproduce normally.
- ✓ Affective state: This perspective emphasizes the animal’s capacity to suffer or experience positive emotions, focusing on the presence or absence of distress and pleasure.
- ✓ Natural state: This view highlights the difference between captive and wild environments, and considers welfare in terms of the animal’s ability to express natural behaviors typical of its species.

2.1. Function of Laboratory Animals

Laboratory animals serve as experimental models in scientific studies to investigate biological processes, understand disease pathways, and evaluate the safety and effectiveness of pharmaceuticals and medical procedures prior to human clinical trials [13]. Laboratory animals are used for various types of research including development of vaccines both in the developing as well as developed nations [3]. In many countries, it is mandatory to categorize the level of discomfort inflicted on animals,

generally categorized as minor (e.g., single blood sampling), moderate (e.g., recovery from anesthesia), or severe (e.g., toxicity testing). In Europe, data shows that approximately 50% of animals experience minor discomfort, 30% moderate discomfort, and 20% severe discomfort.

The distribution of animal use in research varies by purpose. Approximately 2% is allocated to cardiovascular research, 12% to cancer research, 23% to drug development, 21% to vaccine and biological testing, 1% to education, 9% to toxicity testing, and the remaining 32% to other research activities. In terms of species distribution, mice account for the largest proportion at 44%, followed by rats (33%), birds (10%), fish (7%), guinea pigs (2%), rabbits (1%), and other species (3%). Basic biomedical research frequently relies on animals to investigate the functioning of human tissues and organs, as well as the mechanisms underlying disease. Landmark medical advances, including the development of polio and diphtheria vaccines, insulin therapy for diabetes, and kidney transplantation, were all achieved with the support of animal experimentation [14].

Genetically modified (GM) animals, particularly mice and rats, are widely used in genetic and biomedical research to investigate gene function and disease mechanisms. A wide range of transgenic models has been developed, including *Drosophila* (fruit flies), frogs, fish, and mammals such as mice, rats, and livestock. These models are invaluable for advancing our understanding of gene expression, development, and the genetic basis of disease [15].

2.2. Types of Laboratory Animals

2.2.1. Laboratory Rats

Rats have been utilized predominantly in biomedical and scientific research for more than a century, playing a pivotal role in numerous advancements. They are followed in frequency of use by mice, rabbits, dogs, pigs, and primates, particularly in *in vivo* studies. Laboratory rats are domesticated derivatives of wild brown rats, with no genetic evidence of hybridization with black rats (*Rattus rattus*) [16]. Despite their extensive use, conventional housing systems often fail to accommodate their behavioral and physiological requirements [17], thereby raising concerns about animal welfare. Social stress in rats produces marked behavioral alterations within colonies and during experimental procedures. Subordinate individuals, for instance, display diminished locomotor activity, heightened anxiety in elevated plus maze tests, increased vigilance, reduced aggression, and enhanced defensive responses. Such stress also induces modifications in neurotransmitter activity and neural architecture. Furthermore, relocating rats close to parturition or with neonates can lead to development of cannibalism [18]. Consequently, minimal disturbance is recommended during late pregnancy and nursing. Conversely, routine cage cleaning may foster habituation to human handling and reduce stress [19].

2.2.2. Laboratory Mouse

Mice are naturally active and exploratory, and spend

significant time in the wild foraging for varied foods, constructing intricate nests and burrows, and maintaining complex social systems. These strong natural drives remain evident in laboratory mice, and restricting their expression can lead to frustration and poor welfare. Isolation stress, also referred to as isolation syndrome, manifests in behaviors such as aggression, convulsions, nervousness and handling difficulties [20]. Unlike laboratory rats, laboratory mice often carry genetic contributions from multiple related species and subspecies [21].

Forming stable single-sex groups requires careful selection of compatible individuals, a task particularly difficult in males. Compatibility is shaped by internal factors including age, sex, and dominance rank, as well as external influences such as space and resource distribution [22]. The role of space in welfare outcomes is complex; although male aggression often declines with higher stocking density, some studies indicate that crowding may exacerbate stress-related responses [23].

Group housing provides social buffering; wherein social interactions mitigate stress in challenging situations. Research demonstrates that socially housed mice show better postoperative recovery and require less analgesia [24]. Nevertheless, keeping mice in barren, unstimulating cages consistently promotes abnormal behaviors [25]. In addition, nest-building behavior is widely regarded as a sensitive marker of health and well-being in mice [26].

2.2.3. Laboratory Rabbits

Rabbits are the most widely used species in atherosclerosis research. Among enrichment options, food-based items, particularly hay, are most effective, as hay sustains their attention longer than non-food materials [27]. Timothy hay, in particular, not only promotes natural foraging behavior but also provides essential dietary fiber necessary for maintaining normal gastrointestinal motility. Continuous access to hay has the added benefit of reducing barbering behavior in socially housed rabbits. In addition to dietary enrichment, safe chew toys encourage natural gnawing activity. Suitable options include untreated cardboard tubes, wooden blocks, hard plastic dumbbells, Kong toys, balls, stainless steel rattles, large chains, and rings, as well as certain repurposed baby toys like oversized plastic keys [28]. Wooden blocks or sticks have been shown to significantly increase locomotion and feeding behaviors [30].

Enrichment materials must be carefully selected to avoid risks of injury or entrapment for other species. Chains should be short enough to prevent tangling, and items with sharp edges or easily destructible parts should be avoided. Adequate housing, with at least 6 square feet per rabbit, is recommended to facilitate stable social housing. Moreover, the inclusion of hiding areas and visual barriers allows rabbits to regulate contact with conspecifics, supporting their behavioral needs [30].

Beyond the laboratory, rabbits hold commercial importance as a source of lean, low-cholesterol meat, making them suitable for health-conscious consumers [31]. In Spain, for instance, a survey revealed that 20.54% of respondents consumed rabbit meat weekly, while 34.63% reported no consumption at all [32].

2.2.4. Laboratory Pigs

Swine are widely employed in research across disciplines such as cardiovascular, integumentary and behavioral studies, and are also regarded as a primary model for surgical training in complex procedures, including organ transplantation [33]. They are hardy, intelligent animals that adapt well to laboratory settings. However, caregivers must consider their natural behaviors and dispositions to prevent stress caused by inadequate housing and handling. Poor management can lead to stress, making pigs more difficult to handle and compromising research data reliability. Owing to their intelligence, swine can be readily trained to cooperate in various husbandry and research tasks, such as standing for biophysical assessments, undergoing physical examinations, electrocardiography, dermal dosing and even nasal dosing. However, pigs that are fearful of humans exhibit elevated corticosteroid levels regardless of human presence, suggesting chronic stress [34].

Pigs are opportunistic omnivores that spend much of their day rooting for food and eating multiple small meals. Rooting is a key behavioral need, often more significant than food intake itself, and pigs also engage in extensive environmental exploration. They are most active in the morning and evening, spending the remainder of the day resting, rubbing against objects, and wallowing in mud or water. These behaviors not only regulate body temperature but also alleviate skin irritation and reduce parasites.

Solid, rough flooring combined with a substrate like straw is ideal as it reduces physical discomfort throughout the pig's life stages, stimulates foraging, promotes activity, and decreases abnormal behaviors such as stereotypies and social withdrawal. Conversely, rough handling has pronounced negative effects on pig physiology and behavior [34]. When individual housing is unavoidable, maintaining sensory contact (visual, auditory, olfactory, and tactile) is critical to reducing stress associated with isolation [35].

2.2.5. Laboratory Guinea Pigs

The laboratory guinea pigs (*Cavia porcellus*) were likely introduced to Europe from South America about 400 years ago. A detailed account of their history and nomenclature is provided in *The Biology of the Guinea Pig* [36], a comprehensive reference considered essential for those engaged in their care or research use. Domesticated guinea pigs are docile and nonaggressive, becoming highly responsive to gentle handling and regular interaction. They readily recognize familiar caretakers, often vocalizing with characteristic whistles when approached, and may lick handlers; a behavior generally interpreted as a sign of acceptance or affection. Because of their strong social nature, they require companionship to maintain physiological and behavioral well-being. Housing them with conspecifics reduces stress associated with confinement, as bonding is central to their welfare. Indeed, both sexes exhibit lower cortisol levels in stressful situations when accompanied by a familiar partner [30].

Guinea pigs are cursorial rodents that do not burrow, although in the wild they may use shelters made by other

animals. To meet their behavioral needs in captivity, cages or pens should be enriched with structures such as tubes or shelters that allow hiding and climbing, thereby reducing fear responses. Hay provides both roughage and a source of environmental enrichment, while wooden sticks are suitable for gnawing. Owing to their social tendencies, guinea pigs should be housed in compatible groups, such as pairs, harems, or all-female groups. Males, in particular, are best kept in pairs to encourage stable social relationships [37].

2.2.6. Laboratory non-human Primates

Nonhuman primates often experience considerable fear when subjected to forced handling. Although procedures such as injections and blood sampling may be perceived as minor, they are unlikely to cause “little or no discomfort” unless the animal is neither restrained against its will nor removed from its home cage. Unlike certain rodent species selectively bred for laboratory adaptation, primates have not undergone such processes, which may render them more vulnerable to distress and potentially capable of experiencing greater suffering than other laboratory animals [38]. Nonhuman primates play a central role in biomedical research, particularly in β -cell replacement strategies such as islet transplantation. With appropriate training using counter-conditioning and positive reinforcement, primates can learn to cooperate with medical procedures and even facilitate their own care, while remaining in the familiar environment of their home cages [39].

As highly social species, primates derive significant security from group living, especially within kin-bonded or otherwise compatible groups. Solitary housing, in contrast, predisposes them to abnormal behaviours, where as social housing markedly reduces their occurrence [37]. Enrichment strategies, including provision of fruits, nuts, coconuts with intact shells, frozen grapes, puzzle feeders and foraging substrates such as sawdust mixed with dried fruits or mealworms, have proven effective in minimizing stereotypies and stimulating activity [40]. Data from European research institutions show that primates are primarily employed in toxicology and safety testing, applied biomedical and veterinary studies, and fundamental biological research [38].

Passive forms of refinement for captive primates include television, videotapes, mirrors, and auditory stimuli such as music or natural sounds. Mirrors can be particularly beneficial, allowing primates to see their own reflection as if it were a conspecific or to observe activities outside their enclosure. Refinement strategies should be tailored to the species, sex, age, and ideally the individual animal. Among various enrichment strategies, providing social housing stands out as the most successful approach for refinement, particularly in reducing the occurrence of atypical behaviors in young primates [37].

2.3. Measures for Laboratory Animals Welfare

Assessment of animal welfare should focus on positive indicators, such as pleasure, which can be measured through preference tests or behavioral observations in the home cage. Anticipatory behavior, expressed as

heightened activity before a predictable reward is particularly useful in evaluating welfare by reflecting both positive and negative experiences. It represents a form of arousal with goal-directed activity during the appetitive phase, when the reward itself (e.g., food, water, sexual contact, enriched housing) is not yet available [14]. Evaluating welfare requires consideration of environmental impacts on the individual, the challenges of coping with these impacts, and the extent of positive experiences. Since welfare encompasses both physical health and emotional states, and as many coping mechanisms are brain-regulated, assessing changes in the brain is critical when evaluating the effects of environmental stressors [41]. Motivated behaviors, combined with physiological data, provide valuable insights into animal priorities, physical conditions, and the consequences of husbandry or experimental procedures. Observations in the home cage are particularly informative, as changes in behavioral repertoire following modifications such as enrichment or the addition of a social partner reveal important welfare outcomes. A broad behavioral range generally signifies good well-being, but accurate evaluation requires detailed knowledge of the species-specific behaviors and biological needs [14].

Welfare assessment employs a wide range of indicators, including avoidance responses, behavioral changes, physiological parameters and pathological conditions. Short-term problems, such as those occurring during handling or transport, are best measured through acute stress markers like heart rate and plasma cortisol. In contrast, long-term welfare issues are more accurately assessed using behavioral patterns, immune function and disease prevalence. Welfare over extended periods is often described as “quality of life,” which is compromised when coping is difficult or impossible, resulting in harm to the individual [42]. Indicators of discomfort can be classified into behavioral, physiological and post-mortem parameters. Behavioral signs include stereotypies, abnormal postures, sudden fear or aggression, vocalizations, reduced grooming, and chromodacryorrhea (red secretions around the eyes and nose) in rats and mice. Physiological markers include weight loss, decreased food intake, diarrhea, altered cardiovascular or respiratory function, and changes in stress-hormone or immune parameters. Post-mortem findings provide retrospective insights valuable for improving welfare in surviving animals, with relevant parameters including organ size, fatty deposits, infections, stomach ulcers, and dehydration [14].

Measurements of animal welfare comprises of the following:

- Physiological indicators of pleasure
- Behavioral indicators of pleasure
- Extent to which strongly preferred behaviors can be shown
- Variety of normal behaviors shown or suppressed
- Extent to which normal physiological processes and anatomical development are possible
- Extent of behavioral aversion shown
- Physiological attempts to cope, immunosuppression, disease prevalence
- Behavioral attempts to cope, behavior pathology
- Brain changes, body damage prevalence, reduced

ability to grow or breed

- Reduced life expectancy

Some signs of poor welfare arise from physiological measurements. For instance increased heart rate, adrenal activity, adrenal activity following ACTH challenge, or reduced immunological response following a challenge, may all suggest compromised welfare compared to unaffected individuals. These alterations often reflect what has been described as a pre-pathological state, where physiological systems are strained but clinical disease has not yet developed. More recently radio-telemetry has enabled heart rate, blood pressure and body temperature to be measured in stress-free, awake and freely moving animals [14]. Experiments must only be conducted when scientifically required, with avoidance of over-use, unjustified duplication, and unnecessary harm or killing [43].

2.4. Requirements of Laboratory Animals Welfare

2.4.1. Housing and Care

Housing systems for laboratory animals have traditionally been designed with primary consideration for economic and ergonomic factors such as cost, equipment, space, workload, ease of observation and maintenance of hygiene, rather than for the welfare of the animals themselves. Yet, an animal's environment encompasses a wide range of stimuli, including both the social environment (conspecifics, other species, and human interactions) and the physical environment (cage structure and its contents). Structural changes like nest boxes, tubes, partitions, and nesting materials improves how animals mentally evaluate the controllability and predictability of their surroundings [45].

In practice, however, laboratory animals are usually housed throughout their lives in relatively barren cages, and given unrestricted access to food. This frequently results in adverse effects on the behaviour and physiology of the animals, and in a shortened lifespan due to overfeeding and inactivity [46]. Rodent housing conditions in laboratories represent an important potential welfare problem. Most animals used in research and testing spend their lives in small cages. Although there has been a gradual shift toward including some enrichment, a significant proportion of laboratory housing remains devoid of enrichment, despite widespread agreement on the necessity of reform within the scientific community [47].

Housing systems for animals kept in captivity are frequently designed primarily considering economic and ergonomic factors including equipment, expenses, spatial requirements, workload, observation capabilities and hygiene maintenance, often with minimal attention to the animal's well-being. Standardization of the animal cage was considered essential to reduce variation, resulting in a shoebox-shaped cage and standard bedding material for rodents. Over the past ten years, there has been increased awareness of the need to create environments tailored to the specific needs of different species. This includes ensuring environmental complexity and social housing, which has contributed to enhancing their overall well-being [48]. One effective way to enhance the living

conditions of captive animals is environmental enrichment, which involves modifying their environment to promote both physical and psychological health by offering stimuli tailored to the needs of each species [14]. Enrichment not only supports the expression of natural behaviours but also reduces stress, abnormal behaviours and physiological signs of poor welfare, thereby improving both animal well-being and the reliability of experimental outcomes.

2.4.2. Dietary Requirement

Farm animals and laboratory animals have similar nutritional requirements. All require adequate amounts of energy, protein, carbohydrates, lipids, macro-minerals, vitamins and trace elements, provided in diets that are palatable and free from chemical or biological contaminants. Fiber is generally not listed among basic nutrient requirements. However, depending on the species, including fiber or supplementing the diet with it can support overall health. Rabbits and guinea pigs benefit from additional fiber, which promotes proper biological functioning [49].

Many species also engage in coprophagy, directly consuming feces even when housed on grid floors. This behavior allows them to obtain vitamins synthesized by intestinal bacteria, particularly vitamin K and B12. However, germ-free animals lack these microbial populations and therefore require diets supplemented with additional vitamins [50]. Another consideration is variability in dietary composition; both between different feed manufacturers and between batches from the same producer. Such inconsistencies may sometimes induce pathological changes, leading to compromised welfare through impaired biological functioning.

Palatability, defined as the acceptability of feed in terms of taste and texture, is also a critical factor. Animals must ingest sufficient quantities to meet their essential nutrient requirements. When experimental substances are incorporated into the diet, palatability may be altered. To prevent reduced intake, it is advisable to conduct pilot studies in a small number of animals before large-scale experiments. For instance, when dietary fish oil was introduced to herbivorous rabbits, a habituation period of approximately six months was necessary before they consumed the required amounts for maintaining good health [51].

2.4.3. Veterinary Medical Care

Veterinary care requires assessment of animal well-being and effective management of clinical care for research and non-research related health conditions. It must include a mechanism for direct and frequent communication with the veterinarian to ensure animals receive appropriate treatment, relief from pain or discomfort, or euthanasia, if indicated. Certain aspects of veterinary care can be delegated to trained personnel. However, establishing effective systems for direct and frequent communication is crucial to ensure that veterinarians are promptly informed about animal health, behavior, and welfare issues, enabling timely treatment or euthanasia when necessary. An emergency medical plan should be established to ensure prompt and suitable

veterinary care for any animal that is injured, distressed, or exhibiting signs of serious illness [52]. Recognition and prompt management of pain are essential to prevent unnecessary suffering. Untreated pain not only compromises animal welfare but also leads to adverse physiological effects, including weight loss, muscle catabolism, hypertension, and delayed recovery. Failure to provide adequate analgesia is ethically and scientifically unacceptable [53]. The veterinarian is responsible for administering anesthesia and analgesia when needed to alleviate pain and suffering in animals, unless scientific reasons approved by the institutional animal care and use committee prohibit such actions. Their primary role in a research setting includes monitoring animals' health and well-being, providing treatment for those that are ill, and overseeing or conducting euthanasia when necessary to ensure the animals welfare [54].

Rounds should be conducted by a trained individual at least once every 24 hours to visually check and monitor each animal's health and well-being. This monitoring should include observing food and water intake, urination, defecation, attitude, behavior, movement and any signs of illness or other issues [55]. Animal monitoring should precede enclosure cleaning to assess food intake, housing condition, and excreta (feces, urine, vomit). For animals housed in groups, monitoring should also take place during feeding time, so that appetite (food intake) or conflicts around food may be observed. Any animal showing signs of pain, suffering, distress, rapid health decline, life-threatening issues, or suspected zoonotic conditions must be promptly assessed and properly managed [56].

In Canada, veterinarians hold primary responsibility and authority for ensuring the implementation of a comprehensive veterinary care program and for overseeing all aspects of animal care and use. The Canadian guidelines further require that the institution's senior administration and the animal care committee delegate to the veterinarian the authority to treat, remove from a study, or euthanize an animal, as warranted by their professional judgment [54].

2.5. Legislative Aspect of Housing and Care of Laboratory Animals

2.5.1. Policies, principles and regulations for the use of Laboratory Animals

The recognition of animals as possessing moral standing that their interests merit ethical consideration; forms the basis of animal ethics. This perspective underlies the development of animal welfare science and continues to shape debates on the ethical use of animals in biomedical research [57].

In Europe, housing specifications for laboratory animals were first formalized in 1986 through two key documents. The most influential document is the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Convention ETS 123) issued by the Council of Europe, which features Appendix A: Guidelines for the Accommodation and Care of Animals [58]. In the United States, revisions to the Animal Welfare Act (AWA) in

1970 mandated the provision of "adequate veterinary care, including the appropriate use of anesthetic, analgesic, or tranquilizing drugs, when such use would be proper in the opinion of the attending veterinarian" [59]. This approach was later reinforced by the National Research Council (2009), which emphasized that laboratory animals "need not experience substantial or ongoing pain," framing prevention and alleviation of pain as an ethical imperative rather than solely a veterinary responsibility [60]. The guiding ethical principle in laboratory animal welfare policy is that while the infliction of pain or distress on sentient animals may be permissible under certain circumstances, it requires compelling scientific justification [61].

In Europe, Directive 2010/63/EU on protecting animals used in scientific research sets unified rules to reduce differences among Member States in laws and administrative procedures related to laboratory animal welfare [62,63]. The Directive explicitly recognizes animal welfare as a core value of the European Union, as enshrined in Article 13 of the Treaty on the Functioning of the European Union (TFEU). Adopted on 22 September 2010, it replaced Directive 86/609/EEC and is firmly grounded in the principle of the Three Rs—Replacement, Reduction, and Refinement—to guide the ethical use of animals in scientific research.

2.6. Alternative Approaches to the Use of Laboratory Animals

Increasing attention within both academia and industry is directed toward developing alternative approaches that replace animal use while providing more accurate models of human biology and disease. Simultaneously, there is increasing focus on improving the design, conduct, and analysis of in vivo research, particularly by aiming to reduce animal usage and enhance overall quality welfare. Knowledge of animal's physical and behavioral needs, as well as the welfare implications of experimental procedures, is expanding rapidly and is being translated into practical refinements aimed at minimizing pain, distress and variability, thereby strengthening scientific reproducibility. Refinements in laboratory practice include low-stress mouse-handling methods to reduce anxiety and variability, use of grimace scales for pain assessment and relief, and environmental enrichment to promote species-typical behavior, minimize abnormalities, and improve construct validity [57].

The principle of the Three Rs provides a foundation for ethical animal research. Replacement involves substituting animal models with non-animal alternatives, such as tissue cultures, computer simulations, or other innovative systems, whenever scientific objectives can still be met. Reduction emphasizes minimizing the number of animals used to the lowest possible level that still ensures reliable results and adequate statistical power. Careful experimental design and appropriate statistical analysis are critical in achieving this aim. However, reduction must be balanced carefully, as lowering numbers may in some cases, increase the level or duration of discomfort experienced by individual animals. Refinement focuses on optimizing study design and procedures to maximize

scientific value while minimizing pain, distress, or lasting harm. This includes strategies such as the use of humane endpoints, where interventions are applied to prevent unnecessary suffering if adverse outcomes occur [64].

3. Conclusion and Recommendations

Laboratory animals have long been central to experimental research in the biological and medical sciences. There is broad agreement that improving animal welfare not only benefits the animals but also enhances the quality and translational value of research. The principle of the Three Rs contributes to this goal by promoting both welfare and scientific validity. Routine laboratory procedures, such as handling, can be a significant source of stress for species like mice and rats, and dietary requirements must also be met to ensure well-being. For instance, feeding guinea pigs a rat diet results in vitamin C deficiency, leading to disease and death within 14 days. Promoting welfare is crucial because animals can suffer not only during experiments but also through inadequate husbandry and health management. Poor care and stressful housing conditions may induce both physiological and psychological distress, which in turn can compromise research outcomes. Stress alters biological responses, thereby reducing reproducibility and reliability of data. Providing appropriate nutrition, including all essential nutrients, is necessary for maintaining normal biological function, safeguarding welfare, and ensuring sound scientific results. Furthermore, cultivating a considerate and empathetic attitude toward animals, alongside the application of refined experimental techniques and the use of humane endpoints, strengthens both ethical standards and research integrity.

Based on the above conclusion the following recommendations are highlighted:

- Stress-free handling methods, such as tunnels or cupped hands for mice and rats, are preferable to tail or body lifting, benefiting both animal welfare and research reliability.
- Species-appropriate nutrition is fundamental for both animal welfare and experimental validity.
- Positive human-animal interactions markedly reduce stress and suffering.
- Euthanasia should minimize pain and distress, with veterinary oversight ensuring protection from injury and discomfort.

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Contribution of Authors

All the authors contributed to the conceptualization, writing, reading, and approval of the final manuscript for its submission for publication.

Conflict of Interest

The authors declare no conflict of interest.

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