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### **Systemic failures and suffering in animal-based research: Why a human-relevant paradigm is urgently needed**

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#### **Abstract**

Animal experimentation is often portrayed as scientifically indispensable and ethically well policed; however, contemporary evidence indicates otherwise. This chapter identifies six systemic weaknesses within the current animal-research paradigm: (1) sustained high animal use and large “surplus” killings in breeding colonies; (2) routine under estimation and under treatment of pain; (3) selective transparency that omits ≈95% of US laboratory animals and keeps harm–benefit and retrospective assessments confidential; (4) public-funding bias that allocates ≤1% of health-research budgets to human-biology-based nonanimal methods (NAMs); (5) welfare and translational shortcomings driven by barren housing, chronic stress, and interspecies differences; and (6) fragmented regulatory oversight characterized by low refusal rates and limited accountability. Severe procedures now cluster in neuroscience, high-dose viral challenge, and fish ecotoxicity, while ≈90% of drug candidates that appear promising in animals fail in human trials. A decisive pivot to human-relevant science is both an ethical imperative and a strategic investment in biomedical innovation.

**Keywords:** Animal experimentation; Nonanimal methods; Funding bias; Transparency gap; Pain and severity assessment; Surplus breeding; Translational failure; Regulatory reform

## **Introduction**

Animal experimentation remains one of the most contested practices in contemporary science. Proponents depict it as essential to biomedical progress, while critics point to intolerable ethical costs and persistent scientific shortfalls. The debate has been sharpened by

rapid advances in human-based technologies, including organoids, organ-on-chip systems, high-throughput omics, and in silico models—that offer mechanistic insight without the confounding effects of interspecies biology.<sup>1–3</sup> In this chapter, new approach methodologies (NAMs) are understood as human-relevant methods—that is, approaches that are human-derived and/or demonstrably predictive of human biology. Platforms sometimes marketed as “NAMs” but based on animal tissues, animal-trained systems, or hybrid animal–human platforms fall outside this definition. Advocates of human-biology-based replacement argue that a paradigm shift is overdue.<sup>4–6</sup>

This chapter contends that six systemic failure points in the current animal-research paradigm undermine both the ethical and the scientific justification for continued reliance on animals: (1) the scale of animal use and surplus killing; (2) persistent underrecognition and undertreatment of pain; (3) selective transparency and weak public accountability; (4) funding allocations that marginalize human-relevant approaches; (5) welfare shortfalls and poor translatability rooted in species differences; and (6) fragmented, often rubber-stamped regulation. Drawing on peer-reviewed literature, official statistics, regulatory reports, and economic analyses, the chapter shows how these domains converge on a single conclusion: the justification for animal use is weaker than ever.

This analysis is global in scope but focuses on jurisdictions with comparatively detailed reporting systems—the European Union (EU-27 + Norway), the United Kingdom (UK), the United States, Canada, and Australia. The chapter closes with evidence-based recommendations to accelerate the adoption of NAMs—defined here as human-derived or human-validated methods—so that biomedical research becomes both more humane and more predictive for human biology and health.

## **Scale of animal use and suffering**

### **Regional trends**

In the European Union, overall animal use declined by 11% between 2002 and 2022,<sup>7</sup> but numbers have remained largely stable since 2015. The most recent statistics reported a further decrease in 2023, with nearly 8 million animals used in scientific procedures across the EU and Norway, representing a 4.9% reduction compared with 2022. In addition, almost 1 million animals were used for the creation and maintenance of genetically altered (GA) lines. GA animals accounted for 22.3% of all reported animal uses in 2023, offsetting reductions in traditional toxicity testing.<sup>8</sup>

The United States publishes audited figures only for species covered by the Animal Welfare Act (AWA), thereby excluding purpose-bred mice, rats, fishes, and, until recently, all birds—groups that together constitute over 90% of laboratory populations. A regulatory change finalized in 2023 brought only nonpurpose-bred birds under the AWA for the first time;<sup>9</sup> this change was first reflected in the 2024 reporting cycle, when 63,396 birds were included in the national statistics.<sup>10</sup> Purpose-bred birds remain excluded from federal reporting, leaving total avian use unknown. In that year, 1014 research facilities reported using 775,297 AWA-covered animals in 2024.<sup>10</sup> Nevertheless, these figures capture only a small fraction of total animal use. Extrapolating from institutional data, Carbone<sup>11</sup> estimated that around 111 million rats and

mice are used annually in US laboratories, with an estimated 40–45 million subjected to painful experiments. Estimates vary depending on methodology and assumptions; however, even conservative extrapolations imply tens of millions of mice and rats annually, with a substantial fraction falling into potentially painful categories.

Canadian statistics likewise indicate persistently high levels of animal use. In 2024, the Canadian Council on Animal Care (CCAC) reported 3.71 million animals used in research, teaching, and testing. Fishes (46.2%) and mice (32.4%) accounted for the majority of reported animal use, while cattle represented 10% of all animals used. GA animals dominate biomedical studies, and regulatory testing involving farm animals has increased alongside the intensification of animal agriculture.<sup>12</sup>

In Australia, the absence of a national reporting scheme means figures must be assembled from state reports, surveys, and freedom-of-information (FOI) requests. Available data indicate that approximately 6 million animals are used each year, a figure that has plateaued rather than declined.<sup>13</sup> Australia is unusual in counting free-living animals observed in ecological studies, which inflates totals but still demonstrates the continuing reliance on vertebrate models.

In Brazil, by far the biggest user in Latin America, regulatory authorities reported that approximately 2.5 million animals were used in research and teaching activities in 2021, with more than 11.3 million animals used between 2019 and 2023 combined.<sup>14</sup> These figures included rodents, birds, fishes, horses, pigs, and smaller numbers of nonhuman primates and reptiles. While the Brazilian reporting system captures a broader range of species than many international reporting systems, important limitations remain. While the Brazilian reporting system captures a broader range of species than many international reporting systems, the published statistics do not provide disaggregated information on genetically altered animals, surplus breeding, or severity categories.<sup>14</sup>

China, Japan, and South Korea have begun publishing partial statistics, but incomplete coverage of GA animals, fishes, and cephalopods leaves continental estimates uncertain.<sup>15</sup> Given China's scale in biomedical research, the unreported population is likely substantial. Taylor and Rego Alvarez estimated that China used approximately 20.5 million

animals for scientific purposes in 2015, making it one of the world's largest users of laboratory animals.<sup>15</sup>

Across these regions, official statistics reveal plateauing trends in headline numbers but conceal extensive undercounts and hidden deaths. The drivers behind this persistence are explored in the next section.

### **Drivers of persistent use**

The plateau in overall animal-use numbers coincides with a dramatic rise in genetically engineered mice for disease modeling.<sup>8</sup> These models require large breeding colonies and generate substantial “wastage” when offspring do not possess the desired genotype. According to the European Commission's report on the statistics for the use of animals for scientific purposes in the EU,<sup>16,17</sup> the number of animals killed as surplus—i.e., bred but not used in procedures—was estimated at 10.58 million in 2020, 10.13 million in 2021, and 9.57 million in 2022.<sup>16,17</sup> These figures represent animals killed without being used in experiments, including animals deemed unsuitable because of genotype, sex, age, or overbreeding.

The fact that surplus killings consistently exceed or rival the number of animals used in scientific research (e.g., 9.24 million animals used in procedures in 2022) underlines a largely hidden dimension of animal use in science. These deaths remain largely invisible in public discourse and are not scrutinized through the same harm-benefit assessments required for research procedures, despite their substantial welfare implications.

At the same time, regulatory chemical safety programs—such as Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) in the EU and the Toxic Substances Control Act in the United States—continue to rely heavily on animal tests despite validated *in vitro* and other nonanimal approaches. Analyses highlight structural barriers, including entrenched career incentives, established supply chains, and institutional inertia that favor familiar animal protocols over novel NAM platforms that require additional validation.<sup>18-20</sup> The FDA's Potential Approaches to Drive Future Integration of New Alternative Methods to Animal Testing roadmap likewise acknowledges that, although NAMs are advancing, animal tests remain the default in many preclinical safety domains.<sup>21</sup> The result is an ethically paradoxical landscape: technological capacity for replacement is advancing rapidly, yet systemic inertia continues to sustain animal demand.

## **Pain, distress, and severity assessment**

### **Regulatory frameworks**

Under Directive 2010/63/EU and the Animals (Scientific Procedures) Act 1986 (UK, as amended),<sup>22</sup> every procedure must be assigned a prospective severity classification—mild, moderate, severe, or nonrecovery—before work begins, and an actual severity classification once the procedure ends. Whenever real-world harm exceeds the authorized limit, the

incident must be reported; in the United Kingdom, such a deviation automatically triggers a compliance investigation.<sup>23,24</sup>

### **Prospective underestimation of severity**

Licensing files reveal a persistent optimism bias in prospective severity assessment. In a retrospective review of 506 German project-licence applications, covering 684 rodent recovery surgical procedures, 6% of procedures a severity classification lacked any severity classification, despite this being a legal requirement.<sup>25</sup> Among the remaining procedures, researchers classified almost 40% as “mild,” even though all involved surgery under general anaesthesia. Using Directive 2010/63/EU and relevant guidance documents, Herrmann and Flecknell classified all procedures as at least moderate and 28% as severe, compared with only 7% classified as severe by the applicants themselves. Overall, 59% of researcher-provided severity classifications deviated from the authors’ assessment, and in 58% of cases the applicants’ classification was lower. Only two procedures were classified more severely by the applicants than by the authors.<sup>25</sup> The authors concluded that researchers frequently underestimated the pain, suffering, distress, and lasting harm associated with the proposed procedures, and that planned health-monitoring strategies were often inadequate, reflecting persistent deficits in pain recognition, refinement implementation, and training.<sup>25</sup>

### **The harm-benefit ledger: structural bias**

Licensing decisions are meant to pass a simple utilitarian test: projected human benefit must outweigh animal harm after the latter has been “reduced to the absolute minimum.”<sup>26</sup> In practice, however, neither side of the equation is measured with scientific rigor, and both are routinely tilted toward approval. Applications regularly downplay the real severity of procedures. Independent reviewers rescoring German rodent protocols found that one-third of procedures labeled “mild” should have been classified as “severe,” including thoracotomies, craniotomies, and intraabdominal implantations performed under minimal or conditional analgesia.<sup>25</sup>

Underestimation extends beyond surgical pain. Monitoring plans are frequently vague or delayed, allowing distress to go undetected for hours or days; anesthesia and analgesia are often limited to a single 24-hour dose despite evidence of longer-lasting pain; preventive multimodal regimens are rare. Humane endpoints are frequently late or poorly defined, and killing methods still include techniques known to be aversive or unreliable (e.g., CO<sub>2</sub> exposure, cervical dislocation, and decapitation without adequate sedation).<sup>27,28</sup> Because severity is underestimated prospectively, the “cost” column in the harm–benefit ledger begins artificially low. Retrospective reviews—an important mechanism for detecting this bias—cover only a minority of projects and are seldom published.<sup>29</sup>

Just as harms are discounted, anticipated benefits are often overstated. Most interventions that show promise in animal studies ultimately fail during human clinical development,<sup>30,31</sup> a pattern linked to poor reproducibility, selective reporting, and weak model validity.<sup>2,3</sup> Oversight committees may lack the time, expertise, or data access to

interrogate these claims in depth, further entrenching optimism bias.<sup>32</sup> Cultural attitudes can also contribute: speciesism—privileging human interests over equivalent animal interests—predicts reduced empathy for nonhuman pain,<sup>33</sup> and long-term exposure to animal suffering can create a “moral callus,” normalizing severe practices.<sup>34,35</sup>

Cumulative suffering is likewise underrecognized. Although Directive 2010/63/EU requires retrospective review of severe projects, repeated procedures, chronic recovery, and barren housing are rarely captured from a lifetime perspective.<sup>36</sup> Multiple audits confirm the gap, including findings of 58% underestimation in German rodent surgery proposals.<sup>25</sup> Public summaries also remain uneven: only 15%–18% contain sufficient detail to reconstruct lifetime experiences, and reporting quality varies substantially across jurisdictions.<sup>37</sup> EU methodological notes further emphasize that statistics are reported per “use,” not per animal, meaning that reused animals can appear multiple times and cumulative burden cannot be reconstructed from official datasets.<sup>16,17</sup> New approaches, such as the cumulative severity assessment -scores, have therefore been proposed to quantify lifetime burden more systematically.<sup>38</sup> Transparency analyses likewise show that lay summaries often highlight a single intervention while obscuring the broader lived experience of animals.<sup>18</sup>

Taken together, these patterns indicate a structurally biased harm–benefit assessment in which harms are systematically underestimated while anticipated benefits are overstated. Addressing these shortcomings requires independent severity audits, mandatory NAM expertise on review boards, outcome-based reporting of cumulative suffering, and a strategic shift toward human-relevant methods.

### **Actual severe use in the EU and the United Kingdom**

According to official EU statistics, across the EU-27 plus Norway, the absolute number of severe procedures declined from 976,445 (10.9%) in 2018 to 784,048 (9.2%) in 2022. Regulatory safety testing once accounted for almost one-third of these cases,<sup>39</sup> but contributed only 18% in 2022<sup>16,17</sup> after EU-wide adoption of in vitro pyrogen assays<sup>40</sup> and the near-elimination of mouse-ascites antibody production.<sup>16</sup> Severe cases are now predominantly associated with basic and translational research—representing approximately 77% of all severe procedures—particularly in neuroscience, oncology, and infectious-disease models. The mouse ascites method nevertheless still caused about 24,000 severe procedures in 2022, almost all in France.<sup>41</sup> Ecotoxicology remains a hotspot, with 42% of fish toxicity studies still classified as severe in 2020.<sup>42</sup>

Great Britain shows an even steeper decline: severe experimental procedures accounted for approximately 8% of all work in 2014 but only 4% in 2022, and the latest Home Office data indicate a further decline to around 3% in 2023.<sup>43,44</sup> When breeding of GA lines is included, severe classifications represent about 2% of all procedures. In 2022, mice experienced 45,500 severe procedures, followed by zebrafish (≈4600), guinea pigs (≈1300, mostly batch-safety tests), and rats (≈1000). Acute LD50-type studies remain the single most severe category: 47% of such UK tests were logged as severe in 2022.<sup>24</sup>

## **Analgesia deficiencies**

Pain management continues to lag behind best practices. A systematic review of more than 2000 rodent craniotomy studies found that only approximately one quarter reported any perioperative analgesia, while fewer than one in six mentioned postoperative pain control, underscoring persistent deficiencies in both pain management and reporting.<sup>45</sup> A German review found 30% of almost 700 planned rodent surgeries included no postoperative analgesic plan, while genuine multimodal regimens were virtually absent.<sup>28</sup> Inadequate pain relief not only harms animals but also distorts immunological, endocrine, and behavioral data, compromising reproducibility.<sup>46</sup>

The foregoing analysis shows that severe suffering has declined in some domains but remains structurally underrecognized and undertreated. The next section examines why widely promoted mitigation strategies—refinement and partial replacement—have not delivered the expected welfare gains in practice, and how these limitations may also compromise scientific validity.

## **Welfare shortfalls and scientific validity**

### **Environmental enrichment and the standardization fallacy**

Barren, tightly standardized housing has not resolved the reproducibility problem: in a coordinated three-laboratory study, behavioral phenotypes differed more between sites than within a given protocol, revealing substantial lab-to-lab effects despite meticulous standardization.<sup>47</sup> At the same time, adding enrichment improves welfare and promotes normal species-typical behavior without inflating within-group variability or producing conflicting outcomes, countering the long-standing assumption that “richer” cages compromise

precision.<sup>48</sup> Together, these findings expose the standardization fallacy—the assumption that increasingly strict homogenization automatically yields more reliable data.<sup>49</sup>

A more effective strategy may be systematic heterogenization: preplanning modest, theory-driven variation in factors such as housing, handling, timing, or apparatus—and, where feasible, distributing experiments across sites—to sample the real-world noise that otherwise blindsides replication. Conceptually and in proof-of-principle demonstrations, this structured variation produces effects that are more stable across contexts without demanding larger sample sizes, thereby improving external validity within animal research (i.e., generalizability across labs/batches/handlers) and between-lab reproducibility—while preserving internal validity and statistical efficiency.<sup>49</sup>

### **Chronic stress and epigenetic change**

Routine stressors—handling, restraint, cage cleaning, olfactory cues from predators—activate the hypothalamic–pituitary–adrenal axis, altering cytokine profiles, tumor growth kinetics, and cognitive performance.<sup>46</sup> Epigenetic modifications can persist for

generations, meaning that stress experienced by parent animals may confound phenotypes in offspring even before experiments begin.

### **Species differences and translational failure**

Even with improved husbandry, the biology of nonhuman species imposes a ceiling on predictive value. A large comparative study by Seok and colleagues found that genomic responses to acute inflammatory insults—including trauma, burns, and sepsis—were highly reproducible across different mouse strains but bore little resemblance to the corresponding human responses.<sup>50</sup> This striking divergence underscores how even well-controlled animal models can systematically misrepresent human disease pathways.

Persistent failures in human translation reflect fundamental limitations in the ability of animal models to reliably predict human physiology, disease mechanisms, and therapeutic responses.<sup>51,52</sup> Sun and colleagues trace many of these failures to an optimization culture that, during preclinical development, prioritizes nanomolar potency and plasma pharmacokinetics while overlooking whether a compound actually reaches the relevant human tissue at tolerable doses.<sup>52</sup> They argue that this “structure–tissue–exposure gap” explains why efficacy signals collapse once compounds are tested for effectiveness in Phase II trials, and why toxicity resurfaces when drugs are tested for safety and tolerability in Phase I trials or during the larger confirmatory Phase III studies. Crucially, this gap cannot be bridged by animal testing, since tissue exposure, metabolism and selectivity profiles differ across species. Instead, it demands human-relevant methods—such as organoids, organ-on-chip systems, and *in silico* ADME (absorption, distribution, metabolism, and excretion) models—that can directly measure or predict human tissue distribution and selectivity.

As Pound and Ritskes-Hoitinga emphasize, fundamental species differences in immune pathways, metabolic enzymes, and receptor pharmacodynamics impose limits on the translational validity of animal models—limits that cannot be overcome by better husbandry or incremental refinements.<sup>3</sup> These challenges are therefore not merely methodological but biological in nature. As later chapters show, NAMs—including organoids, organ-on-chip systems, multiomics platforms, and computational disease models—offer opportunities to study human biology directly while avoiding many of the translational uncertainties associated with cross-species extrapolation.

### **Human costs: moral injury and secondary trauma**

Animal experimentation inflicts not only suffering on nonhuman animals but also significant psychological costs on the humans tasked with harming them.<sup>53</sup> Ethnographic studies first highlighted this unease: Arluke documented the moral ambivalence of laboratory technicians,<sup>54</sup> while Chang and Hart showed how bonds formed with laboratory animals deepened distress when the same animals were later subjected to painful procedures or euthanasia.<sup>55</sup> Reeve et al. described this as the caring–killing paradox: when individuals are both caregivers and executioners, the resulting guilt, grief, and emotional exhaustion create a distinct form of occupational strain.<sup>56</sup>

Philosopher Bernard Rollin was among the first to call this phenomenon moral stress, identifying the harm done to individuals compelled to kill animals for reasons not in the animals' own interests.<sup>35</sup> Later, Johnson and Smajdor argued that animal research institutionalizes a "failure of recognition," systematically reducing animals to objects and thereby eroding the moral agency of the humans tasked with harming them.<sup>57</sup> Rohlf and Bennett described this process as perpetration-induced traumatic stress, noting symptoms akin to posttraumatic stress disorder, including intrusive thoughts, emotional withdrawal, and hyperarousal.<sup>58</sup>

Empirical data confirm the scope of these burdens. Randall et al., in a survey of 801 laboratory-animal professionals across Europe and Asia, found that compassion fatigue and secondary traumatic stress were widespread, particularly among staff who named or trained animals.<sup>59</sup> The systematic review by Rumpel et al. synthesized twelve studies and identified euthanasia as the most consistent and distressing stressor for laboratory-animal personnel.<sup>60</sup> Across contexts, killing animals produced both acute strain (grief, sadness, guilt) and chronic strain (burnout, compassion fatigue, secondary traumatic stress). Importantly, the review distinguished between stressors (such as euthanasia), strain (psychological outcomes), and moderators (such as social support), showing that while institutional coping programs may buffer some effects, they do not resolve the ethical conflict at the root of the problem.

Institutions, however, frequently reframe these experiences using the language of compassion fatigue. Coping strategies—such as rotating staff between tasks, providing workshops, or holding symbolic memorial rituals—are aimed at sustaining staff resilience rather than interrogating the harms done to animals.<sup>61,62</sup> Analyses of hidden curricula suggest that researchers are socialized through stepwise desensitization—beginning with minor procedures, then progressing to surgeries, and finally euthanasia—so that empathy is gradually suppressed and emotional detachment becomes professionalized.<sup>63</sup> This dynamic can be understood as a form of engaged followership—a concept from social psychology describing conformity to authority<sup>64</sup>—in which loyalty to laboratory norms and the perceived demands of "science" overrides individual moral instincts.

Recognizing moral injury and secondary trauma underscores that animal experimentation harms both nonhuman animals and human personnel. Transitioning toward nonanimal research methods would not only spare animals but also protect the mental health and moral integrity of the people compelled to harm them.<sup>57</sup>

### **Refinement and replacement: the implementation gap**

Despite decades of research on refinement—including multimodal analgesia, non-aversive handling, enriched housing, and earlier humane endpoints—uptake remains inconsistent. Literature reviews continue to show poor reporting of analgesia, monitoring, and humane endpoints.<sup>65-67</sup> A multinational trainee survey ranked refinement as both urgent and feasible, yet respondents cited few concrete changes actually implemented.<sup>68</sup> Retrospective reviews of German animal-research licence applications further revealed substantial gaps between refinement principles and actual practice.<sup>25,27,28</sup>

Approximately 30% of surgical procedures planned no postoperative analgesia, while another 10% proposed analgesia only “if needed,” despite lacking structured pain-assessment protocols.<sup>27</sup> Preventive analgesia was frequently omitted when volatile anaesthesia was used, and multimodal analgesic regimens were exceedingly rare even for highly invasive surgeries such as thoracotomies.<sup>27</sup> Humane endpoints were often vague, implemented only at advanced stages of suffering, or omitted altogether, with nearly half of reviewed surgical procedures lacking explicit humane endpoints.<sup>28</sup> Researchers also frequently provided incomplete or inadequate health-monitoring strategies, and some applications lacked legally required information, including severity classifications.<sup>25</sup>

Together, these findings suggest that concerns about experimental standardization and potential confounding effects often continued to outweigh concerns about animal suffering, despite longstanding legal and scientific recognition of refinement obligations. Restricted access to project dossiers and retrospective assessments has limited independent evaluation of whether refinement practices have improved over time. In short, refinement remains the most extensively discussed of the 3Rs, yet its implementation is often assumed rather than systematically demonstrated.

Replacement shows a similar asymmetry. While regulatory toxicology has adopted several validated non-animal methods,<sup>69</sup> some training programs have transitioned toward simulation-based and other animal-free approaches,<sup>70</sup> biomedical research—where most animals are used—still defaults to live models.<sup>7</sup> Contributing factors include weak enforcement of the hierarchical 3Rs (replacement before refinement), limited awareness, and scant incentives for deviating from legacy pipelines.<sup>71</sup>

The mitigation tools that should reduce suffering and bias in data are not consistently implemented; when they are, they cannot overcome fundamental species limits.

### **Where severe suffering persists—and how to target NAMs**

When alternatives become binding regulatory standards, the welfare dividends dwarf incremental refinements. The monocyte-activation test and other in vitro pyrogen assays enabled the EU’s phase-out of the rabbit pyrogen test—preventing more than 140,000 severe procedures annually.<sup>40,72</sup> Similarly, moving monoclonal-antibody production from mouse ascites to cell culture eliminated tens of thousands of severe cases each year in some countries.<sup>8</sup>

Yet severe classifications persist. Fish LD50 ecotoxicity, aggressive neuro-oncology models, and high-dose viral challenges remain major contributors in the EU and UK. Public datasets such as ALURES (ANIMALUSE REPORTING - EU SYSTEM) and the UK Home Office Portal

now allow mapping of these pain points to prioritize NAM development and implement sunset clauses once validation is achieved.<sup>42,73</sup> The challenge is to pair intense refinement research with equally vigorous programs that convert validated alternatives into compulsory practice—realizing the ethical hierarchy envisioned by Russell and Burch in 1959.<sup>26</sup>

Outside Europe, severity frameworks are weaker, and outcome audits are rare. In the United States, the US Department of Agriculture's (USDA) pain categories C–E record only whether analgesia was provided and cover less than 10% of laboratory animals.<sup>74</sup> Canada's CCAC grades projected invasiveness but lacks compulsory outcome audits.<sup>12</sup> Australia's national A–E scale leaves retrospective review to state discretion, producing variable enforcement.<sup>75</sup> These gaps blunt incentives to implement refinement, obscure cumulative severity, and slow the shift to replacement. Measured reductions in headline “severe” numbers show that targeted replacement works.

However, persistent optimism in prospective assessments, under-analgesia, and the invisibility of lifetime burden mean the ethical ceiling is still far off—and the scientific one is already visible.

## **Funding bias, economic opportunity costs, and regulatory momentum**

Public funding for biomedical research continues to favor animal models, despite growing recognition of their scientific and ethical shortcomings. In the European Union, only about 0.7% of the €7.3 billion Europe Health Pillar has been directed toward NAM-exclusive projects,<sup>76</sup> with initiatives such as the Animal-free Safety assessment of chemicals: Project cluster for the Implementation of novel Strategies (ASPIS) and the Partnership for the Assessment of Risks from Chemicals (PARC) partnership representing important but still marginal investments.<sup>77,78</sup> In the United States, the Government Accountability Office estimates that around US\$ 5.5 billion annually supports animal research compared to just US\$ 69 million for organoid and organ-on-chip work—a disproportion of roughly 80 to 1.<sup>79</sup> Australia shows a similar imbalance, with less than 0.2% of its combined public health research budget earmarked for NAMs.<sup>13</sup> Across these regions, systemic bias is clear: NAMs receive only a fraction of total funding, and in many cases, grant systems do not even track method use, further obscuring the true scale of investment.

The economic opportunity costs of this imbalance are significant. Animal-based pipelines underpin a 90% attrition rate in drug development, where most compounds that pass animal tests ultimately fail in humans.<sup>3,50,80</sup> The US Food and Drug Administration notes that nonhuman-primate safety studies can cost around US\$ 50,000 per animal and delay programs by months.<sup>21</sup> By contrast, organ-on-chip systems, 3D-bioprinted tissues, and AI-driven in silico simulations can reduce timelines and direct costs while improving predictive accuracy.<sup>81,82</sup> Even modest reallocations—such as redirecting 5% of the ongoing animal-research budgets—could inject hundreds of millions annually into NAM science in the EU, the United States, and Australia. Modeling studies suggest that such reallocations could yield a five- to tenfold return on investment.<sup>13</sup>

Regulatory momentum is beginning to align with these arguments. In the EU, the One Substance, One Assessment package<sup>83</sup> anchors NAMs within a new cross-agency data

platform to harmonize chemical-safety evaluations, while the European Medicines Agency has introduced annexes for organ-on-chip data in regulatory guidelines. In the United States, public and political pressure for change is mounting, with polls showing large majorities in favor of phasing out animal testing.<sup>84</sup> The FDA has pledged to reduce primate use in biologics development, while the NIH has created the Office of Research Innovation, Validation, and Application (ORIVA) to coordinate policy across agencies.<sup>85</sup> In June 2025, the NIH terminated funding for Harvard neuroscientist Margaret Livingstone’s infant-macaque deprivation studies, citing its new strategic focus.<sup>86</sup> The same freeze also suspended US\$ 2.2 billion in Harvard grants—including Don Ingber’s flagship organ-on-chip program—prompting the university to sue the US Department of Health and Human Services.<sup>87</sup> Advocates hailed the termination of the primate studies as an ethical victory, yet the broader, politically charged cuts risk undermining the very NAM projects ORIVA is intended to support.

The EPA’s 2019 plan to eliminate mammalian testing by 2035 was later rescinded before being reaffirmed in January 2026, underscoring how politically contingent animal-testing transition policies remain.<sup>88</sup> For the first time, the FDA,<sup>89</sup> NIH, and EPA now all have policy initiatives pointing toward reduced reliance on animal testing, offering a potential tipping point—if sufficient funding, implementation capacity, and clear milestones are sustained.

## **The transparency deficit**

The much-publicized UK-based “Concordat on Openness on Animal Research” was drafted in 2014 and promoted by the lobbying group Understanding Animal Research (UAR)— an organization whose explicit mission is to defend and normalize animal experimentation.<sup>90</sup> Over 120 universities, charities, and industry bodies voluntarily signed the document, pledging clearer communication about their use of animals. Independent evaluations, however, show that most “openness” outputs celebrate refined husbandry practices and promised medical breakthroughs while omitting failed studies, unexpected deaths, and detailed adverse-event data.<sup>18</sup> Meanwhile, in the United Kingdom, Section 24 of the Animals (Scientific Procedures) Act 1986 (as amended)<sup>22</sup>—the Act’s confidentiality clause—continues to criminalize the unauthorized release of project information, severely limiting external scrutiny despite the Concordat’s rhetoric of transparency.

Across the EU, Directive 2010/63/EU requires the publication of nontechnical project summaries (NTS) and annual severity statistics. Yet, a review of 400 German and UK NTS found that only 44% adequately described predicted adverse effects, and 28% were rated “poor” or “very poor” overall.<sup>38</sup> Because severity data are aggregated only by species and overall purpose, it is impossible to hold individual projects accountable for the harms they inflict.

In North America, transparency gaps persist in different forms. Canada’s Canadian Council on Animal Care issues annual national summaries, yet universities frequently redact license

details when responding to provincial FOI requests, citing researcher safety and intellectual-property concerns.<sup>12</sup> In the United States, the Freedom of Information Act enables public access to USDA inspection reports, but more than 95% of laboratory animals—mice, rats, and birds—are excluded from the AWA and thus from routine public reporting. Furthermore, ClinicalTrials.gov registration rules do not cover preclinical studies, so negative animal results remain largely unpublished, perpetuating publication bias.<sup>46</sup>

Australia regulates animal research through state-based Animal Ethics Committees, whose minutes and license applications are rarely released. A review of public inquiries found that responses typically provided only high-level statistics with little contextual information or ethical justification.<sup>91</sup> The lack of national-level reporting impedes ethical oversight and prevents cross-jurisdictional benchmarking of scientific outcomes.

These patterns contribute to broader concerns about public trust. Sociologists describe this selective transparency as a “promissory discourse,” in which institutions amplify hypothetical future benefits while downplaying present harms.<sup>18</sup> Public polling indicates that acceptance of animal research is conditional on trust that suffering is strictly necessary.<sup>92</sup> When institutional disclosures appear incomplete or self-congratulatory, that trust erodes, fueling citizen petitions, ballot initiatives, and calls for outright bans (e.g., the European Citizens' Initiatives “Stop Vivisection” and “Save Cruelty-free Cosmetics”).

## **Regulatory track record**

The EU’s 2018–2022 implementation report shows that 20 Member States met the legal target of inspecting one-third of establishments annually, but 18 still missed the target in at least one year. Eight carried out no unannounced inspections across the entire five-year cycle.<sup>93</sup> Cyprus and Portugal have never conducted an unannounced visit since the Directive entered into force.

The UK Home Office recorded 169 breaches in 2023, involving approximately 155,000 animals; 57% related to basic husbandry failures—food deprivation, faulty ventilation, and overcrowding.<sup>94</sup> These numbers emerged despite mandatory training, designated veterinary care, and a public commitment to a “culture of care.”

Approximately 15,000 project licenses are authorized each year across the EU and Norway; refusal rates hover at approximately 4.5%, and eight countries reported zero refusals in 2022.<sup>93</sup> Only Ireland requires that at least one evaluator possess demonstrable expertise in NAMs. Elsewhere, applicants often face panels lacking sufficient specialist knowledge to rigorously challenge the necessity of using animals.

Empirical analyses confirm that approval bias is entrenched: over 95% of submitted protocols are ultimately approved, raising concerns that review processes function more as technical checks than as genuine harm–benefit evaluations.<sup>95</sup> Oversight committees are frequently dominated by animal researchers, whose professional background and career

incentives may predispose them toward accepting animal use rather than scrutinizing its necessity. In this “rubber-stamp” culture, discussions tend to focus on technical refinements or minor procedural adjustments, while claims of benefit are rarely interrogated with equal rigor.<sup>95</sup>

The lack of mandatory NAM expertise further compounds this bias, creating an uneven playing field in which proposals for human-relevant alternatives are disadvantaged from the outset. Requiring at least one accredited NAM specialist on every review and licensing panel would help counterbalance this bias and ensure that replacement options receive equitable consideration. Directive 2010/63/EU also allows each EU member state to determine how many licensing/inspection bodies—so-called competent authorities (CAs)—it will operate. The result is a patchwork: Denmark runs just one CA, Germany has 573, Poland 328, and Spain 112.<sup>93</sup> With hundreds of separate offices interpreting the same law, decisions on severity, housing standards, or project approval can vary substantially; multinational firms may therefore “forum-shop,” submitting their protocols in whichever country is known for the quickest or most permissive review.

Together, approval bias and fragmented governance reinforce systemic inertia, ensuring that the Directive’s stated principle of prioritizing replacement over refinement remains more aspirational than operational. This stands in direct contrast to Russell and Burch’s original hierarchy of the 3Rs, which placed replacement as the first priority.<sup>26</sup> In practice, however, committees largely default to approving animal use with minor refinements, leaving the Directive’s ethical foundation unfulfilled.

### **United States: Legal void post-Jarkesy ruling**

In June 2024, the US Supreme Court’s decision in *SEC v. Jarkesy* limited agencies’ use of in-house proceedings to impose civil penalties, prompting reassessment of available enforcement pathways.<sup>96</sup> Although not about animals specifically, the ruling has affected AWA oversight: during this period of reassessment, USDA’s Animal and Plant Health Inspection Service has not been issuing civil fines for AWA violations in research settings. Trends reported by the Animal Welfare Institute indicate long-standing concerns about weak deterrence and reduced penalties under the AWA, which predated but may now be compounded by this legal uncertainty.<sup>96,97</sup> Reporting on high-profile laboratories has likewise highlighted how weak enforcement cultures can manifest in practice, underscoring the need for transparent and legally durable sanction mechanisms.<sup>98</sup>

## **Ethical, scientific, and societal implications**

The licensing calculus that permits animal experiments depends on two premises: (1) that suffering is minimized and (2) that any remaining harm is outweighed by reliable human benefit.<sup>26</sup> Evidence reviewed throughout this chapter undermines both claims. Severity is habitually underpredicted, pain undertreated, and cumulative distress underreported,

while the anticipated benefits are undermined by irreproducible data, publication bias, and a ≥90% failure rate when animal-derived leads enter human trials.<sup>2,3</sup>

The scientific shortfall carries a measurable economic drag. The member countries of the Organisation for Economic Co-operation and Development spend an estimated US\$10–12 billion each year on regulatory and exploratory animal studies, even though validated NAMs are already matching—or exceeding—predictive value at lower cost and with faster turnaround.<sup>81</sup> Capital and early-career talent continue to be channeled into an R&D architecture that generates high clinical attrition, while organoids, microphysiological systems, and AI-enabled modeling continue to receive only a small fraction of public funding.<sup>6</sup>

A further distortion is now visible: federal and industry roadmaps increasingly market NAMs as complements to legacy animal pipelines rather than replacements. Initiatives such as NIH’s Complement-ARIE funding call<sup>99</sup> and FDA-endorsed “combinatorial NAMs”<sup>100</sup> actively promote murine or canine organ-on-chip systems and hybrid in silico platforms that are trained on animal data. Framed as synergy, the approach underwrites the animal-testing supply chain, siphons resources from fully human-based systems, and hard-wires the very translational failures NAMs were meant to solve.<sup>101–105</sup> Recent analysis has described this dynamic as a form of path dependency, in which institutional investment in animal-based

infrastructures and regulatory paradigms perpetuates the integration of animal systems into emerging NAM frameworks rather than enabling a genuine transition toward fully human-relevant science.<sup>106</sup>

Public tolerance is already conditional. In the UK, fewer than half of respondents support animal use “for any research as long as it is well regulated”<sup>92</sup>; in the United States, 80% favor a federal plan to phase it out.<sup>84</sup> Secrecy provisions, such as the United Kingdom’s retained Section 24 and the continued exclusion of purpose-bred mice, rats, and fish from US national statistics, erode that fragile social license to operate.<sup>18</sup>

Several policy measures are therefore critical for establishing a credible harm–benefit framework:

- Ring-fence ≥ 5% of public biomedical budgets for NAM development and validation.
- Legally define NAMs as human-derived or human-validated only; exclude animal-based “complementary” systems.
- Embed accredited NAM specialists on every review, funding, and regulatory panel.
- Publish outcome-based metrics—reproducibility indices, clinical translation rates, and surplus-breeding numbers—in open databases.
- Consolidate inspection into well-resourced agencies with ≥ 50% unannounced visits.
- Mandate colony-management tools (cryopreservation, genotype forecasting, regional sharing) to eliminate surplus kills.
- Require protocol-specific pain-recognition training and biomarker-supported humane endpoints.

- Attach binding sunset dates to each animal test once a NAM is validated (EU pyrogen-test precedent).

Almost seven decades after Russell and Burch coined the 3Rs, incremental refinement cannot compensate for structural scientific limits. Redirecting funds, embedding NAM expertise, enforcing outcome-based transparency, tightening enforcement, and inoculating human-based science against “complementary” co-option would steer biomedicine toward a paradigm that is humane by design and demonstrably more predictive for human health. The opportunity cost of inaction is no longer merely moral; it is scientific, economic, and societal.

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