Ending the use of dogs in research and testing

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Executive summary

This white paper focuses on the large amount of research and testing using dogs that is funded with U.S. taxpayer dollars by the National Institutes of Health (NIH), the U.S. Department of Health and Human Services' biomedical research agency, as well as the role various other U.S. government agencies play in maintaining reliance on the use of dogs in research and testing. There are **serious ethical and scientific issues associated with the use of dogs as experimental models**, which highlights the **need for a proactive approach to fully replace dogs** with non-animal technologies that are more human-relevant, effective, and efficient.

To investigate the context of dog use in biomedical research and testing, we undertook an in-depth analysis of NIH-funded projects using dogs. We also evaluated the likely experience of these dogs and estimated the ultimate impact of the research. Our analysis revealed that between 2015 and 2019, over **$200 million have been awarded by NIH to 200 individual institutions[1] for 303 separate projects using dogs as experimental models,** and yet **there appear to be few, if any, appreciable benefits to humans**. The dogs in these projects may have been subjected to multiple surgeries, fitted with devices to impair their heart function, had one of their lungs removed, or killed so that their organs could be examined—with very little return on this investment for human or animal health. To ensure that their tax dollars provide the greatest return to our citizens, we call for future **funding to be redirected toward projects employing more human-relevant methods for understanding disease and evaluating the safety of drugs and other products**.

Significant progress has been made in the development of these non-animal technologies, the so-called new approach methodologies (NAMs). These methods continue to evolve and improve, thus moving us closer to the elimination of dogs as experimental models for biomedical research and testing. The U.S. Environmental Protection Agency's (EPA) has recognized this, and has a stated commitment to end reliance on mammalian testing by 2035 [1] . We are calling for NIH and other federal agencies to adopt similar timelines for reducing animal use and adopting or supporting non-animal methodologies.

The Humane Society of the United States and Humane Society Legislative Fund are in agreement with provisions detailed in the 2019 budget approved by Congress and signed by the president [2] that requires the reduction or elimination of dogs from the U.S. Department of Veterans Affairs' (VA) research programs, as well as the related recommendations arising from the National Academies of Sciences, Engineering and Medicine, Institute for Laboratory Animal Research (ILAR) report, "Necessity, Use, and Care of Laboratory Dogs at the U.S. Department of Veterans Affairs" [3]. We are also supportive of the announcement from the Food and Drug Administration (FDA) that it is investigating opportunities to eliminate dog use in some bioequivalence studies [4]. These actions are welcome and indicative of a shift away from an animal-dependent research paradigm but are limited in scope and do not adequately address a glaring scientific issue: Research using dogs is not predictive of human responses [5-7]. We believe **there is much more that U.S. federal agencies can do to reduce dog use and eventually eliminate dogs in research and testing**.

We urge policymakers, funders and regulators to consider the recommendations summarized below, explained in detail on pages 17-18 and rationalized throughout this white paper. We call for the introduction of strict criteria for the use or funding of dogs in research and shifting focus and resources to non-animal

[1] Between May 2015 and September 2019, NIH awarded $202,427,478 to 200 individual institutions for 303 projects involving purpose-bred dogs. The majority of the awards (114 awards worth almost $92 million) went to 29 companies. Forty-two public universities were awarded 87 grants worth $42 million, with 23 private universities awarded 59 grants worth $32 million. Additionally, seven federal facilities, 12 hospitals, four Veterans Affairs facilities and 13 non-profits were awarded NIH funding for research using purpose-bred dogs.

approaches that lead to scientific progress, which benefits humans while sparing animals from suffering. Implementation of these actions will enable the move away from dog use in research and testing.

Recommendations

The HSUS and HSLF propose the following recommendations to reduce reliance on dogs in biomedical research and testing, to improve the development and application of non-animal, human-relevant methodologies that can replace dogs, to improve the lives for dogs while they are in laboratories and to ensure that these dogs are offered the chance of a loving family life when they leave the laboratory:

1. Federal agencies should follow the example of the EPA, defining a timetable for phasing out animal use, and the EPA under the Biden Administration should reaffirm its previous commitment made under the Trump Administration to end reliance on mammalian testing by 2035.

2. Veterans Affairs should implement ILAR's recent recommendations to prohibit "unnecessary" dog use and improve conditions for dogs in laboratories.

3. The FDA's Center for Drug Evaluation and Research (CDER), and its other centers, should undertake retrospective analyses of all dog studies submitted to, or generated by, the agency to assess the continued need for dogs.

4. The FDA's Center for Veterinary Medicine (CVM) should support the development and use of microphysiological systems using dog cell lines as replacements for live dogs.

5. NIH should:
   - Review the results of past and current NIH-funded projects using dogs to determine whether benefits to public health were realized.
   - Promote collaborative research between veterinary clinics and researchers to facilitate use of companion dogs in clinical trials.
   - Apply strict criteria before funding or carrying out research on dogs.
   - Redirect funding toward the development and use of non-animal approaches.
   - Commit to a timetable to phase out the use of dogs in laboratories.
   - Prohibit the use of dogs for any category E research (experiments in which dogs may be subjected to unrelieved pain and/or distress).
   - Improve the minimum welfare standards for dogs in laboratories.

6. All U.S. states should pass laws:
   - Ending the use of dogs in toxicity testing not required by federal law.
   - Requiring the adoption of dogs from research facilities until there is no further use of dogs in these laboratories.

Introduction

Why it's time to end animal testing

The HSUS and HSLF advocate an end to the use of **all** animals in biomedical research and testing. Accordingly, we strive to decrease and eventually eliminate harm to animals used for these purposes. Our concern encompasses all aspects of laboratory animal use, including their housing and care. We carry out our work on behalf of animals used and kept in laboratories primarily by promoting non-animal research methods (new approach methodologies or NAMs) that have the potential to reduce animal use, with the objective of replacing animals in laboratories. Replacement, reduction and refinement (improving welfare for the animals) are known as the 3Rs and this approach, rigorously applied, will benefit both animal welfare and biomedical progress.

There is growing recognition within the scientific community that rodents, dogs, monkeys, and other animals are not necessarily the optimal models for understanding human biology and disease [8-11]. It is evident that a move away from animals need not negatively impact research and that using human biology-based tools could accelerate understanding of human disease. For example, patient-derived organoids (PDO) are laboratory-based models of individual patient tumors that have emerged over the past decade and have been shown to recapitulate key features of the original cancer [12]. PDO offer a pathologically realistic platform against which to screen drugs and find effective treatment regimens [13]. The human biology-based models show impressive predictive abilities compared to animal-based approaches; a recent analysis of 55 cancer drugs showed that the drugs that were effective in treating PDO also had a positive impact for the patient [14]. In contrast, relying on animal models reduces the likelihood of success for cancer drugs to less than 4% [15], meaning that at least 96 out of every 100 possible cancer treatments fail when they reach the patient, despite appearing promising after animal testing. To capitalize on the potential for human biology-based tools such as PDO, the U.S. federal government should dedicate more financial support to these approaches to accelerate the move away from reliance on animals. There is evidence that this is ongoing but is at a nascent stage. Recent analysis indicates that NIH is starting to support new research grants that use PDO (this may be instead of, or in addition to, animals) but this funding still lags behind support for animal-based research. In breast cancer research, for example, between 2014 and 2019 NIH awarded less than $5 million to projects using PDO, with an average of two projects a year, compared to an annual average of 20 projects and over $54 million for breast cancer projects using animals [16].

The need for more dedicated support for new methods is not isolated to cancer research. The past decade has seen significant increases in the development of NAMs, with a slow rise in financial support for non-animal approaches. NIH's National Center for Advancing Translational Sciences (NCATS[2]) has awarded over $80 million for research on tissue chips, devices intended to serve as models of the structure and function of human organs. A total of almost $350 million has been allocated from other agencies, including the Defense Advanced Research Projects Agency (DARPA), the NIH Common Fund and other NIH Institutes, for the development and application of tissue chips for toxicity testing, understanding human disease, and drug screening [17]. This shift in funding is a promising start but still only represents a tiny fraction of the annual budget estimated at around $41 billion, approximately 50% of which is allocated to animal-based testing.

[2] National Center for Advancing Translational Sciences. (n.d.). https://ncats.nih.gov

Use of animals for safety testing

Animals are used for safety testing that is regulated by several federal agencies, including the EPA and the FDA. The EPA is charged with protecting human and environmental health through the analysis of safe exposure levels for various environmental chemicals, including pesticides. The EPA commitment to reducing animal use is apparent, with various initiatives supporting the 2019 directive calling for the agency to reduce animal testing and funding 30% by 2025 and to eliminate animal testing entirely by 2035.[3] The EPA has already committed over $4 million for the development and "use of alternative test methods and strategies that reduce, refine, and/or replace vertebrate animal testing."[4] Reaffirming its commitment to replacing animals within the timeline presented in 2019 would be a welcome sign that the EPA remains dedicated to animal replacement.

The mission of the FDA is stated as "protecting the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, and medical devices; and by ensuring the safety of our nation's food supply, cosmetics, and products that emit radiation."[5] The FDA recently started an Alternatives Methods Working Group[6] that aims to support the development and use of new technologies for regulatory toxicity testing, a step towards FDA's stated objective of "reducing, replacing and/or refining the use of animals in research, whenever possible."[7]

While this working group represents the right intentions, more needs to be done to enable drug developers to move away from animals. The current regulatory guidance may be non-legally binding, but it overwhelmingly describes animal testing, and there are many areas in which the FDA requires animal data prior to approval of products. This is despite abundant evidence that non-animal methods, including cell-based tests or computer modelling, are more predictive of human responses than animal testing [18-20]. The route to acceptance of non-animal data, and therefore replacement of animals in testing strategies, is not straightforward. Non-animal methods are subject to complex and extensive validation and qualification processes to which the animal tests have never been subjected. This can lead to a reluctance to develop or use non-animal approaches.

As a first step toward replacing dogs, we request that **the FDA carry out a retrospective analysis to evaluate what regulatory data from dogs were vital to decision-making**. Identifying where dogs are still necessary is an important first step in defining the areas in which NAMs need to be further developed and helps map out a timeline for replacing dogs in toxicity testing. NAMs developers have to be made aware of these areas of greatest need and funding should focus on these areas to promote the development of novel, and evolution of existing, NAMs and enable animal replacement.

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[3] U.S. Environmental Protection Agency. (2020, October 7). *EPA Announces Guidance to Waive Toxicity Tests on Animal Skin* [Press Release]. https://www.epa.gov/newsreleases/epa-announces-guidance-waive-toxicity-tests-animal-skin

[4] U.S. Environmental Protection Agency. (2019, September 10). *Administrator Wheeler Signs Memo to Reduce Animal Testing, Awards $4.25 Million to Advance Research on Alternative Methods to Animal Testing* [Press Release]. https://www.epa.gov/newsreleases/administrator-wheeler-signs-memo-reduce-animal-testing-awards-425-million-advance

[5] U.S. Food & Drug Administration. (2018, March 28). *What We Do*. https://www.fda.gov/about-fda/what-we-do

[6] U.S. Food & Drug Administration. (2021, January 21). *Advancing Alternative Methods at FDA*. https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda

[7] U.S. Food & Drug Administration. (2018, November 16). Statement by FDA Commissioner Scott Gottlieb, M.D., on efforts to reduce animal testing through a study aimed at eliminating the use of dogs in certain trials. https://www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-efforts-reduce-animal-testing-through-study-aimed

Ethical and scientific issues associated with the use of dogs

PHOTO BY BRYAN MITCHELL/AP IMAGE FOR THE HSUS

The breed most commonly used in research is the beagle [21]. This preference is not attributable to any close physiological comparison to people or any other scientifically defensible reason. The origin of beagles as experimental models dates back to the 1950s [22] and the horrific radioactive testing program that these dogs were subjected to because of their longer lifespan compared to other dog breeds. Their use persists over 70 years later, with beagles being used mainly because of their small size and gentle, trusting, docile nature. These dogs are purpose-bred for a life in the laboratory with some companies offering "pathogen-free" dogs [23]—animals bred and raised in sterile, barren, unnatural environments. Often dogs born with genetic (inherited), debilitating conditions are maintained in "colonies" in research institutes so that they can be bred for use in specific experiments. The dogs are frequently inbred, with father-daughter or father-granddaughter matings used to maintain the disease. This inbreeding can lead to further complications, with research from the dog colonies indicating a positive correlation between inbreeding and mortality [24]. The original "founder" dogs who are used to start these colonies will be used extensively for breeding, despite the issues with their own health. One report states that a sick dog was used to breed five litters before she died at only 5 years old [25]. Genetic conditions may impact dogs differently than humans. For example, Duchenne muscular dystrophy, a disease primarily found in males, leads to very high mortality before the age of 6 months in golden retrievers but not in human males born with this condition [26].

Dogs not only differ from humans, but they also differ from one another. For example, greyhounds (another docile breed used for research) exhibit significant differences in metabolism compared to beagles [27]. Recent research has shown that testing on purpose-bred beagles is liable to give significantly different results when compared to companion beagles [28]. Dogs purpose-bred for research and testing are so inbred and lacking in genetic diversity that their responses are unlikely to be an accurate representation of other companion dogs of the same type. Results from laboratory beagles do not accurately predict companion beagle dog responses [29], and yet data from these inbred beagles are being used to inform human health research, and drug and chemical safety decision-making.

While dogs have been used historically as models of human diseases, this does not justify their continued use. This point was recently acknowledged in the ILAR review of dog use in VA research [3]. Researchers now accept that the use of dogs as experimental models originated because of their size, availability and ease of use—not because of scientific validity [5]. In fact, **there are few close physiological similarities between dogs and humans**, as would be vital for accurately extrapolating adverse effects from one to the other [5]. Besides the obvious anatomical disparities between dogs and humans, there are other important physiological differences, which means that the response in a dog—to a drug, chemical or disease—can be very different from a human response to the same stimulus.

For example:

- Dogs have significantly higher heart rates than people [30].
- There are important electrical differences between dog and human heart function [31].
- Dogs have a specialized circulatory system that protects them from blood vessel blockages [32].
- Digestion is different in dogs; gastric acid levels are higher and food remains in the stomach longer [33].
- There are significant differences in liver enzyme activity between dogs and humans [27].

- Extensive analysis of saliva has revealed that dog secretions are highly anti-microbial when compared to human [34].
- There are important differences in the composition of dog blood compared to human blood [35, 36].

The need to move away from dogs: Dogs do not predict human responses

There is a growing body of scientific evidence showing the substantial disconnect between animal data and human data [37-49]. NCATS declares that, "Approximately 30% of promising medications have failed in human clinical trials because they are found to be toxic despite promising preclinical studies in animal models. About 60% of candidate drugs fail due to lack of efficacy.[8]" This equates to approximately 90% of drugs that appear safe and effective in preclinical animal testing to be either unsafe or ineffective in humans [50].

A drug tested in animals is not guaranteed to be safe and effective in a person: Animal data do not predict human responses. The best-case scenario is that we are losing drugs that may be useful for humans (these are discarded due to toxicity observed in animals), but the worst-case scenario is that drugs that appear safe in animals may go on to cause serious harm, and even death, in people [51]. This is also true for drug efficacy: There are numerous examples that show animal models of disease fail to recapitulate the human condition [52-54] and that animals genetically engineered to show symptoms of a human affliction do not offer a route to effective treatments. For example, Vioxx (rofecoxib) was originally designed to treat pain related to osteoarthritis and was approved by the FDA in 1999. Vioxx use was linked to over 38,000 cardiovascular-related deaths and myocardial infarctions (MI) in human patients and withdrawn from the market in 2004 [55]. Animal studies (including those performed on dogs) failed to indicate increased risk of cardiovascular events. Instead, it took four years of long-term clinical studies in patients, and several complex meta-analyses of these data, to show that Vioxx was responsible for increasing risk of heart attack and stroke in humans [56].

Researchers have referred to the translational failure inherent in preclinical animal testing as an "insurmountable problem of species difference" [45] and these so-called translational failures, together with the continued reliance on poorly predictive animal models, has pushed drug development into crisis. Development costs are increasing [41], clinical trial failures are reported on an almost daily basis [57] and the number of new drug approvals is stagnating: In the last decade, fewer than 40 drugs, on average, received FDA approval each year [58]. Overall, the likelihood of approval for a new drug is less than 10% [59]. This means that more than nine out of every 10 drugs in development—which are being tested on hundreds of thousands of animals, including dogs—are ultimately failing patients in need.

The EPA has committed to ending reliance on mammalian testing by 2035 [1] and has carried out several retrospective analyses that have ultimately enabled the elimination of several toxicity tests previously required for pesticide registration, including the one-year dog test [60]. But the FDA has yet to follow suit with similar retrospective analyses, which are vital to determining dogs' possible role in providing essential information for drug approval. Therefore, we request the FDA carry out retrospective analyses, in conjunction with independent experts at the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), as a matter of urgency to clearly determine whether the use of dogs is necessary, and, if so, under what circumstances dog testing is vital for regulatory decision making. We also ask that NIH carry out similar retrospective analyses of the research using dogs it has funded to define areas that fail to progress or benefit from the use of dogs. These analyses would help develop a set of criteria detailing conditions where dog testing is unnecessary, which would enable companies

[8] Efficacy refers to whether a drug has the desired therapeutic effect.

to avoid testing on dogs and allow NIH to refine research project funding to ensure that the most suitable methods are supported, therefore driving impactful science that benefits human and animal health.

The extent of dog use in research and testing

Thousands of dogs suffer and die every year

Analysis of the United States Department of Agriculture (USDA) annual reports of registered research facilities shows that more than 55,000 dogs were used or held by U.S. research and testing facilities in 2019 alone, with almost 50,000 of those dogs used for research or testing and most likely killed. These data have not changed significantly over the past 20 years, showing no sign of a sustained decrease (Figure 1), thus indicating continued use of dogs and a reluctance to shift to more advanced, human-relevant methods currently available.

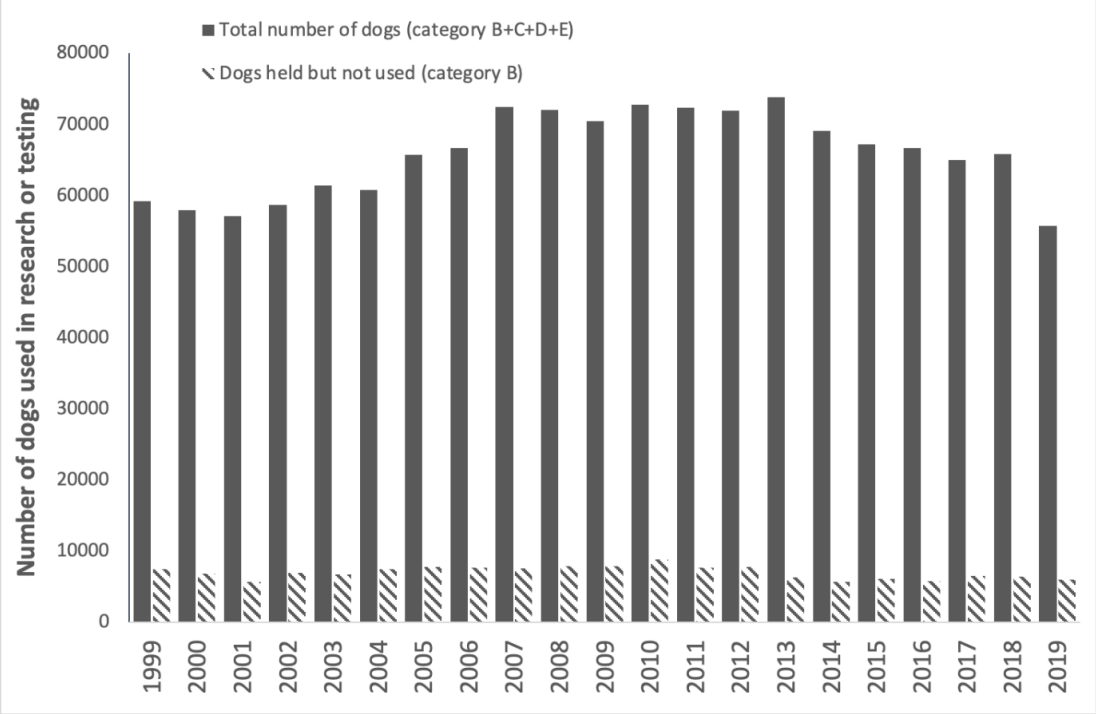

Figure 1. Dog use for research and testing in the U.S. has remained above 50,000 dogs per year since 1999.
The figure shows the total numbers of dogs used in research and testing in the U.S. annually (USDA data - categories B, C, D and E[9]; gray bars) and the numbers of dogs kept in laboratories but not used in experiments in that year (category B; hatched bars). These data show that dog use is not undergoing any sustained decline despite the continued development and evolution of non-animal methods and public outcry over the use of dogs for experimental research.

[9] USDA annual reports of animal use require that animal numbers are returned in categories B through F. The criteria for each category are defined as follows: "Column B: the number of animals (regulated species) being bred, conditioned or held for future use; Column C: the number of animals that are used in procedures that do not involve pain and/or distress and for which the use of anesthetics, analgesics or tranquilizers was not indicated; Column D: the number of animals that are used in procedures which would involve more than slight or momentary accompanying pain or distress and for which appropriate anesthetic, analgesic or tranquilizing drugs were used; Column E: the number of animals subjected to procedures involving more than slight or momentary accompanying pain or distress in which appropriate anesthetics, analgesics, or tranquilizing drugs are withheld because their use would have adversely affected the teaching, testing, or experiments; Column F: the sum total of the animals listed in Columns C, D, and E."

There are regulatory constraints that may limit the current use of non-animal methods for safety testing, but this could be addressed by **using retrospective analyses to identify where more NAMs development is necessary** and then dedicating funding to enable this progress.

On average, since 1999, (the first year for which records are publicly available), almost 65,000 dogs a year have been utilized by research facilities, including more than 7,000 held in laboratories and not currently used for research/testing. While the number of dogs used for biomedical research and testing has gradually declined since the 1970s, with over 86,000 dogs used in 1973 [3], annual numbers in this century have remained fairly steady.

It is concerning that there is no sustained downward trend in these data. Employing the annual difference in the numbers of dogs used between 2013 and 2019, we calculated an average decrease of around 1,000 dogs per year. At this rate, it **would take until the year 2114, 96 years from now, before dog use reaches zero**.

Dogs used in Category E: Hundreds of dogs every year are subject to severe, unrelieved pain

Using USDA data from 1999 to 2019, we calculated that an average of 660 dogs a year have been subjected to procedures that cause unrelieved pain and/or distress (Category E). All research using dogs is concerning, but subjecting dogs to pain or distress without any relief raises serious ethical issues, particularly because most research fails to demonstrate appreciable benefit to humans or animals. One analysis revealed that the vast majority (over 76%) of experiments in which animals experienced pain were rated by expert reviewers as having little or no clinical or applied value and over 70% had little or no scientific value [61][10].

As defined by the USDA, the following are examples of procedures that may be performed on animals without the use of anesthetics, analgesics, tranquilizing drugs or other measures to relieve pain or distress:

- "Drug, pesticide or radiation toxicity testing involving extremely high doses of these substances.
- LD50 determinations (LD50 refers to the amount of test substance required to kill 50% of the animals) or any other studies involving death as an endpoint.
- Multiple survival surgeries.
- The exposure of an animal to an agent that produces unrelieved pain or distress.
- The exposure of an animal to electrical shocks that are generally accepted as causing pain in humans." [62]

Current use of dogs in taxpayer-funded projects

Table 1 summarizes our analysis of NIH-funded research projects retrieved through a search of the publicly available database RePORTER.[11] From 2015 to 2019, 387 projects that were funded by, or received continued funding from, NIH were associated with the keywords "canine", "dog" or "Canis familiaris." Of these, 303

[10] This study used animal research protocols from U.S. Institutional Animal Care and Use Committees (IACUCs) and thus only refers to those animals covered under the U.S. Animal Welfare Act, defined as "any live or dead dog, cat, monkey (nonhuman primate mammal), guinea pig, hamster, rabbit, or such other warm-blooded animal, as the Secretary may determine is being used, or is intended for use, for research, testing, experimentation, or exhibition purposes, or as a pet; but such term excludes (1) birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research."

[11] National Institutes of Health. (n.d.). https://reporter.nih.gov

projects used purpose-bred laboratory dogs for research or testing procedures with varying degrees of invasiveness that often ended with euthanasia.

To summarize, NIH awarded over $202 million in funding to 200 individual institutions for 303 projects involving purpose-bred dogs. The majority of the awards (114 worth almost $92 million) went to 99 companies, 42 public universities were awarded 87 grants worth $42 million and 23 private universities were awarded 59 grants worth $32 million. Additionally, seven federal facilities, 12 hospitals, four VA facilities and 13 non-profits (mostly research institutes) were also awarded NIH funding for research on dogs.

Despite over $202 million being awarded to projects using purpose-bred dogs, there were very few significant outputs (i.e. patents or clinical trials) associated with this research, according to our analysis of data from the RePORTER database. This means that **millions of tax dollars are repeatedly awarded to projects that fail to provide any appreciable benefits to humans, while causing considerable harm to these dogs**.

We would echo the recommendation of the ILAR committee on dog research carried out by the VA, that is, to track the impact of past and current NIH-funded research requiring dogs for such time as this is still permitted: "Specifically, the VA should: Establish a mechanism for tracking the impact and translation of research using dogs. Such a retrospective reporting mechanism should use objective and state-of-the-art methods (e.g., bibliometrics or citation in regulatory documents and patents) to track the relationship between dog experiments and translated interventions for veterans. Such performance assessment should be required to establish, and if need be correct, risk–benefit and welfare assessments used in the authorization of research." [3]

It is apparent from our analysis that the majority of studies using purpose-bred dogs are failing to achieve the level of benefit to humans that would justify the harms caused to the dogs, or at least that these benefits are not captured by the database or in the scientific papers published by the grant holders. We urge **NIH to review the results of past and current NIH-funded projects using dogs to determine whether benefits to public health were realized**. To maximize returns on investment, NIH could adjust funding criteria to ensure that projects are not continually receiving funding for invasive research on dogs that fail to translate to effective treatments for human or animal health.

However, our analysis revealed one notable exception: Cancer research appears to be undergoing somewhat of a paradigm shift such that client-owned dogs are recruited as so-called parallel patient populations. This refers to research in which companion dogs with spontaneously occurring tumors are recruited to clinical trials for testing novel treatments that may directly help the dogs and translate to effective treatments for corresponding human cancers, benefiting both the human and canine patient [63-66]. It is encouraging that fewer than half of the 65 cancer projects funded by NIH used purpose-bred dogs. This shift toward the use of companion animals, whose owners' consent to their use in the research and who may benefit from participation, was recognized in the ILAR review and articulated as a formal recommendation: "Recommendation 5: Establish long-term external collaborations to optimize the use of companion dogs and humans in biomedical research." [3]. We urge NIH to consider this approach more widely across its biomedical research portfolio to enable more rapid acceleration of the phasing out of purpose-bred dogs and to promote research that truly benefits animals and humans.

Table 1: NIH-funded projects retrieved using the keywords "canine" or "dog" or "Canis familiaris"

For projects receiving funding between 2015 and 2019, total costs are given and the number of projects using dogs as experimental models was assessed to allow calculation of the costs of research on purpose-bred dogs. Projects with a primary purpose of 'Animal-assisted therapy' look at the relationships between dogs and people, and do not use purpose-bred dogs, so are not included in the final cost analysis. In contrast, projects under the category of 'Animal model facility funding', may not explicitly state the use of purpose-bred dogs in all the applications, but the mention of 'large animal models' indicates that dogs are likely to be part of the research at some level and therefore these projects were included in the cost analysis.

Primary purpose of research or testing	Total number of projects using dogs	Number of projects using purpose-bred dogs[12]	Total costs (USD)	Costs for projects using dogs as experimental models
Animal-assisted therapy	13	N/A	3,023,186	N/A
Animal model facility funding	12	12	3,771,648	3,771,648
Cancer	64	25	56,555,849	14,916,474
Cardiac	38	38	20,800,077	20,800,077
Dental	2	2	590,212	590,212
Diabetes	8	7	3,926,060	3,780,920
Diagnostic tools	14	6	4,436,335	3,232,484
Gene therapy	32	30	15,100,498	14,612,675
Genetics	9	7	9,589,148	8,238,196
Hematology	6	5	2,730,265	1,806,080

[12] Some projects were designated as clinical and only used client-owned companion dogs. We did not include these in this analysis and instead specifically calculated the number of projects that were using purpose-bred dogs who are likely to have to live in the laboratories/testing facilities throughout their lives.

Immunology	6	3	4,990,493	2,236,671
Infectious disease	9	7	3,228,702	2,277,190
Medical devices	8	8	3,833,700	3,833,700
Muscular dystrophy	1	1	315,700	315,700
Neurological	6	3	2,614,551	1,862,605
Ocular	7	7	2,666,432	2,666,432
Orthopedics	6	6	1,906,951	1,906,951
Other	6	2	6,330,164	1,128,832
Pharmaceutical	123	116	111,987,640	108,243,091
Pulmonology	3	3	984,242	984,242
Stem cells	5	5	2,229,036	2,229,036
Vocal	8	8	3,185,277	2,994,262
TOTAL	**388**	**303**	**264,796,166**	**202,427,478**

Non-animal approaches to replace dogs in research and testing

New approach methodologies are superior to animals

One of the advantages of NAMs is that they can fully exploit scientific advances to continually evolve and improve. For example, researchers are creating microphysiological systems for every human organ and are connecting these to create the human-on-a-chip (for understanding normal function and development) and the patient-on-a-chip (for developing new drugs and testing personalized medicine approaches) [67-71]. In contrast, animal models of human disease may only be tweaked as technologies improve; the animals themselves do not alter and so these model systems retain the inherent species differences that hinder their ability to predict the human response: Animals will never be valid models of humans [45]. The introduction of human genes into animals, creating "humanized" animals (invariably mice), is claimed to give greater human-relevance to these models [72], but this process is both technically and ethically challenging, costly and inefficient, and humanized animals still bear the species differences that contribute to the lack of predictive ability [73, 74]. However, the scientific advances occurring in the creation of sophisticated human cell-based systems, like organoids and microphysiological organ chips using human-induced pluripotent stem cells, are ongoing, cumulative and offer a more realistic platform to model healthy human organs, understand human diseases and disease progression and even mimic individual patients [75-78].

Life in a home after time in the laboratory

Most dogs used in research are normal, healthy and very young, between 1 and 5 years old, and the majority are killed at the end of the experiment, even if they survive the testing, e.g. [79-81]. Until dogs are fully replaced for research and testing, they should be afforded the opportunity to live a happy life in a home as a companion instead of being euthanized, whenever possible. As a step in the right direction, the FDA has an initiative to investigate whether analyzing blood samples from live dogs, instead of euthanizing animals for tissue analysis in bioequivalence studies of anti-parasitic products, is an approach that could be applied more widely. This pilot project will save several dogs' lives and allow them to be adopted [4], but it has the potential for saving many more dogs. For example, these data could be used to develop computer-based models for drug absorption that may replace dogs in future tests. **We welcome this preliminary advance, but it represents a very first step of many that should be taken**.

Some federal agencies, such as VA, NIH and FDA, have put dog adoption policies in place [82], demonstrating that it is possible for purpose-bred laboratory dogs to lead normal, happy family lives. There are numerous examples of successful adoptions following a dog's time in the laboratory, including a group of 32 dogs used in pesticide testing and successfully placed in homes by an animal shelter [83]. State adoption laws are already in place in 12 states (California, Connecticut, Delaware, Illinois, Maryland, Minnesota, Nevada, New Jersey, New York, Oregon, Rhode Island and Washington), setting precedents for the remaining states and ultimately federal legislation.

Phasing out dogs in laboratories

In a research landscape where funds are scarce and competition is fierce, NIH and other funding bodies should more carefully scrutinize projects that are reliant on outdated animal models and have limited benefit

to humans. Recent news articles revealing the plight of dogs in labs [84] indicate the public's clear displeasure over their tax dollars being used to support these experiments, and opinion surveys increasingly show that people would fully embrace a modern research strategy that prioritizes funding for and use of non-animal, human-relevant methods [85, 86].

In order to enable the full replacement of dogs in biomedical research, we encourage NIH, the largest funder of biomedical research in the world, as well as other agencies, to follow the example given in the ILAR review of VA research using dogs [3], and similar principles and criteria found in the Institute of Medicine's report, "Chimpanzees in Biomedical and Behavioral Research: Assessing the Necessity*"*[87]. The conclusions of the 2011 chimpanzee report were driven by scientific advances in models and methods that eschewed chimpanzee use. The committee developed three principles to assess research on chimpanzees and additional criteria for determining the necessity of using chimpanzees in biomedical research (Table 2). We recommend that NIH develop similar principles and criteria for the use of dogs.

Table 2 – Principles and criteria for the use of chimpanzees in biomedical research

Principles Guiding the Use of Chimpanzees in Research
The knowledge gained must be necessary to advance public health
There must be no other research model by which the knowledge could be obtained, and the research cannot be ethically performed on human subjects
The animals used in the proposed research must be maintained either in ethologically appropriate physical and social environments or in natural habitats.
Criteria for the Use of the Chimpanzee in Biomedical Research
There is no other suitable model available, such as *in vitro*, non-human *in vivo*, or other models, for the research in question
The research in question cannot be performed ethically on human subjects
Chimpanzees are necessary to accelerate prevention, control, and/or treatment of potentially life-threatening or debilitating conditions

Reduce and refine dog use in research and testing - until dogs are fully replaced

The ILAR report on the use of dogs in VA research recognized the need to improve the welfare of dogs in laboratories, setting out various recommendations for dogs during the time that the research is deemed necessary. These recommendations act as a useful starting point to improve the lives of purpose-bred dogs

in laboratories and should be made a prerequisite for any NIH-funded research. In addition, we urge NIH to take a more stringent approach and, until such time that a complete phaseout of dog use is implemented, **NIH-funded research should require that the following criteria and welfare standards be met to permit the use of dogs** (using the IOM chimpanzee report as a model).

Criteria for dog use in research:

- The knowledge gained must be novel, of benefit to wider society and necessary to advance understanding of public health issues.

- Repeat applications, where funding was previously awarded to studies using dogs, must be carefully scrutinized against these criteria. If dog use is still required, this must only be accepted with a defined timeline for phasing out dog use.

- There are no other suitable models, or combinations of models, available, in this order of priority: non-animal methods, including but not limited to *in vitro* (cell lines, primary cells, organoid cultures, microphysiological systems, stem cells), *in silico* (mathematical modeling using existing data sets, computational modeling), *ex vivo* methods (tissue slices, explant cultures), *in vitro* models employing animal cells or animal tissues, human volunteers, or, where all other possibilities have been exhausted, a non-canine animal model. Where research questions cannot be answered by one or more of the non-animal methods, the necessity for using whole animals should be clearly justified and the use of immature forms (embryos) or invertebrates should be considered. In general, NIH-funded research should commit to the use of the least sentient organism, as per currently available scientific information, including but not limited to *Dictyostelium discoideum* [88], *Caenorhabditis elegans* [89], *Lymnea stagnalis* [90], and zebrafish larvae [91].

- The study cannot be performed ethically using human subjects.

- Where it is determined that the research necessitates the use of dogs, NIH should encourage collaboration between laboratory animal veterinarians and companion animal veterinarians and contribute to the rise in veterinary clinical trials [92] by first considering the use of client-owned animals enrolled in clinical trials instead of requiring purpose-bred research animals.

- Researchers have consulted with statisticians to design experiments with greater measurement precision, improving the signal-to-noise ratio of the data analysis and enabling a reduction in the number of animals required, and should not rely on power analysis to calculate required numbers of animals. They should also consider the most appropriate statistical analysis to ensure that all studies using dogs are reproducible [93].

- Because there is no current standard in U.S. law that restricts the level of harm an animal used in research may experience, NIH should adopt a similar policy to the European Directive 2010/63/EU: "... the performance of procedures that result in severe pain, suffering or distress [that] is likely to be long-lasting and cannot be ameliorated should be prohibited" (art. 23).

Welfare standards:

- The dogs used in the proposed research must have all their basic needs met. This includes, but is not limited to, the needs previously described by Dr. David DeGrazia in his presentation to the National Academy of Science, Engineering and Medicine as part of the ILAR review [94]:
    - Nutritious food and clean water
    - Appropriate shelter
    - Adequate stimulation, exercise, opportunities for canine-typical functioning to promote good health and psychological wellbeing in all animals including access to outdoor runs
    - Sufficient rest for health
    - Veterinary care
    - Access to compatible dogs or social group members ensuring dogs are not single-housed for more than for hours at a time[13]
    - Freedom from significant experiential harm
    - Freedom from disease, injury and disability
    - Freedom of movement with adequate space

- The dogs should never be subjected to unnecessary harm including through negligence or lack of care in handling, transport, housing, etc.

- Laboratories must develop new, or enforce existing, policies requiring adoption of dogs after their use in research/testing has ended.

Recommendations to protect dogs today and to ensure an end to their use in research and testing

1. Following the example of the EPA, the VA, FDA and NIH should define timetables for phasing out the use of dogs and other mammals, stating the year in which they will no longer be required for research or testing. Agencies should commit to shifting research funds to the development of non-animal methods to enable wider replacement of animal use. Additionally, the EPA should reaffirm its commitment to 2035 as its end date for relying on mammal testing.

2. The FDA's CDER, in conjunction with an independent entity such as NICEATM, should launch a thorough retrospective analysis to evaluate how data from dog studies has historically impacted risk assessments during the drug approval decision-making process and adjust decision-making processes based on findings. Similar analyses should be conducted in regard to other products regulated by FDA.

3. The FDA's CVM should support the development and use of microphysiological systems using dog cells as replacements for live dogs. Initially, data from organ-chips using dog cells

[13] A recent report from the European Union concluded that, for the metabolic studies required in some toxicity testing regimes, "pair housing of dogs … does not compromise the scientific integrity, and therefore is a major progression in the design of these studies, enhancing welfare" [95].

could be submitted alongside data obtained from the use of live dogs, with the ultimate aim of replacing data from testing on live dogs with chip data.

4. The Department of Veterans Affairs should implement the recommendations from the report to:
   - Prohibit the use of dogs in areas identified as unnecessary [3].
   - Improve living conditions for dogs when they are used in research funded or carried out by the agency.
   - Continually measure the impact of dog research to determine necessity.

5. NIH should:
   - Review the results of past and current NIH-funded projects using dogs to determine whether benefits to public health were realized.
   - Use a parallel patient population approach, promoting collaborative research between veterinary clinics and researchers to facilitate use of companion dogs in clinical trials that directly benefit the dogs and potentially humans.
   - Apply strict criteria before funding or carrying out research on dogs (see page 16 for details on criteria).
   - Redirect funding toward the development and use of NAMs, which are more human-relevant and provide better understanding of human disease, indicate more appropriate human drug targets and enable faster, more cost-effective drug development. Further, grant proposals that appropriately use non-animal approaches over the use of dogs should be given priority in terms of funding.
   - Commit to a timetable to phase out the use of dogs in laboratories. This must include a declaration that NIH will not accept or fund any new grant applications that require the use of dogs as laboratory subjects and that projects with dogs currently funded by NIH will actively pursue application of NAMs.
   - Prohibit the use of dogs for any Category E research (where the dogs may be subjected to unrelieved pain and/or distress).
   - Improve the minimum welfare standards for dogs during the time that they are subject to laboratory conditions (see pages 16-17 for details on these standards), and at the end of the experimental period, dogs must be re-homed following the creation of new (or adherence to existing) adoption programs.

6. All states should pass laws:
   - Limiting the use of dogs in toxicity testing not required by federal law in agreement with recent legislative efforts in California, the Prevent Extraneous Testing Act.
   - Requiring the adoption of dogs from research institutions until there is no further use of dogs in these laboratories. Adoption laws should also be federal policy. For states that already have laws in place, they should be expanded to include all facilities using dogs and require public disclosure of the numbers of dogs that are made available and ultimately adopted into loving homes.

Conclusion

Every year, millions of U.S. taxpayers' hard-earned dollars are being used to fund painful, distressing experimental procedures on dogs, when an increasing number of non-animal methods are becoming available with the potential to replace the use of dogs.

Our research revealed that over a recent period of four years alone, NIH awarded more than $202 million to 303 projects that ultimately resulted in the deaths of thousands of dogs. We could not attribute any great advances in human health research (few new patents or new drugs, or obvious advances in treatment strategies, etc.) to any of these projects and this calls into question whether there are any tangible human benefits to this controversial, harmful, and harrowing research.

The HSUS and HSLF are calling for the elimination of the use of dogs as laboratory models, an end to the deliberate breeding of dogs with debilitating genetic conditions and an end to the infliction of severe, unrelieved pain. We lay out our recommendations for change (pages 17-18), offering a win-win for science and society. Science benefits from more human-relevant methods to progress our understanding of human disease and evaluate possible treatments; safety testing benefits from a more effective, efficient and predictive approach to assessing chemical safety; and ultimately, society benefits through development of safe and effective drugs using non-animal, human-relevant methods that are more cost-effective and reach the patients in need in a more timely fashion.

The failure of dog models coupled with advances in NAMs are such that progress in science and biomedical research will not be negatively impacted by moving away from the use of dogs as test subjects and will, in fact, benefit people and animals. We believe that having federal agencies formally set goals and timetables to eliminate the use of dogs will ultimately lead to an acceleration in the development of appropriate NAMs to replace them.

About us

The Humane Society of the United States is the nation's most effective animal protection organization, fighting for all animals for more than 60 years. Together with millions of supporters, we take on puppy mills, factory farms, trophy hunts, animal testing and other cruel industries. With our affiliates, we rescue and care for thousands of animals every year through our animal rescue team's work and other hands-on animal care services.

The Humane Society Legislative Fund's mission is to ensure that animals have a voice before federal and state lawmakers by advocating for measures to eliminate animal cruelty and suffering, to educate administrative and elected officials, as well as the public on animal welfare issues, and to elect humane candidates to public office.

Glossary of acronyms

3Rs	Replacement, reduction and refinement of animal use in research and testing
CDER	FDA's Center for Drug Evaluation and Research
CVM	FDA's Center for Veterinary Medicine
DARPA	Defense Advanced Research Projects Agency
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
HHS	U.S. Department of Health and Human Services
HSLF	Humane Society Legislative Fund
HSUS	Humane Society of the United States
ILAR	Institute for Laboratory Animal Research
IOM	Institute of Medicine
NAMs	New approach methodologies
NAS	National Academy of Sciences
NCATS	NIH's National Center for Advancing Translational Sciences
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
PDO	Patient-derived organoids
USDA	U.S. Department of Agriculture
VA	U.S. Department of Veterans Affairs

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