Policy Brief

Understanding the Global Gain-of-Function Research Landscape

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Executive Summary

Gain-of-function (GOF) and loss-of-function (LOF) research are two valuable methodologies that allow scientists to study pathogens. These interconnected research approaches alter pathogens' genomes to add or subtract functionality, allowing scientists to examine and better understand how pathogens function and develop new vaccines and therapies.

Despite its widely recognized value for science, gain-of-function research has attracted attention and concern from U.S. policymakers due to what some see as inherent risks in this methodology, particularly following the outbreak and debated origins of the COVID-19 pandemic. The risk that gain-of-function research could inadvertently contribute to pandemics or widespread illness has sparked discussion about new regulations. LOF research results in weakened pathogens—and thus does not impart the same risks as GOF research—and is rarely mentioned in policy debates in the same way as GOF research.

In this report, we map the gain- and loss-of-function global research landscape using a quantitative approach that combines machine learning with subject-matter expert review. We identify about 7,000 PubMed research papers related to our criteria for GOF and LOF research, published between 2000 and mid-2022. Our research shows that GOF and LOF research are intertwined; they are conducted using the same experimental procedures and thus would both be impacted by any future regulations. As such, throughout this report, the two types of research are often discussed in tandem. Our aim is to help policymakers understand the research landscape in order to more effectively mitigate risks without impacting beneficial GOF and LOF research.

Our key findings include:

1. **Gain- and loss-of-function research is ongoing, global, and collaborative** with U.S.-affiliated researchers contributing to approximately half of identified publications between 2000 and mid-2022.
2. **Gain- and loss-of-function research frequently co-occur in the same study**. That said, LOF research appears in more publications than GOF research.
3. **Gain- and loss-of-function research is conducted over a range of different experimental methodologies, pathogens, and applications.**
    - Methodologies: GOF and LOF research does not require cutting-edge gene-editing technologies; 21 percent of all publications we identified for this report use serial passaging instead of other more technically

sophisticated techniques such as CRISPR. The use of serial passage is more frequent in GOF publications than LOF publications.

- Pathogens: GOF and LOF research involves pathogens that span the four biosafety levels (BSLs), with nearly all research being conducted on pathogens that are categorized as BSL-2, BSL-2+, or BSL-3.
- Applications: a range of research topics involve GOF and LOF research. For example, approximately 24 percent of the identified publications were related to vaccine development and the most-studied pathogens are those that cause high global health burdens.

Based on our analysis, we assess that GOF and LOF research will be difficult to regulate because:

1. **Gain- and loss-of-function research are widely used in public health applications.** Regulations will need to target the types of research that cause the most risk without impeding disease research or therapy development.
2. **Gain- and loss-of-function research are intertwined.** Regulations that restrict GOF research will also restrict less risky LOF research, potentially delaying public health developments without achieving the desired safety enhancements.
3. **Researchers cannot always predict whether an experiment will cause a pathogen to become more or less virulent.** Experiments that were not anticipated to be GOF research may not be prevented by proactive regulatory requirements.
4. **Gain-of-function research can be conducted without access to gene editing technologies.** Regulating gene editing technologies, including CRISPR or DNA synthesis, would not affect the approximately 21 percent of experiments that were conducted using serial passaging.
5. **Risk varies among GOF studies, and should not be uniformly regulated.** The risk level of GOF and LOF research changes based on experimental factors including the pathogen's biosafety level, methodology, and the animal model(s) used. Regulations will need to target the types of research that cause the most risk rather than impose a one-size-fits-all regulatory policy that does not account for these vital differences.

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Introduction

Altering a pathogen's genome is an established way to study gene function, including how a pathogen's genetic code impacts its ability to cause infection. Experiments that result in pathogens with additional functionality are categorized as gain-of-function (GOF) research, while genetic changes that result in weakened pathogens are categorized as loss-of-function (LOF) research.

GOF and LOF research is not new. In the late 1800s, Louis Pasteur created the first vaccines for chicken cholera, anthrax, and rabies using LOF experiments.[1] Since then, both GOF and LOF research have led to landmark scientific breakthroughs in vaccine development, genetic research, and gene therapy. Today, scientists continue to rely on these methods to augment our understanding of disease transmission and the relationships between genes and physical traits, among other uses.

A subset of GOF and LOF research involves high-risk, highly virulent pathogens that are capable of spreading widely among humans. This research can be scientifically valuable for understanding how to fight and prevent diseases and to study new, virulent pathogens. The COVID-19 pandemic and the associated theory that it originated from GOF research at the Wuhan Institute of Virology in China has sparked renewed policymaker interest and debate about the risks and benefits of this kind of research. In the United States, on a federal level, Congress has considered reducing National Institutes of Health (NIH) funding for GOF pandemic preparedness research while at the state level, Florida has banned GOF research.[2]

In March 2023, the National Science Advisory Board for Biosecurity (NSABB) published a new proposed oversight framework to assess, regulate, and mitigate risks from potential pandemic pathogens. The proposed framework led to debate among scientists about the scope and scale of future regulations with some believing GOF research should be halted altogether and others arguing for more effective regulation to enable safe research with potentially significant societal benefits.[3]

Given the ongoing debate and the benefits and risks involved in GOF research, CSET researchers aimed to map the landscape, including the scale and scope, of both GOF and LOF published research. While LOF research results in weakened pathogens—and thus does not impart the same risks as GOF research—CSET researchers chose to characterize both GOF and LOF research because they are conducted using the same experimental procedures and thus could both be impacted by future regulations. Policymakers seeking to regulate GOF methodologies need to first understand this

landscape in order to more effectively regulate this research without preventing beneficial gain- and loss-of-function research. They must also understand what constitutes cutting-edge of research, where regulatory gaps may exist, and how restricting certain types of high-risk GOF research would impact a wide range of experimental methodologies that are used in all types of biological scientific research.

Structure of Report

In the remainder of this Policy Brief, we provide background on gain- and loss- of function research and why and how researchers conduct this kind of work; describe the current policies and regulations governing this kind of research; describe our methodology and results in greater detail; and conclude with policy considerations and recommendations for U.S. policymakers interested in regulating this kind of research,

Background and Context

What is Gain-of-Function and Loss-of-Function Research?

Definitions for GOF and LOF research vary widely, but, at its core, this research changes an organism's genome to add or subtract biological functions. While scientists can manipulate any organism's genome via GOF or LOF techniques, this report focuses on pathogens, organisms that can cause disease. Experiments that increase a pathogen's ability to cause disease—through increased virulence, pathogenicity, or transmissibility—are classified collectively as gain-of-function research. In contrast, experiments that decrease a pathogen's ability to cause disease are classified as loss-of-function research. These two outcomes are interconnected; GOF and LOF research use the same methods, equipment, and techniques. Moreover, in many cases, researchers may not be able to predict whether an experiment will make a pathogen more or less able to cause disease.

In this report, we used the definition provided by the NSABB to inform our working definition of GOF or LOF research as any study that altered any of the following pathogen characteristics:[4]

1. Pathogen production, replication, or growth rates,
2. Survival rate or symptom severity in infected cells or organisms,
3. Transmission (for example altering the route or rate of spread, or modifying pathogens to infect new cells, tissues, or animals),
4. Susceptibility to immune mechanisms; or,
5. Resistance to drugs, vaccines, therapeutics, or diagnostics.

A subset of GOF and LOF research involves high-risk pathogens that are capable of spreading widely among humans. This subset has garnered increased attention from news media, lawmakers, and other officials, particularly following the outbreak of the COVID-19 pandemic, for the potential societal risks stemming from such research. Gain-of-function research on certain pathogens may also be called dual-use research of concern (DURC), gain-of-function research of concern (GOFROC), or enhanced potential pandemic pathogen (ePPP) research. As we discuss below, existing regulations applicable to GOF/LOF research generally target one of these three.

Box 1. Definitions of DURC, GOFROC, and ePPP

- **Dual-Use Research of Concern (DURC):** "a subset of dual use research defined as life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security." From the United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern.[5]
- **Gain-Of-Function Research Of Concern (GOFROC):** "research that could generate a pathogen that is: 1) highly transmissible and likely capable of wide and uncontrollable spread in human populations; and 2) highly virulent and likely to cause significant morbidity and/or mortality in humans." From the National Science Advisory Board for Biosecurity's Recommendations for The Evaluation and Oversight of Proposed Gain-Of-Function Research (2016).[6]
- **Enhanced Potential Pandemic Pathogen (ePPP):** "a PPP [potential pandemic pathogen] resulting from the enhancement of the transmissibility and/or virulence of a pathogen. Enhanced PPPs do not include naturally occurring pathogens that are circulating in or have been recovered from nature, regardless of their pandemic potential." Defined by The NIH's Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens.[7]

Why Do Researchers Genetically Alter Pathogens?

Researchers conduct GOF and LOF research to learn how pathogens function and to develop new therapeutic treatments and preventative measures. Modifying pathogens can help researchers understand the relationship between specific genes and physical traits, develop altered pathogens with therapeutic benefits, and determine which mutations are likely to make a pathogen more dangerous in order to prioritize public health efforts. For example, researchers may conduct GOF or LOF research to:

- **Learn what a gene does.** A researcher may determine that deleting a specific gene causes a particular virus to replicate more slowly. This loss-of-function experiment would indicate that the deleted gene is important for viral

replication and may be a good therapeutic target. For example, the discovery that a specific viral protein is important for influenza A infection led to the development of the drug peramivir.[8]

- **Study a pathogen in a model organism.** A researcher, who has developed a new antiviral drug, may want to collect more data in living organisms before testing the drug in humans. The researcher may decide to mutate the virus so that it can infect mice in a GOF experiment. Mouse-adapted H1N1 is frequently used to study influenza antivirals in mice.[9]

- **Develop a new vaccine strain.** A pharmaceutical company may develop a weakened strain of a specific virus to use as a vaccine. Researchers in this situation would conduct loss-of-function studies to create a virus strain that causes mild or no symptoms, but protects against future infection—also called a live attenuated vaccine. This is how chickenpox vaccines were developed.[10]

How do Researchers Conduct Gain- and Loss-of-Function Research?

Researchers can genetically alter pathogens using a variety of methods, each of which requires different resources, materials, techniques, and skills. The following are several of the most common techniques that can be used for both GOF and LOF experiments:

- **Serial passaging** involves deliberately passing a particular pathogen through a series of cells, tissues, or animals one after the other, using the pathogen from one round of infection to start the next. Sequentially infecting organisms in this way results in an evolved pathogen with new characteristics, similar to what happens when a pathogen spreads naturally among a population.[11] Researchers can select pathogens that have characteristics of interest during each round of infection; for example, researchers can isolate antibiotic-resistant bacteria by selecting the bacteria that are able to survive in antibiotic-treated mice.

- **Reverse genetics (also called virus rescue)** is a technique that lets researchers engineer new viruses by designing nucleic acids (DNA or RNA) in the laboratory that encode the instructions for a virus. Researchers then introduce this newly designed genome, or viral “blueprint,” into cells, where cellular machinery reads the blueprint and constructs the desired virus.

- **Adding, modifying, or removing genes** from existing pathogens can alter a pathogen's characteristics. For example, researchers can add a new specific

gene to an existing bacterial strain by introducing a plasmid—a circular segment of DNA that carries the desired gene sequence—to living bacteria.[12] Researchers can also mutate individual genes, either to generate a modified version of the gene or to deactivate a gene by disrupting its genetic code.

- **Pathogen recombination** is a technique in which two pathogen strains are combined to create a third that consists of a mixture of genes from the two pathogen parents.[13] Researchers can generate new gene combinations by mixing two pathogen strains in the same environment, for instance, by co-infecting host cells or animals with two pathogen strains. For example, two bacterial strains can pass DNA to each other in a process called genetic transfer, while two viral strains can exchange genes if they both infect the same cell.

Policy Landscape: Current Guidelines and Regulations

Despite its widely recognized value for public health and scientific progress, GOF research has attracted attention and concern from scientists, politicians, and policymakers due to what some see as inherent risks. The possibility of starting a pandemic or causing widespread illness, either deliberately or inadvertently due to accidental release of an enhanced pathogen, has drawn media coverage and sparked debate, particularly given claims that GOF research may have created the SARS-CoV-2 virus that triggered the COVID-19 pandemic.[14] Such concerns, however, predate COVID-19. For instance, in 2011-2012, studies related to H5N1 avian influenza virus transmissibility and other unrelated biosecurity incidents triggered debate and attracted public scrutiny that led the Obama administration to temporarily pause federal funding for GOF research experiments on influenza, severe acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS) viruses in October 2014.[15] During this pause, an evaluation by the National Science Advisory Board for Biosecurity (NSABB) informed the new framework, "*Recommendations For The Evaluation And Oversight Of Proposed Gain-Of-Function Research and P3CO," which* was implemented to guide federal funding decisions when the moratorium was lifted in 2017.[16] Subsequent policy guidance from the U.S. government has attempted to further address safety concerns through methods such as enacting review and reporting processes for GOF and LOF research. Most recently, in 2022, the NSABB was charged by the U.S. government to evaluate the existing regulations; the final report's findings and recommendations have been sent to the Department of Health and Human Services to inform potential new policy guidelines.[17] For a full overview of existing regulations, see Appendix A.

Meanwhile, members of the scientific community have repeatedly pointed out that the politically charged spotlight on GOF has made it difficult to rationally discuss the risks and benefits of such research. For example, in January 2023, over 150 experts signed onto a commentary published by the American Society for Microbiology, calling for a more nuanced, evidence-based discourse.[18] Overall, the scientific community has expressed a range of opinions about how and whether gain-of-function research on potential pandemic pathogens should be regulated. Some researchers are concerned that proposed regulations are too far-reaching and will cause a "chilling effect" that inhibits scientific progress. Others argue that GOF research on potential pandemic pathogens does not provide adequate benefit to justify the risk and should be stopped.[19]

In between these two viewpoints are those who recognize that GOF studies are essential for public health and basic science, and should be allowed to continue but only with additional clarity, risk mitigation, oversight, and an expansion or overhaul of flawed existing regulations.[20] Indeed, there are a number of problems with the current policies and guidelines that regulate GOF research. For one, these various policies do not clearly or consistently define what constitutes GOF research, or which subsets or aspects of GOF research are considered risky.[21] Some of these regulations also hinge on being able to predict the results of an experiment before it is conducted, or in other words, knowing in advance that a particular modification of a pathogen will increase (rather than decrease) its ability to cause disease, which is something scientists are often unable to do. Another challenge is that current policies, other than the list-based Select Agents Program, only apply to federally-funded research and have no bearing on the research conducted in the private sector. Therefore, regardless of whether additional regulations are enacted, the current, ambiguous policies already make it difficult for researchers to know whether or how their work is affected, limited, or entirely prohibited.

Box 2. Attitudes Within the Scientific Community

Given the sensitivity and charged nature of debate around GOF research, CSET researchers conducted a survey to gauge how practicing scientists engage with the topic. Surveyed experts shared their experiences on the impact that this contested discourse has had on their work, citing uninformed regulators, polarized bureaucracy, and inadequate communication of scientific information as constricting mechanisms for their work. Given the survey responses, we believe that researchers might be hesitant to become public advocates or educators of GOF research for fear of losing access to research resources, like funding and public support. The full survey and results are in Appendix C.

Methodology

To better understand the GOF and LOF research landscape, CSET used a quantitative approach that combines a machine learning classifier with subject-matter expert review. Our primary data source was PubMed/MEDLINE, a publicly available database of about 37 million English-language biomedical citations and abstracts provided by the National Center for Biotechnology Information (NCBI).[22]

Subject-matter experts first identified publications that fit our criteria of GOF and LOF research to train a machine learning classifier. This definition was informed by prior guidelines developed by the National Science Advisory Board for Biosecurity (NSABB).[23] As previously noted, we defined GOF and LOF research as experimental work that increases or decreases pathogenic function with any of five changes: altered virulence, pathogenicity, transmissibility, infectivity, or host range.

We then applied machine-learning methods to surface approximately 7,000 publications that are likely to contain our definition of GOF or LOF research, published between 2000 and mid-2022. The subject-matter experts then further analyzed a representative sample of 488 of these publications to determine the proportion of gain- vs. loss-of-function research and additional relevant characteristics including pathogen type, experimental method, vaccine relevance, and animal use. (Appendix B provides more details on methodology.)

Our methodology has a number of limitations. Our classifier surfaced GOF and LOF research from a corpus of English-language publications that does not represent all of the possible experiments that could be occurring globally. This excludes studies from researchers that don't publish their findings in peer-reviewed journals or studies published in a language other than English. For example, industry researchers may not publish their findings in order to protect intellectual property. This analysis is intended as a starting point to understand the GOF and LOF research landscape, while recognizing that it cannot exhaustively describe every experiment that is conducted. The following discussion highlights the findings from our analysis.*

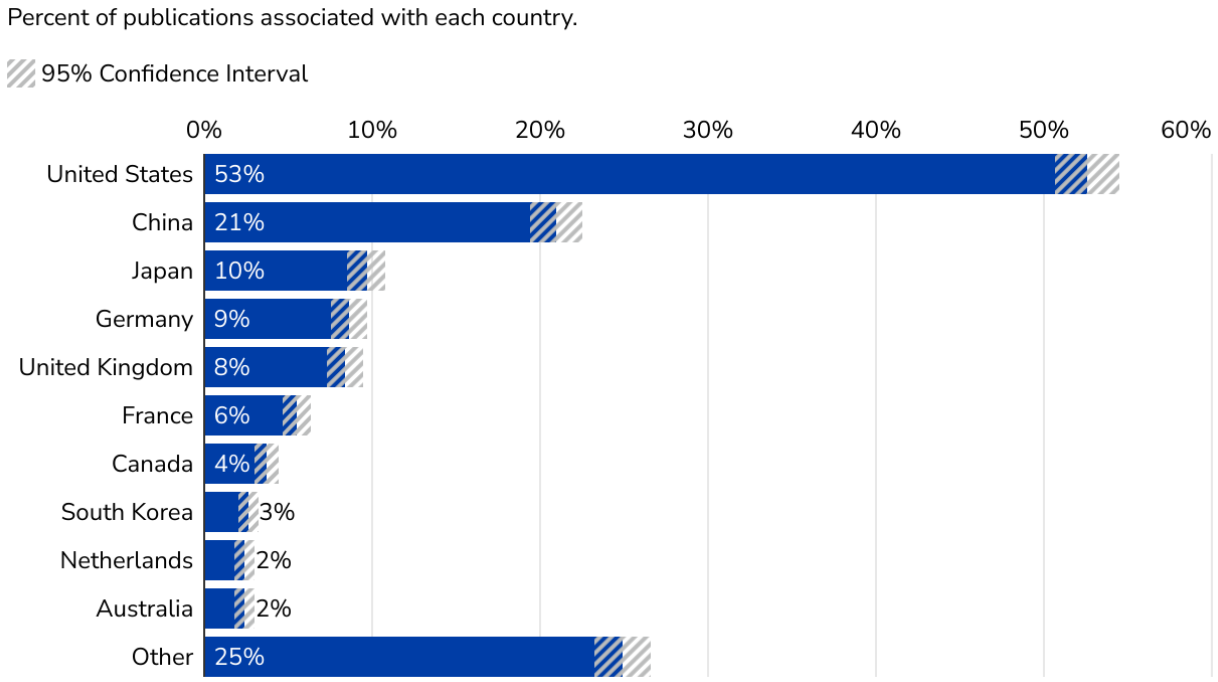
* This analysis captures GOF and LOF research on all pathogens, not just those defined as "high risk" or potential pandemic pathogens (PPP).

Results

Scope and Scale of Gain- and Loss-of-Function Research

GOF and LOF research is ongoing, global, and collaborative, with U.S.-affiliated researchers contributing to approximately half of identified publications. We estimate that there are about 7,000 papers published between 2000 and 2022 that meet our GOF and LOF pathogen research criteria on PubMed (Figure 1). Researchers from U.S.-based institutions contributed to about 53 percent of these publications, while researchers from Chinese institutions authored about 21 percent of the GOF and LOF research papers in our dataset.

Figure 1: U.S.-affiliated Researchers Contributed to More than Half of Gain- and Loss-of-Function English-language Research Publications between 2000-2022

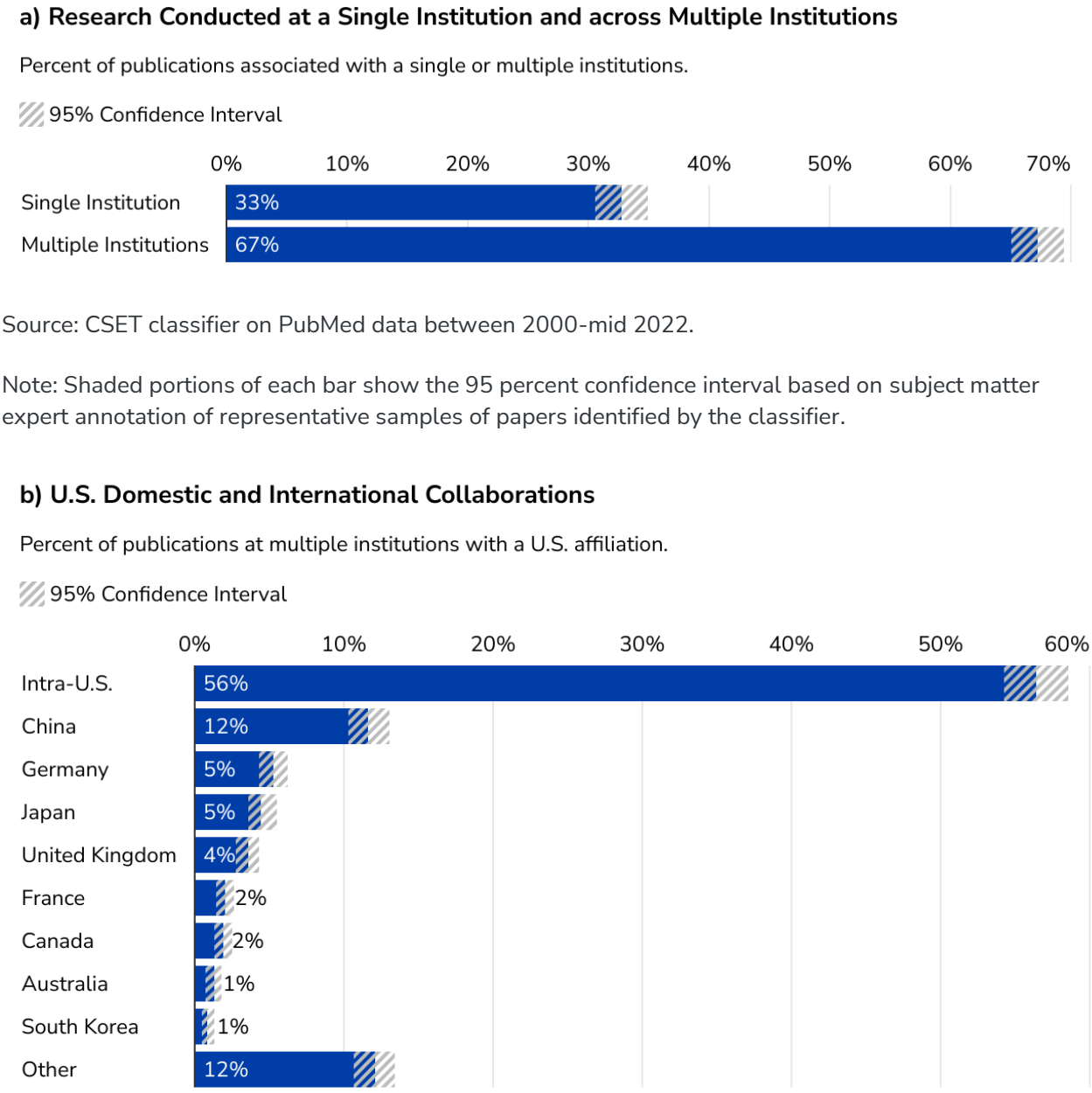


Source: CSET classifier on PubMed data between 2000-mid 2022.

Note: Percentages sum to more than 100 percent because many publications are collaborations between authors in different countries; these publications are counted for each country with a contributing author. Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier. For methodological details, see Appendix B.

GOF and LOF research is highly collaborative, as about 67 percent of identified publications involved a collaboration between researchers at multiple institutions (Figure 2a, next page), with collaborations between authors from at least two U.S. institutions being the most common type (Figure 2b, next page). U.S.-international collaborations occur most frequently between U.S. and Chinese institutions (Figure 2b).

Figure 2: The Majority of Gain- and Loss-of-Function Research was Conducted Collaboratively Across Multiple Institutions



Source: CSET classifier on PubMed data between 2000-mid 2022.

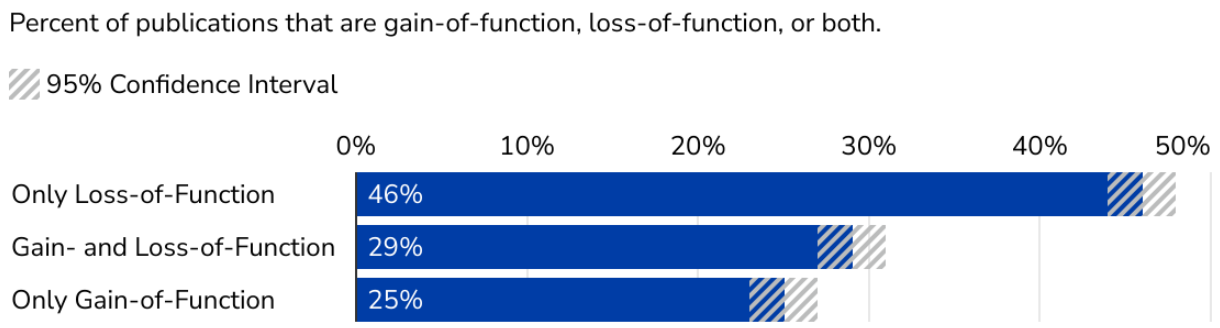
Note: Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

Source: CSET classifier on PubMed data between 2000-mid 2022. Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

Gain- versus Loss-of-Function Research

Gain- and loss-of-function research are interconnected and often conducted in the same study. We estimate that approximately one-third (29 percent) of the 7,000 identified publications in PubMed include both GOF and LOF research. Meanwhile, there are nearly twice as many studies that result in LOF as there are publications that result in GOF (Figure 3). As such, while the public debate is largely focused on GOF, our data indicates that GOF and LOF research are intertwined and that are many more studies focused on LOF research than GOF research.

Figure 3: Distribution of Gain- vs. Loss-of-Function Research



Source: CSET classifier on PubMed data between 2000-mid 2022. Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

Box 3. Examples of Gain- and Loss-of-Function Studies

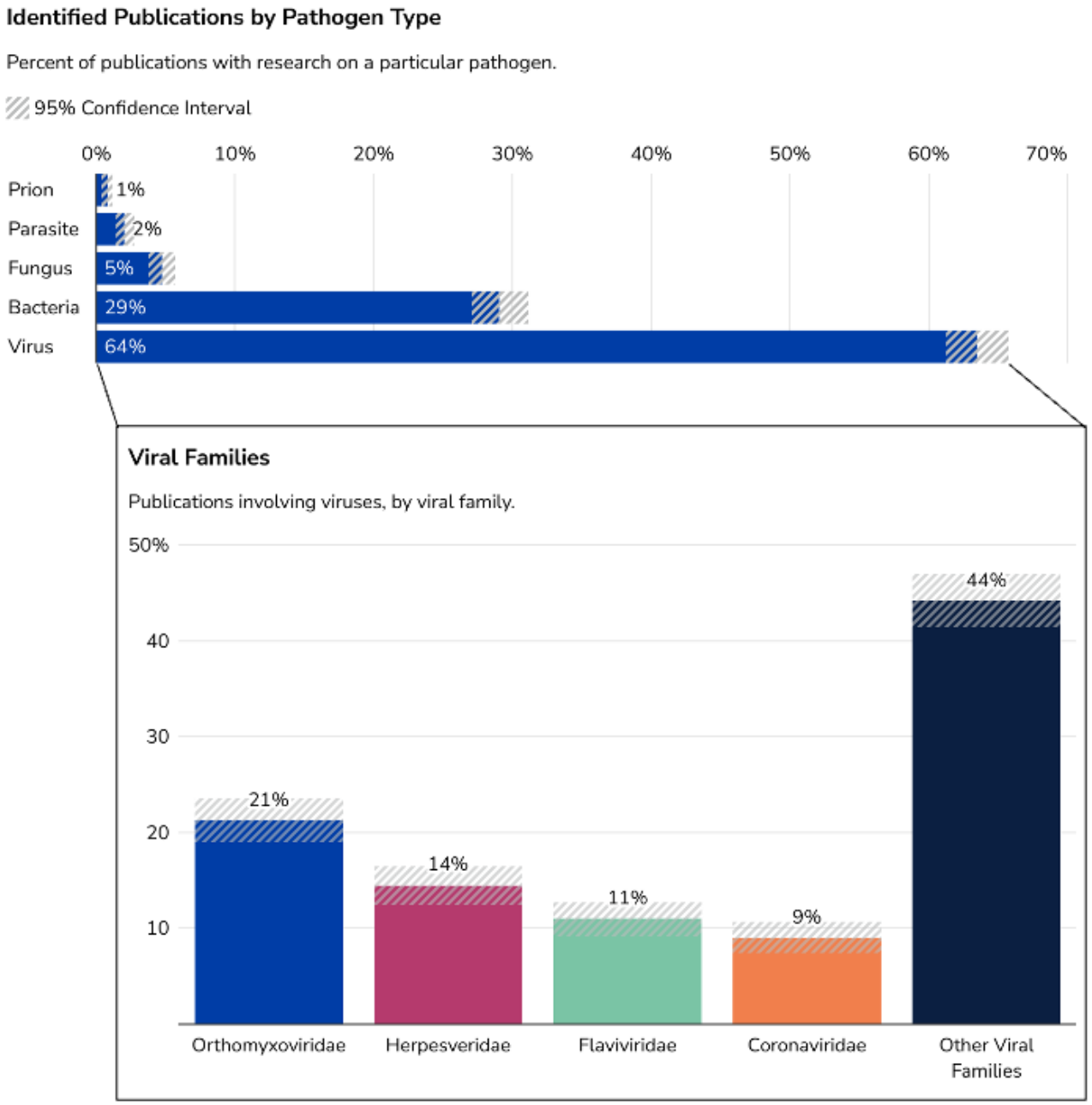
Below are examples of each type of research found during the SME review of selected publications:

- **Loss-of-function**: Researchers deleted genes from African swine fever virus to develop a weakened version of the virus; this attenuated virus fully protected pigs from lethal African swine fever infection when used as a vaccine.[24]
- **Gain-of-function:** Researchers infected mice with the bacteria *Pseudomonas aeruginosa*, that normally does not naturally infect mice, to investigate how the bacteria interacts with a host in an animal system.[25]
- **Gain- and loss-of-function:** Scientists changed the sequence of different strains of the fungus *Aspergillus fumigatus* to determine what causes the fungus to become resistant to antifungal therapies. Some of the mutations caused new fungus strains to not grow at all in mice, while other new strains grew faster in mice than the original fungus stain.[26]

Gain- and Loss-of-Function Research in Public Health

Gain- and loss-of-function research is conducted to better understand how pathogens cause disease and to develop therapies for pathogens with a high global health burden. Accordingly, the pathogens that we encountered most frequently in publications identified for this study are viruses within families that infect a sizeable fraction of the world's population or cause livestock loss, such as orthomyxoviridae (influenza), herpesviridae (herpes simplex virus), flaviviridae (yellow and dengue fever), and coronaviridae (SARS, MERS, COVID-19). We estimate that nearly one-third of GOF and LOF studies in our dataset are conducted on bacteria, which could reflect efforts to combat health concerns posed by antimicrobial resistance (Figure 4).[27]

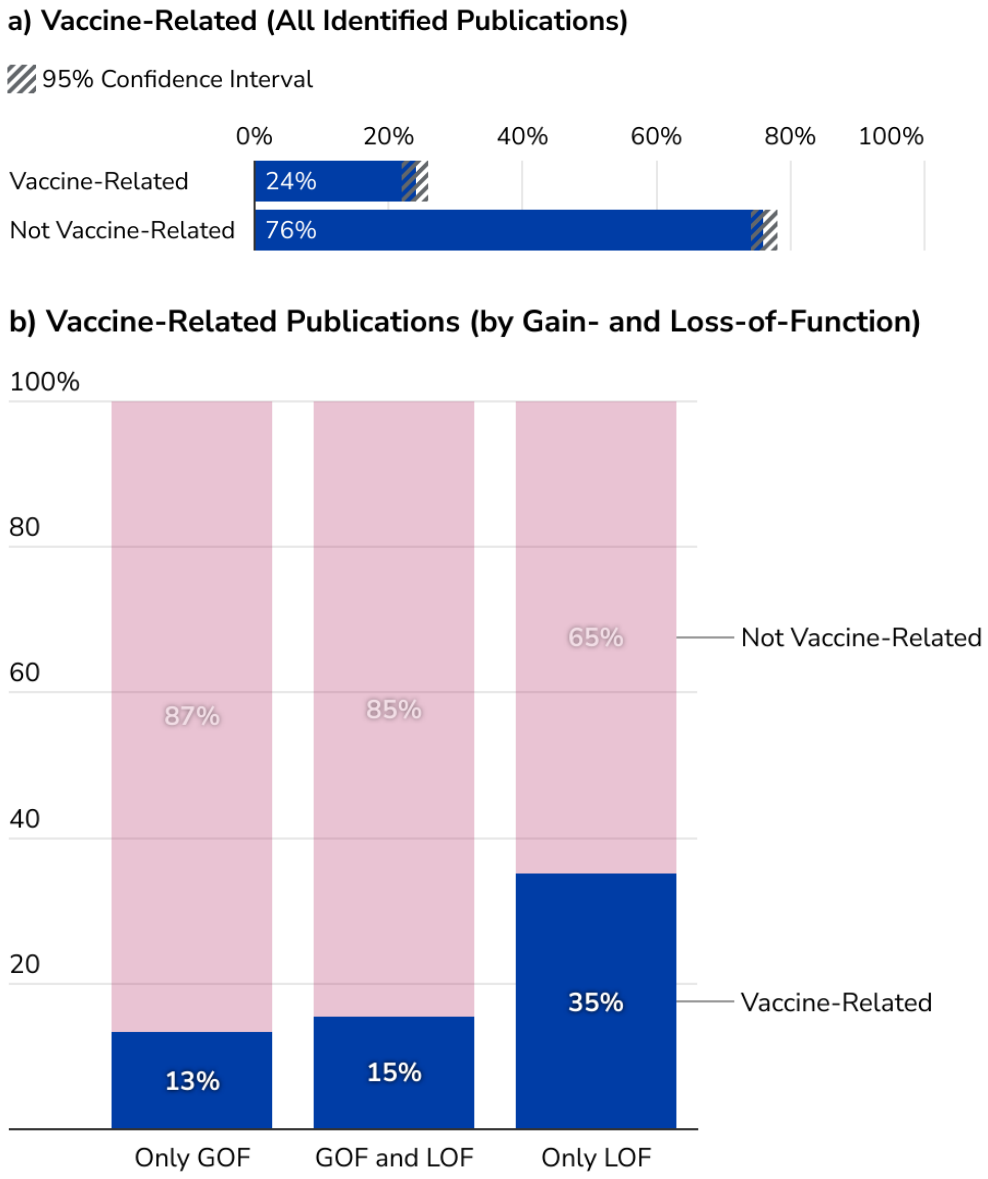
Figure 4: Gain- and Loss-of-Function Research, Categorized by Pathogen Type



Source: CSET classifier on PubMed data between 2000-mid 2022. Percentages sum to more than 100 because some publications use more than one pathogen. Percentages of virus breakdown sum to 99 percent rather than 100 percent of the viruses represented, due to rounding. Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

In addition to studying diseases, researchers conduct GOF and LOF research to develop new preventative medical countermeasures like vaccines. An estimated 24 percent of all identified publications in our dataset are directly involved in research or development of vaccines (Figure 5a). We further find that LOF research is more closely related to vaccine development than GOF research; approximately 35 percent of the publications that result in LOF alone are related to vaccine development, compared to 15 percent of publications that result in both LOF and GOF and 13 percent of publications that result in only GOF (Figure 5b). We only identified publications as "vaccine-related" if they were explicitly developing or testing a vaccine; there are additional foundational research publications that could contribute to vaccine development but may be missed by our specific criteria.

Figure 5: Gain- and Loss-of-Function Publications Related to Vaccine Development



Source: CSET classifier on PubMed data 2000-mid 2022. In Figure 5(a), shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

Gain- and Loss-of-Function Research Methodologies

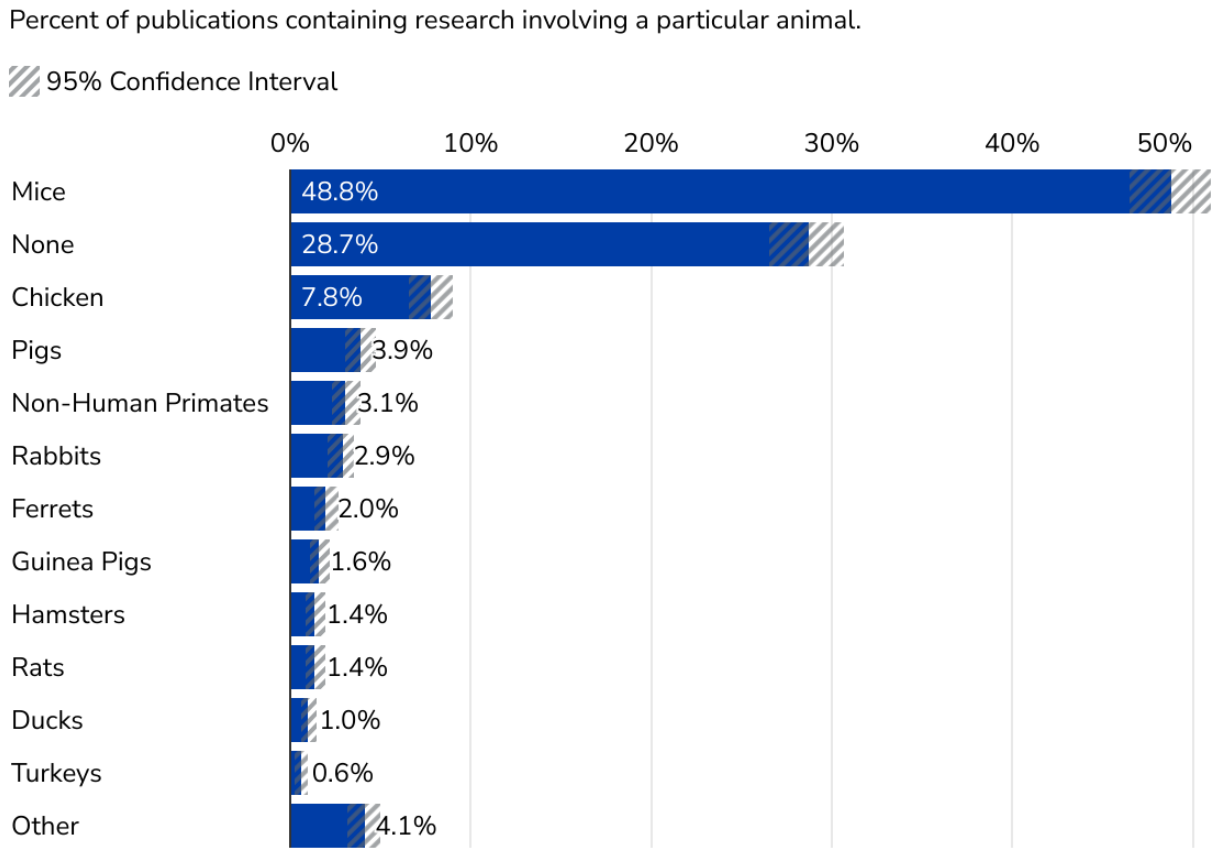
Gain- and loss-of-function studies serve a range of research applications, from basic research in the laboratory to experiments that use animals to test how pathogens function in living organisms. Basic research studies build foundational knowledge, while animal models translate these results to more complex living organisms.

GOF and LOF research are conducted in both basic laboratory settings and in animal studies. Mice, the most commonly-used animal model in research, are used in an estimated 49 percent of GOF and LOF publications (Figure 6).[28] Chickens are the next most-common animal model (about 8 percent), which reflects the frequency of studies on avian flu or studies that use chicken eggs to develop vaccines. We estimate that pigs, non-human primates, rabbits, and ferrets are each used in less than 5 percent of identified publications. About 29 percent of publications do not include research on animal models.

The type of animal model used can increase the risks associated with GOF research. The more biologically similar an animal model is to humans, the greater the likelihood that the research will translate to humans but also the greater the risk of unintended animal-to-human transmission. Non-human primates and pigs are often used to study human diseases because they are physiologically and genetically similar to people.[29] However, this similarity also means that the pigs, nonhuman primates, and humans can be infected by similar pathogens, increasing the risk that these studies may accidentally infect human researchers and handlers.

About 4 percent of the identified GOF and LOF publications involved experiments that infect pigs while approximately 3 percent involved experiments that infect nonhuman primates (Figure 6). Ferret research is frequently cited in policy discussions after two high-profile studies modified H5N1 influenza to spread between ferrets due to the similarities between human and ferret lungs.[30] However, we found that only 2 percent of identified publications relate to research that infects ferrets.

Figure 6: Gain- and Loss-of-Function Research, Classified by Use of Animals

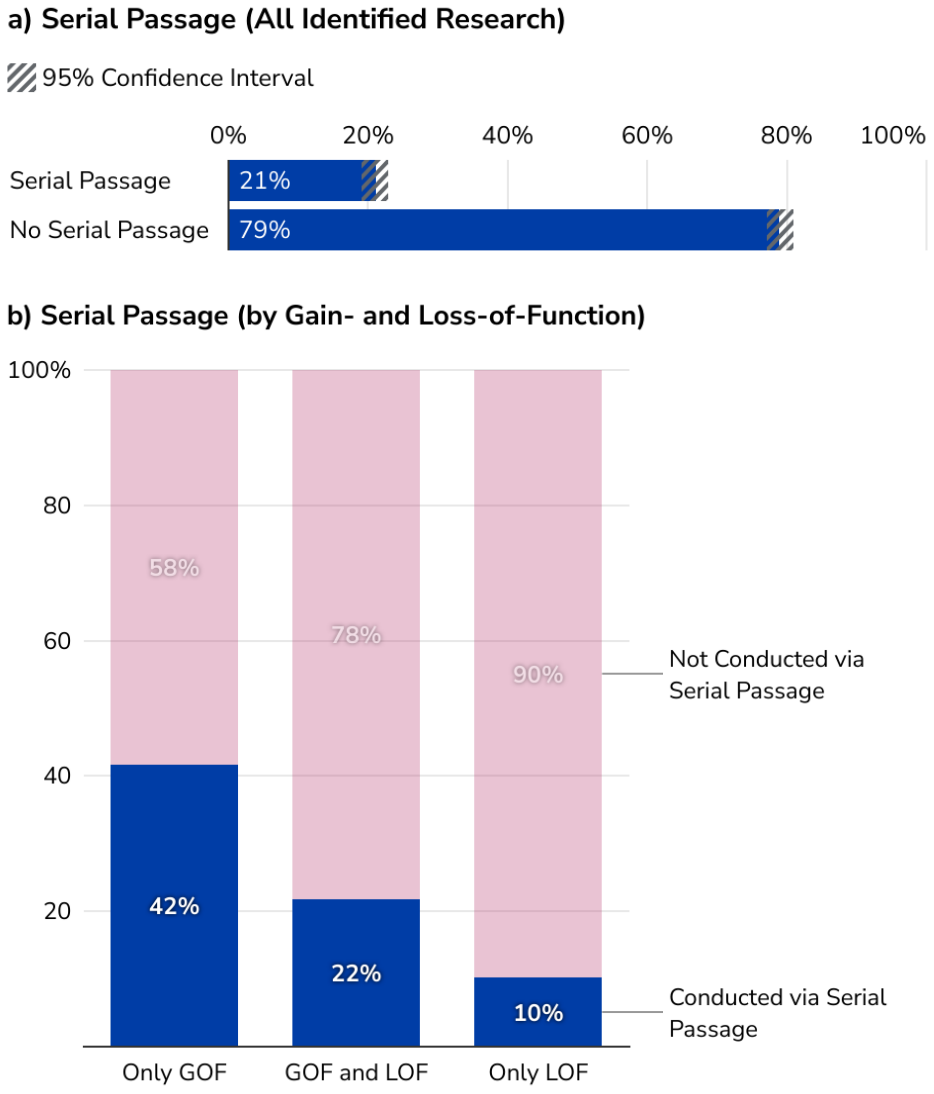


Source: CSET classifier on PubMed data between 2000-mid 2022. Percentages sum to more than 100 because some research is conducted on more than one animal species. Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

While much of the attention has focused on sophisticated gene editing technologies such as CRISPR, it is important to understand that the highest-risk GOF research can be conducted even without access to advanced gene-editing technologies because researchers can use other methods including serial passaging to alter pathogen genomes. Serial passage sequentially infects cells, tissues, or animals with a pathogen over time, and can result in an evolved pathogen with new characteristics under suitable scientific conditions.[31] The method requires basic laboratory equipment and does not rely on more advanced and expensive methods such as CRISPR, DNA synthesis, or genetic engineering. We found that 21 percent of all identified publications used serial passaging (Figure 7a). Serial passaging frequently resulted in GOF genome alterations; 42 percent of identified publications that include only GOF

were conducted using serial passage, compared to 22 percent of publications that result in both GOF and LOF and 10 percent of publications that result in only LOF, respectively (Figure 7b). It is also worth noting that when serial passaging is conducted in animals, the likelihood of animal-to-human transmission, and thus, risk, may increase.

Figure 7: Gain- and Loss-of-Function Research, Categorized by Use of Serial Passaging



Source: CSET classifier on PubMed data between 2000-mid 2022. Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

Box 4. Examples of Serial Passage

Serial passaging is a common technique to genetically manipulate pathogens. Some illustrative examples of its use include:

- **Loss-of-function:** Researchers serial-passaged a strain of Porcine Epidemic Diarrhea Virus, resulting in a virus that induced milder symptoms and exhibited decreased viral shedding in pigs. This new strain is a potential attenuated vaccine candidate, which could help alleviate livestock losses due to the virus's high mortality rate.[32]
- **Gain-of-function:** Researchers investigated how a common antiviral drug inhibits herpes simplex virus by serial-passaging the virus in cells until it was resistant to the antiviral, to better understand why treatments sometimes fail.[33]
- **Gain- and loss-of-function:** Researchers serial-passaged dengue virus in mosquito and mammalian cells to understand how the virus evolved to be able to infect both mosquitoes and humans. Some of the newly-evolved viruses gained the ability to replicate more efficiently, while other viruses replicated less efficiently; the researchers compared the gene sequences of the differently evolved viruses to understand how dengue virus adapts to new hosts.[34]

Types of Pathogens Used in Gain- and Loss-of-Function Research

GOF and LOF research varies in risk level depending on the pathogen that is being manipulated. The CDC stratifies a variety of pathogens into biosafety containment levels (BSLs) 1-4, which represent recommended best practices for safe research based on a protocol-based risk assessment.* We mapped the recommended biosafety levels to the publications based on the pathogen(s) used in each of the 488 publications we inspected manually during our research process. Some papers explored pathogens not assigned a biosafety level by the CDC, which we labeled as "unknown." Note that the CDC biosafety levels are recommended, and that we were unable to confirm whether the research covered in these papers was conducted in the appropriate biosafety level laboratories. Almost all of the publications for which we were able to assign a biosafety level (460 out of 488 manually inspected publications) were performed on pathogens that are categorized at BSL-2 (58 percent), BSL-2+ (10 percent), or BSL-3 (25 percent) (Figure 8). Very few publications involved pathogens that are categorized as BSL-1 (1 percent) or BSL-4 (<1 percent).

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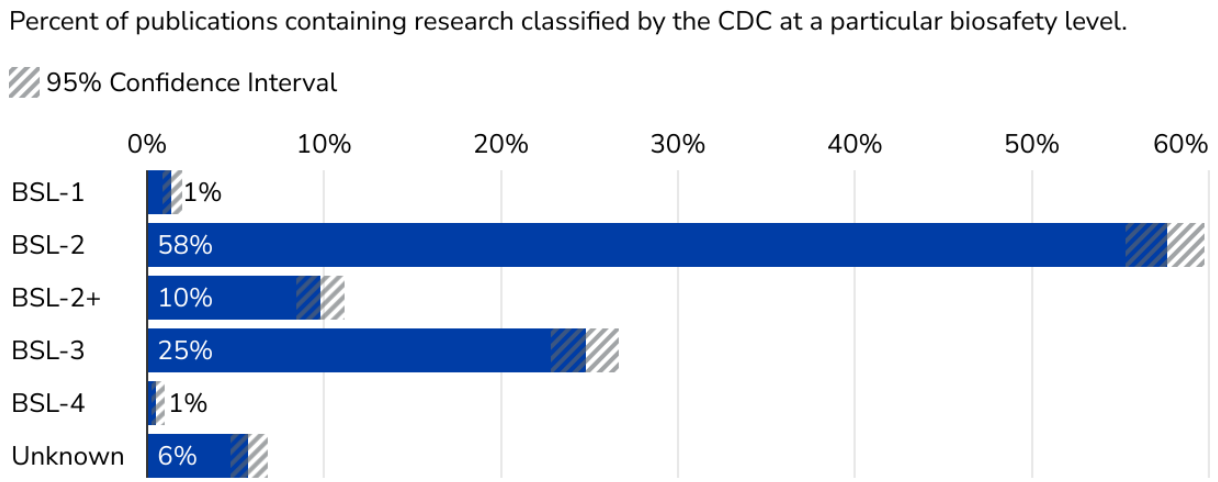
* Center for Disease Prevention and Control Biosafety Level Definitions:
Biosafety Level 1 (BSL-1): appropriate for defined and characterized strains of viable biological agents that are not known to cause disease in immunocompetent adult humans.
Biosafety Level 2 (BSL-2): appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure.
Biosafety Level 3 (BSL-3): appropriate for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections, of indigenous or exotic origin.
Biosafety Level 4 (BSL-4): appropriate for exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available.
"Biosafety in Microbiological and Biomedical Laboratories 6th Edition." Centers for Disease Control and Prevention. https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf.

Figure 8: Gain- and Loss-of-Function Research, Classified by CDC-recommended Pathogen Biosafety Level.



Source: CSET classifier on PubMed data between 2000-mid 2022.

Note: Percentages sum to more than 100 percent due to rounding. Shaded portions of each bar show the 95 percent confidence interval based on subject matter expert annotation of representative samples of papers identified by the classifier.

In summary, we find that gain- and loss-of-function studies are conducted globally, with applications ranging from public health research to vaccine development. These studies vary widely, and it's important to understand that attributes from the type of pathogen used to the research methodology employed can convey different levels of risk. The varied and sometimes interconnected nature of the research ecosystem means that policymakers, particularly potential regulators, must carefully consider the unique benefits and risks of certain types of GOF and LOF research and how restricting one type might affect another.

Regulatory Considerations: Why it Will be Difficult to Regulate Gain-of-Function Research

Our findings highlight the complexities of regulating GOF and LOF research. Based on our analysis, we identified different facets of GOF and LOF research that should be considered when designing or modifying rules, recommendations, and regulations:

1. GOF and LOF research are widely used in public health applications.

- Approximately 25 percent of GOF and LOF studies are involved in vaccine research and development, and the most frequently studied pathogens are those that cause widespread disease.
- Researchers frequently modify pathogens in order to understand how diseases function or to develop vaccines and medical countermeasures. Regulations will need to target the types of research that cause the most risk without impeding disease research or therapy development.

2. Gain- and loss-of-function research are intertwined.

- Approximately 30 percent of the studies in our dataset resulted in both GOF and LOF.
- LOF research does not create enhanced pathogens, and is frequently conducted to develop vaccines and therapies. Regulations that restrict GOF research based on methods, techniques, or resources used in the course of research may also restrict LOF research because the two use the same methods, techniques, and resources. This may delay public health developments without necessarily achieving the desired safety enhancements.

3. Researchers cannot always predict whether an experiment will cause a pathogen to become more or less virulent.

- In addition to GOF and LOF being intertwined, we encountered several studies in which researchers determined what a gene does by deleting the gene and measuring the impact on the pathogen's function. The goal of this experiment is to investigate cause and effect, and thus the output is not always anticipated.
- Scientists do not have complete knowledge of gene function or biological pathways, which prevents them from being able to accurately predict the

results of every experiment. Experiments that were not anticipated to be GOF research may not be prevented by proactive regulatory requirements.

4. Gain-of-function research can be conducted without access to advanced gene editing technologies.

- Over 20 percent of GOF or LOF research we identified for this study was conducted without access to gene editing technologies. For instance, 21 percent of the studies used more basic techniques like serial passage, albeit the use of this technique is more frequent in GOF research than LOF research.

- Researchers have a variety of experimental methods to conduct GOF research, some of which require only basic laboratory equipment. Regulating gene editing technologies, including CRISPR or DNA synthesis, would not prevent all GOF experiments.

5. Risk varies among GOF and LOF studies, and such studies should not be uniformly regulated.

- GOF and LOF publications spanned the full extent of biosafety levels, animal usage, and experimental methods, indicating a range of different risk factors**.**

- The risk level of GOF and LOF research changes based on experimental factors. For example, some pathogens, like common lab strains of *Escherichia coli*, are relatively benign while others can cause severe or fatal disease. Similarly, using research animals that are biologically similar to humans may increase the risk of a modified pathogen accidentally transferring to humans.

Whether or not U.S. policymakers decide to pursue regulations on GOF research, international coordination, transparency, and trust-building between scientific, public, and policy communities will be necessary to build a comprehensive strategy for scientific research governance.

Conclusion

CSET's examination of about 7,000 gain- and loss-of-function research publications, drawing on a database of roughly 37 million biomedical citations and abstracts, indicates that the nature of this research is global, ongoing, collaborative and diverse. While the riskier methods involving gain of function are overrepresented in today's policy discussions, our research shows that it is not as prevalent across the larger research ecosystem examined. This brief aims to help policymakers ground the current discussion and debate in the context of the scale, scope and complexity of this research. It also maps the policy and academic landscapes to help policymakers weigh how best to regulate this research and address regulatory gaps.

Gain- and loss-of-function research methods are complex and nuanced and any regulations of these methods need to be as well. Ultimately, different gain-of-function research approaches contain different levels of risks that are important to understand in order to create effective regulations. For example, some cutting-edge research may rely more heavily on advanced gene editing technologies or animal studies, but these tools are unnecessary for other basic gain- or loss-of-function research. Painting these methods with a broad brush could inadvertently impede scientific progress. As such, one-size-fits all policies aimed at mitigating dangers from one approach could limit other, less-risky research, and overly broad regulations could ultimately limit the scientific community's ability to prepare for future disease outbreaks. Effective policies will need to clearly define the subset of research that poses the greatest risk in order to develop targeted regulations. This brief will hopefully help policymakers address this important topic, armed with knowledge and factual insights.

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Appendix A: Gain-of-Function Research Policies, Regulations, and Guidelines

We provide examples of policies or guidelines that encompass aspects of GOF and LOF research below.

Federal Select Agent Program (FSAP) (Reviewed regularly)[35]
Joint program between the CDC and USDA that oversees the possession, use, and transfer of select agents and toxins that "have the potential to pose a severe threat to public health and safety." The policy only applies to a specific list of agents and toxins, so pathogens that are not on the list at that time are not subject to regulation (the list is updated several times a year).

U.S. Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern (DURC) (Effective 2015)[36]
An institutional oversight requirement for a list of 15 agents and seven types of experiments that are considered "high risk". The policy only applies to a specific list of agents and toxins, so pathogens that are not on the list at that time are not subject to regulation. The policy requires each federal institution to create their own guidelines for regulating DURC-related research funding.

Framework for Guiding Funding Decisions about Proposed Research Involving Enhanced Potential Pandemic Pathogens (2017)[37]
A review process for NIH-funded studies on "enhanced potential pandemic pathogens" (ePPPs), defined as an enhancement to a pathogen that is:

1. Likely highly transmissible and likely capable of wide and uncontrollable spread in human populations; and
2. Likely highly virulent and likely to cause significant morbidity and/or mortality in humans.

PPPs themselves are defined as pathogens that are likely "highly transmissible" and "highly virulent;" the term "highly" is subjective and the requirement to report if a study has "reasonable anticipation" of a risk is unclear. The Framework is guided by the proposed **Recommendations for the Evaluation and Oversight of Proposed Gain-of-Function Research (2016)** by the National Science Advisory Board on Biosecurity. [38]

NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (2019)[39]
NIH guidance, as a condition of receiving funding from the NIH, for laboratory safety and containment practices. The guidelines pertain to research that was developed with NIH funding or conducted at institutions that receive NIH funding on studies that use recombinant or synthetic nucleic acid molecules.

Biosafety in Microbiological and Biomedical Laboratories (BMBL) Guidelines (Most recent revision, 2020)[40]
NIH/CDC laboratory guidance that recommends biosafety and biocontainment procedures to safely conduct research on different pathogens by categorizing pathogens by biosafety levels.

Proposed Biosecurity Oversight Framework for the Future of Science (2023)[41]
2023 updated guidance from the National Science Advisory Board on Biosecurity that evaluates the effectiveness of U.S. policies governing ePPP and DURC research and provides updated recommendations to increase research transparency, expand the pathogens that are subject to review, and further define the roles of research institutions in self-reporting ePPP research. If implemented, the framework would also remove exemptions for vaccine development and pathogen surveillance.

Appendix B: Text Classification Methodology

We applied machine learning methods to develop a classifier that identified the numerous gain- and loss-of-function research and publications available in PubMed. This methodology was chosen because identifying thousands of relevant articles from millions of publications was not a feasible task for manual inspection, and unfortunately there was no reliable way of using the associated Medical Subject Headings (MeSH terms) to simply select gain- or loss-of-function research.

Development of our text-based classifier consisted of several stages. The first was to identify a corpus of candidate research papers, while balancing the benefits of a large dataset with the need to eliminate irrelevant papers. We selected the following criteria from the PubMed metadata to isolate articles of potential interest:

1. An article needed to be published no earlier than 2000;
2. The publication type for an article should be associated with grant-supported research, such as "Journal Article", and not be labeled as "Review", "News" or contain any other non-experimental designation;
3. The MeSH tags for an article should include "Animals" or any of the 221 specific animal and taxonomic categories of animals;
4. The MeSH tags of an article should also include any of the possible 556 viruses, 777 infections, or 115 pathogens terms;
5. The article could not be missing a title or abstract.

As a result of our criteria, 159,227 candidate publications in PubMed were identified as the basis for our corpus.

Based on a literature review of over 300 papers of relevant work, we then developed stringent criteria for gain- or loss-of-function research, along with criteria for specifying non-gain- or loss-of-function research. Seminal papers included Imai et al. 2012 and Herfst et al. 2012,[42] which reported gain of function in the lab-modified H5N1 flu virus among ferrets in the form of new modes of transmission. Documents from the National Science Advisory Board for Biosecurity (NSABB) defined parameters for risk-benefit analysis,[43] provided an annotated bibliography of gain-of-function research and alternatives including loss-of-function,[44] and recommendations for evaluation and oversight.[45] These works allowed us to adopt a narrow but well-specified working

definition for gain- and loss-of-function as experimental research that seeks to either increase or decrease pathogenic function. We slightly modified the definition set forth by NSABB to define gain- or loss-of-function research as any study that:

1. Enhanced (or decreased) pathogen production as a result of changes in the replication cycle or growth.

2. Enhanced (or decreased) survival rate or symptom severity in appropriate cells or animal models.

3. Enhanced (or decreased) transmission (e.g., for example altering the route or rate of spread, or modifying pathogens to infect new cells, tissues, or animals).

4. Evasion of (or reduced ability to evade) existing natural or induced immunity.

5. Change in resistance to drugs or evasion of other medical countermeasures such as vaccines, therapeutics, diagnostics.

The collection of criteria for identification of relevant research lend itself to the use of weak supervision with a large language model.[46] We made use of the Snorkel Flow platform at this stage. Here, we defined a set of heuristics for relevant documents that were encoded as 201 labeling functions. For example, text including the term "recombinant" has a high likelihood of describing gain-of-function research, whereas a phrase such as "human trial" would suggest non-gain-of-function research. These functions, when aggregated for each publication in our corpus, generated a set of weak labels on over 900,000 documents, where each of these documents was weakly labeled as either gain-of-function or non-gain-of-function. The collection of labeled documents served as the training data for our discriminative classification model, a deep neural network.

We first evaluated the performance of our classifier by having a stratified random sample of 200 documents manually annotated by CSET domain experts. Based on this evaluation, we estimate the recall of our model to be 0.80. In addition, each annotator independently analyzed the same 50 documents to evaluate inter-annotator agreement and ensure the robustness of the annotation process.* We conducted a second round of evaluation using a second stratified random sample of 1,000

* Inter-annotator agreement was quite high with Krippendorff's $\alpha = .85$.

documents, which were again manually annotated by CSET domain experts. From this second evaluation, we estimated the precision of our model to be 0.58.

With the collection of relevant research publications identified by our classifier, CSET domain experts annotated the second round sampled research papers to further disentangle gain-of-function research, loss-of-function research, or both; characterize research to identify: pathogen types and families; use of animals, including non-human primates; research methodologies such as serial passaging and genetic engineering; the biosafety level of the pathogen under research; and finally to analyze author affiliations to characterize work conducted at a single institution or collaboratively across multiple institutions, and find country associations.

Appendix C: Survey Methodology

We designed a survey fielded in fall 2022 to ask researchers with expertise in biotechnology how they define and perceive gain-of-function research via email distribution. Our survey sought to garner insight on what experts deem to be GOF research, including identifying GOF research experiments and perspectives on its purposes.

Survey Deployment and Characteristics

We define our population of interest as individuals who authored a GOF-relevant in PubMed and a targeted group of researchers and industry professionals who have been identified by the CSET research team as performing GOF research or have expertise in a field related to gain-of-function research. As this population is fairly small, we supplemented our sample of academic researchers performing gain-of-function research with targeted outreach and a snowball sample of U.S. professionals who work in fields that may include policy, biosafety/bioethicists professionals. In total, we received 14 responses (9 complete and 5 partial) for a response rate of 4.05 percent.[*] The median response time was 10 minutes. We asked questions about:

1. individuals' definitions of GOF research for a variety of audiences,
2. merits and risks of performing GOF research; and,
3. comments on existing definitions of GOF research.

Survey Analysis

Two members of the research team extracted themes from survey open-responses through focused coding and constant comparison methods to analyze these responses. We documented ideas, questions, and comments, and created a list of themes. After initial familiarization with the responses, the same two members of the research team independently engaged in open coding to extract and determine individual themes using thematic analysis. We developed a codebook on the basis of agreement of two members of the research team. We do not report agreement metrics as both researchers coded all responses and resolved any discrepancies. Our extracted themes mapped onto survey topics and included the categories in Table 1.

[*] The response rate for this survey aligns with similar survey response rates.

Table 1: Survey Coding Categories
GOF research implications
public health applications
scientific end means
societal implications
scientific communication
processes and methodologies
GOF research definitions
miscellaneous inflammatory statements
safety concerns

Source: CSET survey analysis

Survey Findings

Generally, respondents agreed upon the following descriptors to characterize GOF research: alterations to the genome of an organism that may or may not result in enhanced functionality. They also typically scoped GOF research to work on viruses as well as gaining characteristics of transmissibility, virulence, and pathogenicity.

Several respondents gave examples of GOF research, "A great example is making a plant more drought tolerant through genetic modification. On the virus and bacteria side of things, there is the potential to understand how they fundamentally do certain things such as invade cells or avoid the immune system." When we asked respondents to note strengths and weaknesses in an existing definition of GOF research as proposed by the National Science Advisory Board for Biosecurity, many respondents expressed that this definition was limited to what they consider GOF research and that this definition has had a negative impact on research regulation. One respondent stated, "While I think that GOF should be defined as noted on the previous page, common usage is not always the same, and the NIH has also changed its definition somewhat. Uniformity and clarity would be very helpful." When we solicited

definitions of GOF research, we anticipated that respondents would provide different definitions or language for different audiences, however, many respondents noted, sometimes explicitly, that they would describe this type of research using the same language to all audiences, “I would use the same definition” or “Please see my former answer. It applies to all audiences.”

To the question about longer terms implications of GOF research, respondents mentioned topics such as advancing science, as well as societal, policy, and public health implications. When discussing the potential merits of GOF research, many respondents noted advancing scientific progress: “We can learn how biological systems work, with the potential benefit that we can improve human, animal, or plant health.” Comments on advancing science ranged from general statements such as “the benefits of gain-of-function research is to understand the function of specific genes and assess whether genes are essential, necessary but not sufficient cell survival, or not necessary for cell survival” to specific scientific advancements in a specific subfield of biology such as, “identifying mutations that make a gene work better; where mapping and studying these changes will identify critical control sites in genes.” Many respondents noted public health implications, including “gain-of-function research…is especially important for understanding how viruses evolve to evade the defense of the immune system. It is also so important for studying how and why bacterial pathogens develop antibiotic resistance. This work will directly inform the development of specific treatments for such superbugs.” One respondent noted directly a potential merit of GOFR is the “ability to design new therapeutics and medical countermeasures.”

We extracted issues of scientific communication from respondents' comments. Many respondents noted the term GOF research was not created by the scientists performing this type of research. This has led to increasing frustration with communicating to decision-makers and the general public about regulation on GOF research, noting that “people who opine on these GOF topics are not qualified to understand how blurry these categories are in practice.” One respondent explicitly mentioned the impact of scientific communication surrounding GOF research, “…forever in biosecurity contexts the more general concept of gain-of-function is linked to research with pathogens that result in new or enhanced functions.” A few respondents mentioned the difficulty of dealing with the ambiguity of performing GOF research, “the problem with the term is that as it wasn't created by scientists, it doesn't adequately describe what scientists might be doing and is super vague.”

The full text of the survey can be found at https://github.com/georgetown-cset/gain-of-function-survey/tree/main.

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