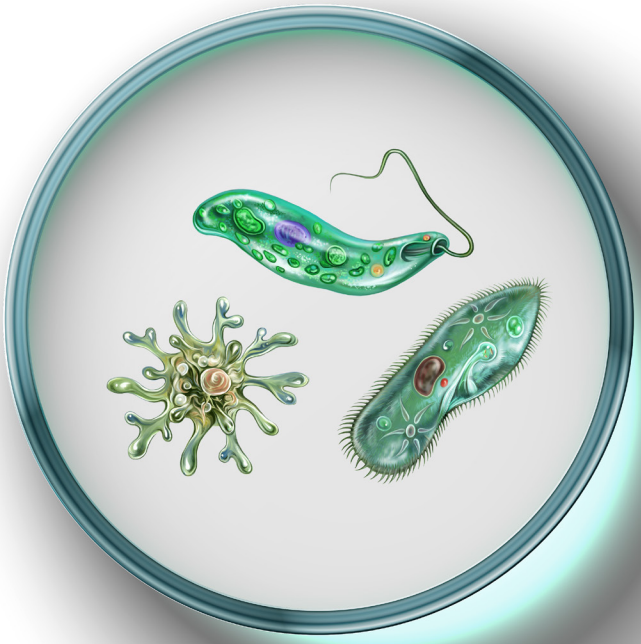
Genetically Modified Microbes



Technological and Legislative Challenges and National Security Implications

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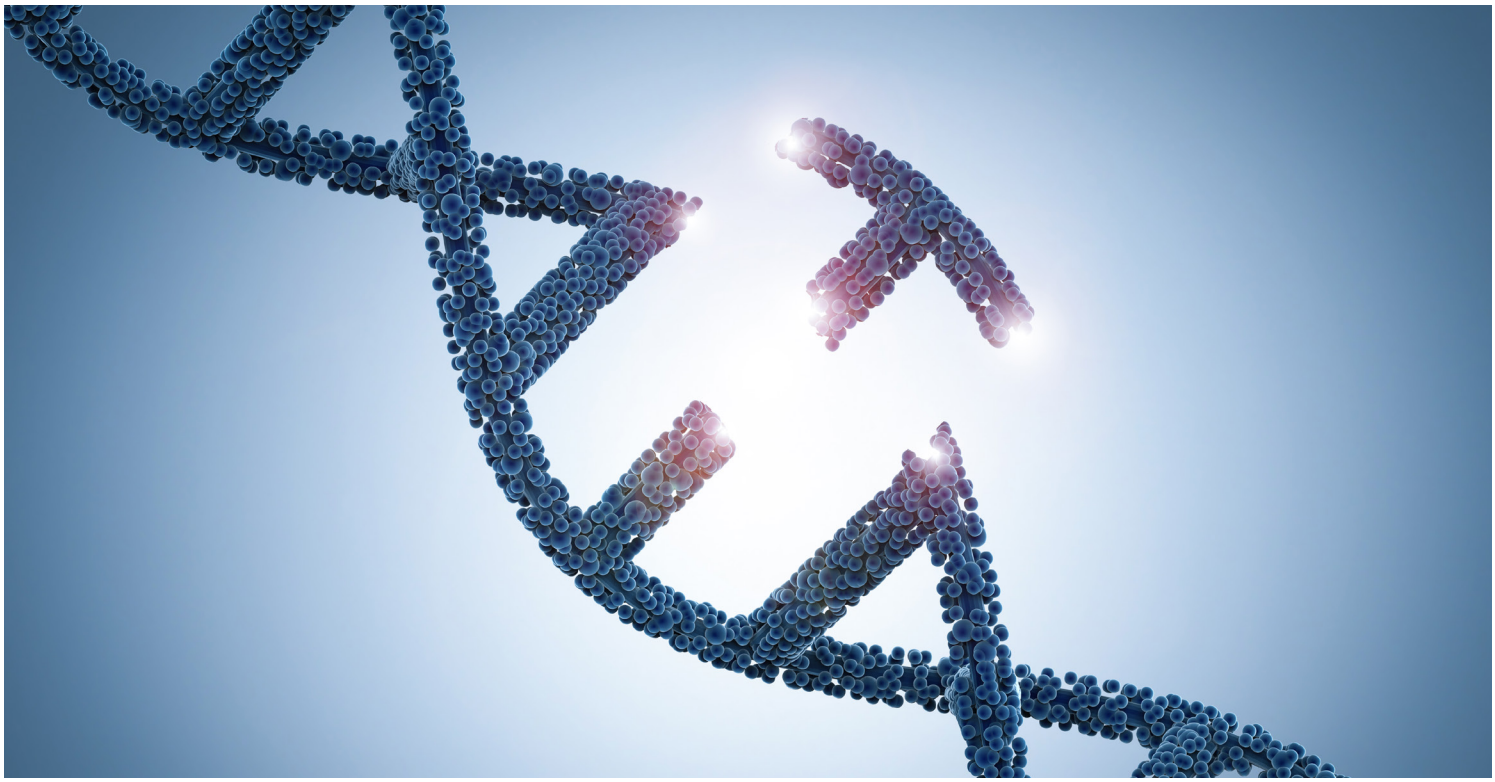
Protect Nature Now
Safeguarding Biological Evolution from GMOs 2.0



Contents

Introduction

Microbes represent the most diverse and numerous class of organisms on Earth. They are everywhere and play a vital role in maintaining environmental balance, as well as contributing to human health. They also represent the simplest organisms, but changes to their genomes can have a much greater impact on their behavior than other higher organisms. Viruses (while not considered living organisms) rapidly share, spread and mutate their genetic code, sometimes interchangeably with other microbial species. (In this paper, we refer to viruses and microorganisms collectively as microbes)

The COVID-19 pandemic illustrates how deadly microbes can spread throughout the world. Although it is often compared to the 1918 Spanish flu pandemic, which infected around a third of the population and caused the death of 17-50 million people (1-4% of the world population), the COVID-19 pandemic is far significantly smaller in scale. COVID-19 was confirmed in just over 1% of the world's population (although it is estimated that the real number is 3-5 times higher), causing several million deaths (around 0.05% of the world population). However, the economic, psychological and social effects of COVID-19 have been staggering, highlighting not only the damage an event like this can cause but also that pathogens like this have the potential to exact a huge toll: both economic and human.

The SARS-CoV-2 virus that causes COVID-19 highlights the dangers inherent to all microbes that undergo genetic changes. It has shown a high capacity for mutation during the pandemic. A new strain of the SARS-CoV-2 virus reported to be 50-70% more transmittable than the earlier strains caused a series of lockdown measures in Europe in late 2020 and early 2021. Although this strain was first observed in late summer, it became the dominant SARS-CoV-2 virus strain within three months in the United Kingdom. This highlights how a new genetic strain with evolutionary advantage (higher transmissibility in this case) can relatively quickly replace other strains to dominate the environment.

Nature is not the only one in the business of genetic alteration. Right now, there are thousands of scientists worldwide engaged in the genetic modification of organisms and viruses for a wide variety of purposes (Regier et al., 2017). Gene-editing has become so inexpensive and easy that the speed and variety of genetically modified organisms (GMOs) have rapidly expanded. Consumers purchase DIY gene-editing kits, while large, automated facilities can generate thousands of gene combinations relatively quickly.

Virtually every type of organism containing DNA is a candidate for modification. Work is being done, for example, on gene-editing of animals, plants, insects, fish, birds, bacteria, algae, fungus, viruses, etc. There are cows engineered without horns, plants that can tolerate herbicides, microorganisms that produce chemicals, including pharmaceuticals, and a variety of species that glow in the dark.

The genetic engineering process, including gene-editing, creates new entities that have not previously occurred in Nature. When these organisms leave the lab either on purpose or by accident and enter our environment, they may replicate and become a permanent part of Nature's gene pool. We don't know how they will interact and impact various ecosystems and typically have no way to recall or remove them without inflicting further environmental damage.

A recent Department of Homeland Security report (DHS, 2020) acknowledged gene-editing technology, including CRISPR, as "a major scientific advance" that gives scientists the ability to "manipulate DNA far beyond previous technology and has opened the door to rapid development in the field of molecular biology." The report states that gene-editing "has the potential to greatly help or greatly harm the United States." It also confirms what many observers have concluded:

"*The speed of innovation has outstripped American regulatory policy and legislation*; given the paradigm-altering potential of CRISPR and related technology, *this disconnect must be closed* (emphasis added)."

Recent research has shown that the gene-editing technologies claimed to be safe and precise often cause errors and deletions in the edited DNA, making the gene-editing process unpredictable and unreliable. The resulting DNA could contain unwanted mutations both in the area intended for changes (target site) as well as throughout the genome (off-target sites). The resulting organism could be substantially different than intended. This introduces significant and often unknown variability into the risk analysis of GMOs, making accurate risk assessment extremely difficult.

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Source: The Department of Homeland Security.

The current Regulatory Framework used to approve and regulate GMOs in the US largely ignores numerous shortcomings and side effects of the underlying technology. While the public mistakenly believes that GMOs, like medicines, are extensively tested and shown to be safe, that is currently not the case. The framework was never designed to properly assess the potential long-term impacts on health or the environment. The massive loopholes in the regulatory policy allow the release of genetically modified organisms into the environment with only cursory consideration of their potential environmental impact; this presents significant and unprecedented risks. These risks are particularly striking in the case of microbes.

Genetic Modification and Gene Editing Technologies: Myths, Limitations, and Latest Developments

When the first GMOs were introduced into the food supply, advocates assured lawmakers, regulators and the public that the process was safe and predictable. Research over the past 25 years, analyzing the DNA, RNA, proteins, and metabolites of GMOs compared to their natural non-GMO counterparts, as well as the results from animal feeding studies and agronomic performance, reveal that the genetic engineering process regularly produces a variety of unpredictable outcomes (Choi et al., 2001; Saxena 2001; U.Roessner et al, 2001; Satu et al., 2005; Zolla et al., 2008; Mesnage et al., 2016).

Gene editing is a relatively new set of technologies used to create GMOs. They typically involve a molecular mechanism that cuts the DNA, and another mechanism determines where that cut is to be made. Gene editing can delete, modify, insert or replace the genetic material in the genome.

Many promoters of GMOs today describe gene-editing as precise, predictable and reproducible. This is not yet the case: recent studies have shown that gene-editing commonly produces errors, including higher rates of mutations in gene-edited cells (Mianné et al., 2017; Kosick et al., 2018; Davies, 2019; Science Magazine, 2019; Wired, 2019; Farris et al., 2020; Modorai et al., 2020 Teboul et al., 2020). The inconsistent and unpredictable outcomes from gene-editing can bring significant dangers. Therefore, the use of this technology requires caution and control to be exercised by scientists and organizations using it and its products.

The most popular gene-editing technique is called CRISPR, short for Clustered Regularly Interspaced Short Palindromic Repeats. It has been at the forefront of the recent genetic revolution that made genetic engineering significantly more accessible. CRISPR uses short DNA sequences found in genomes of prokaryotic organisms (a class of single-cell organisms). These were derived from DNA sequences of bacteriophages that previously infected these organisms and then were used to detect and destroy bacteriophages with similar DNA in subsequent infections.

The CRISPR Cas9 system in the form of CRISPR is most often used. Cas9 enzyme - CRISPR-associated protein 9, is the enzymatic “scissors” that cut the DNA. CRISPR contains the specific DNA sequences that identify where that cut is to be made.

Other common forms of gene-editing used in recent years include (i) engineered meganucleases, (ii) zinc finger nucleases (ZFNs), (iii) transcription activator-like effector nucleases (TALENs), and (iv) oligonucleotide-directed mutagenesis. All of these technologies emerged in the last decade, culminating in a report in May of 2019 that China was introducing human genome editing regulations in response to the reported creation of the first gene-edited humans by He Jiankui (Cyranoski, 2019).

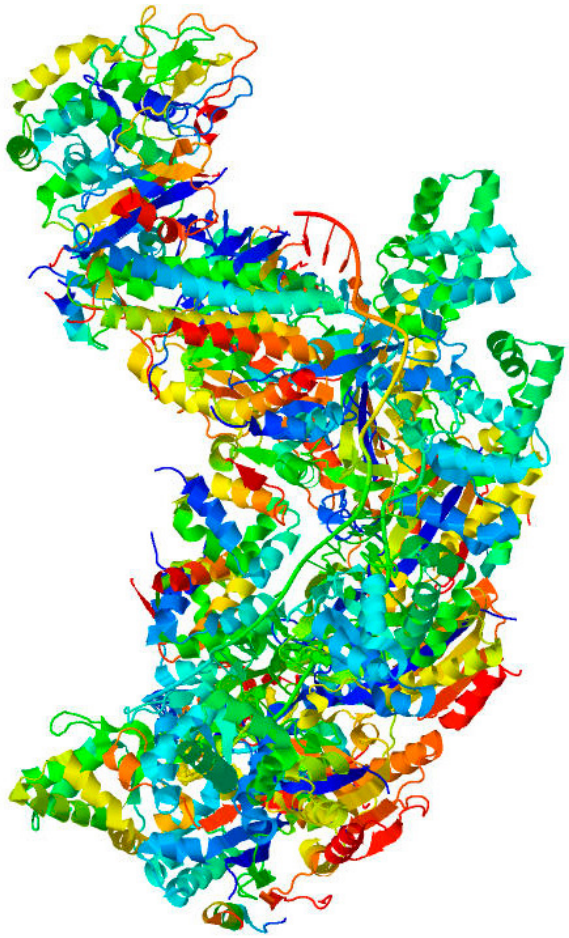

Figure 1. Strand of CRISPR RNA and a short piece of the viral DNA inside Cas9 protein after it has been unwound and recognized.

Gene Editing Can Cause "Chromosomal Mayhem"

A recent article in Nature reported three studies describing experiments using CRISPR-Cas9 gene-editing technology to modify human embryos (Ledford, 2020). All three studies showed "large, unwanted changes to the genome at or near the target site", characterized by the author as "chromosomal mayhem." Earlier research reported that the CRISPR-Cas9 method, in general, has a relatively high rate of "off-target" gene mutations far from the target site, which can be detected using standard methods (Zhang et al., 2015). However, the changes at or near the target site would be much harder to detect, requiring non-standard methods, which are more expensive. The reported results on human embryos show that in one study, "of 18 genome-edited embryos, about 22% contained unwanted changes affecting large swathes of the DNA surrounding the [target] gene." (Alanis-Lobato et al., 2020). In another study, while trying to correct a mutation on a chromosome, "about half of the embryos tested lost large segments of the chromosome — and sometimes the entire chromosome — on which [target gene] is situated" (Zuccaro et al., 2020). Experiments with mouse embryos and other types of human cells showed similar effects – large, unwanted effects caused by chromosome editing (Adikusuma et al., 2018; Kosicki et al., 2018).

Recent reports of genetically edited cattle – looking to create hornless cattle – show that genetic modification using TALENs introduced off-target modifications to the genome (Young et al., 2019). Similar effects were shown to occur in one in four cases of CRISPR-Cas9 edited pig embryos (Carey et al., 2019). The conclusion of the National Academies of Sciences, Engineering, and Medicine is that although gene-editing is not inherently hazardous, all forms of genetic modification "may potentially lead to unintended changes in composition, some of which may have adverse health effects" (NASEM, 2016).

A particularly disturbing finding showed that CRISPR not only alters the sequence of the genome but can also modify molecules that govern or regulate genetic expression (Farris, 2020). Furthermore, these "epigenetic" changes were passed down to the offspring of gene-edited mice for at least ten generations.

These findings indicate that the unintended outcomes of gene-editing are a consistent feature of these technologies and that no particular process is immune to these effects. The structure and function of the new organisms are not predictable and reproducible – the same process will yield different results.

Unfortunately, most scientists who use gene-editing rarely double-check the genomic sequences for off and on-target errors (the latter being particularly difficult to detect) to confirm that the result of gene-editing is identical to their intended sequence. They also rarely analyze the transcriptome, proteome, and metabolome to assess the holistic impacts of the engineering process. The latest research shows the early claims on gene-editing technologies greatly overestimates their capabilities and precision, with potentially damaging consequences.

Numerous regulators, however, are among the overly confident, viewing gene-editing methods as extremely precise—and act accordingly. The regulators do not require testing that could detect unwanted genetic mutations or any unintended consequences of errors and deletions during gene-editing.

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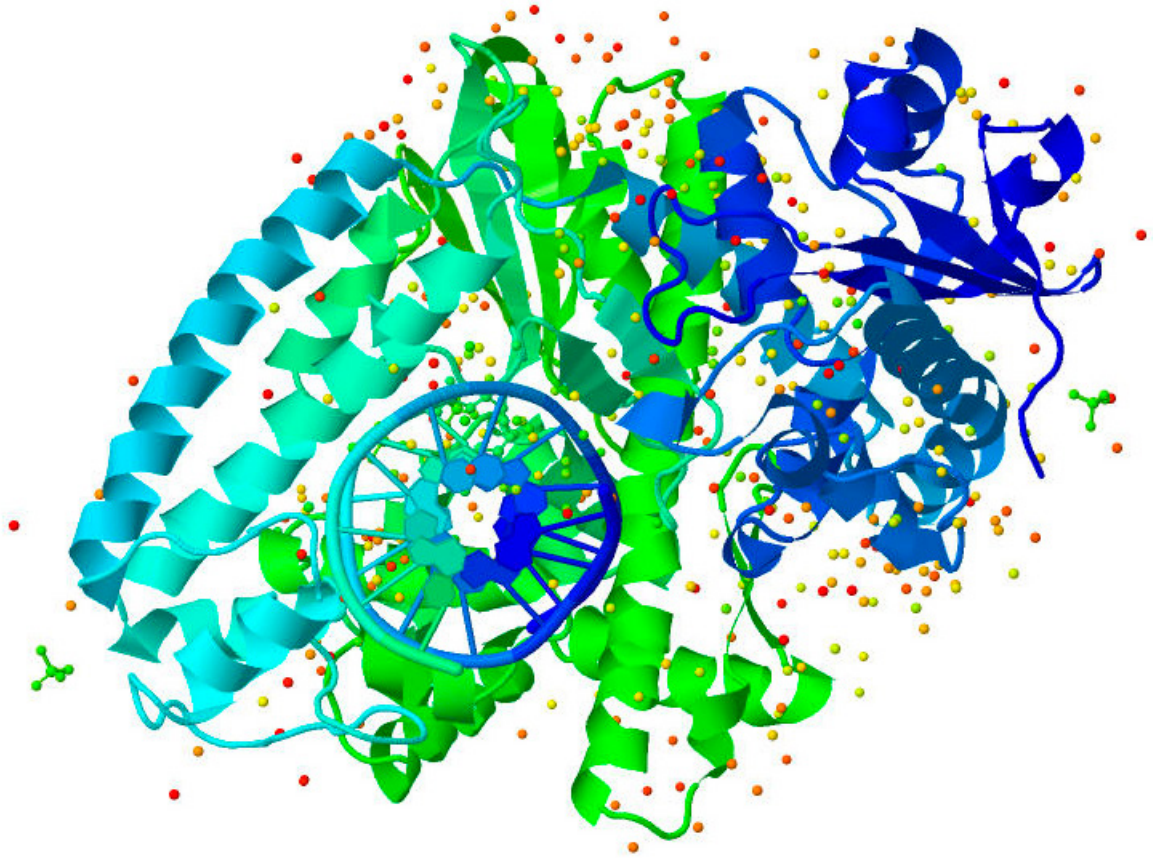


Figure 2. DNA Polymerase I/DNA complex: DNA replication in a catalytically active Bacillus DNA polymerase crystal.

The genetic engineering discipline remains one that requires deep knowledge, experience, and expertise to practice responsibly. Significant advances in the technology, however, have made it more accessible to people who might not have the required knowledge and experience to weigh the risks of their experiments adequately, take the necessary safety measures, or have the skills required to avoid the errors and mistakes in their work that could endanger the environment.

Indeed, DIY gene-editing kits are available for less than $200, and more sophisticated labs with lots of flexible options can be built for $2000. From the public's perspective, this puts very sophisticated and potentially dangerous technology in the hands of people who do not possess the knowledge required to wield it safely and responsibly. Moreover, the introduction of new living organisms into the environment can persist and impact future generations.

Therefore, the use of this kind of technology, which has the potential to do both great good and great harm, at a minimum need to be regulated and monitored to make sure that the people using it have the required knowledge and skills to do so safely and responsibly.

Microbial Interactions are Complex, Fundamental to Health, and Unpredictable

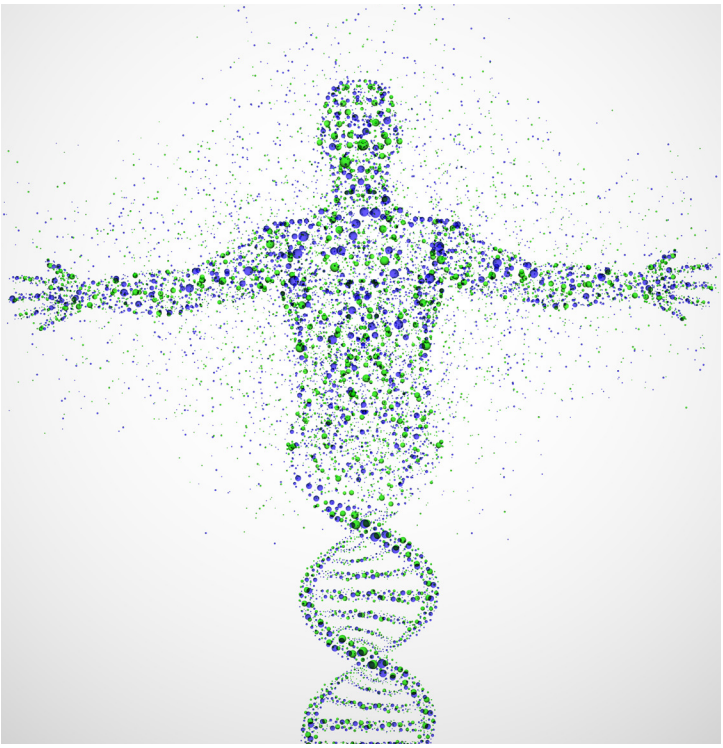
Microbes play a vital role in the environment: they are present everywhere, including in the most extreme environments, such as the North and South Pole, inside volcanos, and deep below the Earth's surface. Although pathogenic microbes that cause diseases may be the most prominent in the public's minds, there is a myriad of microbes that exist in a symbiotic relationship with their environment. The latter perform vital regulatory roles in animal and human metabolism and health and provide plant nutrition in the soil by capturing and converting nitrogen from the air. Without these complex microbial systems – known as microbiomes, life on Earth would wither and die.

The human microbiome (the system of microbes in

and on a human body) has been recognized as a vital element of human health (Scientific American, 2009; Merten et al., 2020). The National Institute of Health launched the Human Microbiome Project in 2007 with an explicit goal of analyzing the genomes of all the microbes that live in the human body (Science Daily, 2010). By 2020, over 200,000 genomes from the human gut microbiome alone have been cataloged and published, along with 170 million protein sequences from 4,600 bacterial species (Science Daily, 2020).

These two databases, the Unified Human Gastrointestinal Genome collection, and the Unified Gastrointestinal Protein catalog, highlight the diversity and complexity of the human gut microbiome: more than 70% of the detected bacterial species had never been cultured in the lab, and their activity in the body is still unknown. However, recent studies also show that the human microbiome is very susceptible to environmental effects and can undergo rapid changes due to outside influence (Vangay et al., 2018; Kaplan et al., 2019).

"The soil microbial community represents the greatest reservoir of biological diversity in the world. The rhizosphere is the soil region in which microorganisms are most abundant because of the richness in plant photosynthates and



secondary metabolites such as flavonoids that inhibit or stimulate targeted microorganisms. Plants may also secrete quorum sensing-interfering compounds that manipulate gene expression in the soil community. The collective genome of the rhizosphere microbiome is referred to as 'the plant second genome'. It has a crucial function for the plant, ranging from the recruitment of essential nutrients to boosting the defensive capacity against pathogens. The functions of the rhizosphere microbiome and its relationship with plants resemble those of the gut microbiome with its animal host" (Merten et al., 2020).

Soil fertility and plant health have been shown to depend on the balance within soil microorganisms, which is commonly disrupted by human activities like the use of antibiotics, heavy metals, and plant protection products like pesticides and herbicides. However, the environmental risk assessment does not fully consider the importance of the soil microbiome, although it is "crucial for the conservation of soil health, particularly under changing environmental and/or management conditions". It remains to be clarified how current environmental risk assessments could capture possible indirect effects of plant and soil microbiomes on soil fertility and plant health" (Merten et al., 2020). The author also adds: "There are no standardized approaches to characterize a healthy soil from a microbiome perspective..." Without sufficient knowledge to understand what is considered healthy, impact assessments and introductions of GM microbes have no meaningful references or metrics.

Biodiversity can be illustrated by some characteristic numbers: "a teaspoon of soil (about one gram) may typically contain one billion bacterial cells (corresponding to about ten thousand different bacterial genomes), up to one million individual fungi, about one million cells of protists, and several hundred of nematodes" (ESDAC, 2020). The soil also contains larger organisms like arthropods, earthworms, and mammals.

Our current knowledge and understanding of both the human and environmental microbiome is rapidly evolving and finally starting to catch up

The soil microbiome also plays a fundamental role in some of the key aspects of an ecosystem, including the delivery of essential ecosystem goods and services:

Ecosystem goods provided by soil biota are:

- food production
- fiber production
- fuel production
- provision of clean water
- provision of secondary compounds (e.g., pharmaceuticals and agrochemicals)

Source: ESDAC, 2020

Ecosystem services provided by soil biota:

- driving nutrient cycling and regulation of water flow and storage
- regulation of soil and sediment movement and biological regulation of other biotas (including pests and diseases)
- soil structure maintenance
- detoxification of xenobiotics and pollutants and regulation of atmospheric composition

with the importance of these systems to human health and ecosystem balance. However, since the concerted effort to analyze and catalog the microbes in these systems only began in the mid-2000s, it will be some time before we achieve the level of understanding that may allow for adequate predictive and risk assessment capabilities.

Cockburn (2002) addresses the issue of when a modified gene in plants leads to a changed metabolic pathway or a new biochemical pathway. He proposes a "full analysis of the gene for open reading frames, ribosome binding sites. Moreover, the cell's metabolic economy may be altered upstream or downstream of targeted change in the pathway affecting the overall nutritional and or toxicological profile of the crop". This general principle can also be applied to GM microbes: genetic modification and properties imparted by it can create new interactions with other organisms in the environment, usually in an unpredictable manner.

Our knowledge of metabolic and chemical processes in the human body is significantly more advanced than the knowledge of the human microbiome and the soil's microbiome. Therefore, if we take the risk of modified or new metabolic or biochemical pathways seriously, we need to acknowledge that the risk of unforeseen interactions of GM microbes with the microbes in the human body or the soil cannot be adequately assessed with our current level of knowledge.

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How A Pandemic Can Be Made Worse

CDC estimates that the seasonal flu epidemic infects between 9 and 45 million people in the US each year (3-13% of the population), causing 140,000-810,000 hospitalizations and 12,000-61,000 deaths annually since 2010. Epidemiologists measure the speed of the spread of an epidemic by using a number called R0 – the number of people on average that catch the disease from one infected person. When R0 is less than 1, the disease dies out on its own because fewer and fewer people get infected in each infection cycle until the number drops to zero.

For seasonal flu, R0 = 1.2-1.4, meaning that a single infected person infects an additional 1.2 to 1.4 people in each cycle, allowing the disease to spread. For pandemic flu, R0 = 1.5-1.8, while for COVID-19, R0 = 2-3.3 (Hilton and Keeling, 2020). The application of widespread epidemiological measures, however, reduced this to around 0.8-1.4 (Battiston, 2020; Dharmaratne et al., 2020; BBC News, 2020; Gov.uk, 2020).

While there are several factors that make COVID-19 more dangerous than flus, e.g. higher mortality, more hazardous and longer-lasting symptoms, its ability to transmit significantly faster than the flu made COVID-19 is critical. Left unchecked, based on its R0 factor without taking into consideration any epidemiological countermeasures, the disease would infect 50-70% of the population within six months – about ten times more than the flu, with millions of hospitalizations overwhelming the healthcare system, leaving many patients without any care (Figure 3).

This would result in hundreds of thousands of unnecessary deaths. R0 is mainly dependent on the modes of transmission of the pathogen – in this case, the flu virus and SARS-CoV-2 virus. The flu virus transmits through droplets, while there is some evidence that the SARS-CoV-2 virus has some ability to be transmitted through air, too. This significantly increases its transmission rates, causing additional infections and requiring aggressive epidemiological measures to reduce the R0, which inflict significant economic damage.

This highlights the importance of the transmission rate of the epidemic and why most research regarding genetic modification of pathogens, like the flu virus, is concerned with their transmission – mostly with how a virus mutates to gain the ability to transmit from human to human. Scientists tend to look for genetic modifications that would allow a virus to become infectious to humans and transmitted from one human to another. Also, they investigate modifications that would allow more effective transmission of the virus between humans, making a virus much more dangerous.

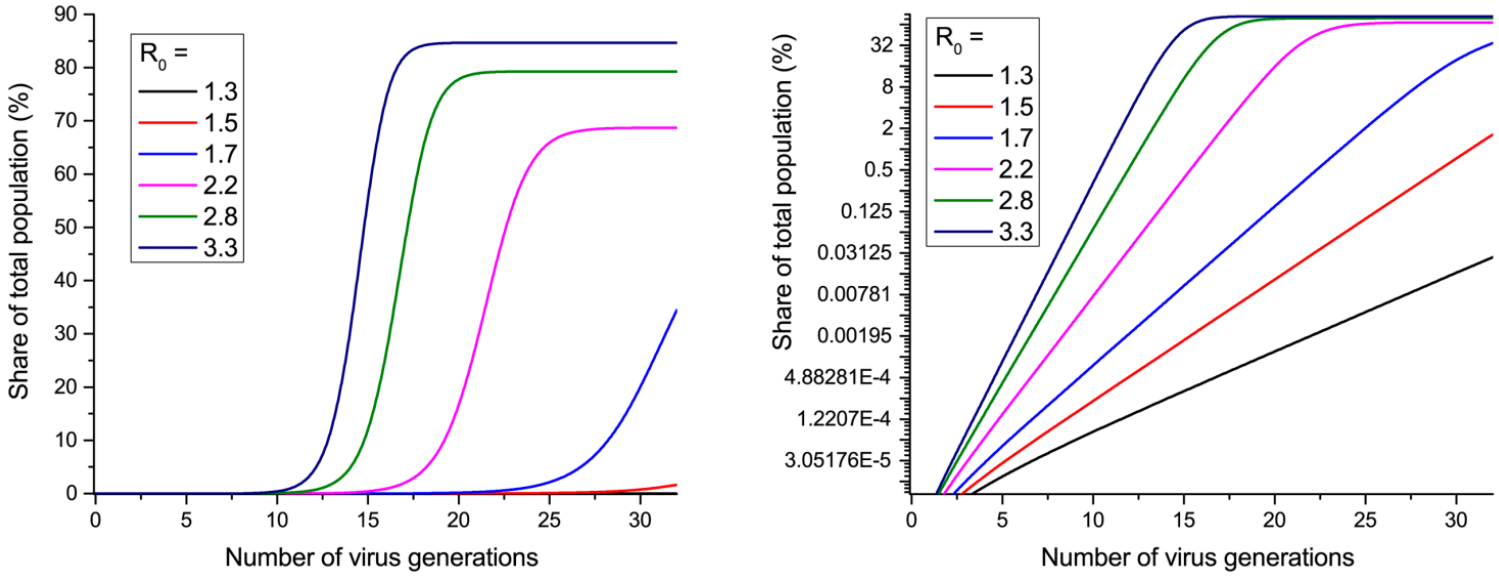


Figure 3. Progression of an infectious disease through the US population (est. 325 million) depending on the R0 factor with an initial pool of 5 infections (one virus generation is assumed to be five days).

In 2012, two studies were published on genetically modified H5N1 avian flu virus (Morens et al., 2012; Nature, 2013). While this virus was not created through gene-editing, this case highlights the potential dangers of genetically modified pathogens. This newly created strain of the H5N1 virus was able to transmit through the air (making it similar in that regard to the SARS-CoV-2 virus), and it spread quickly among ferrets. Ferrets are commonly used in influenza studies because they "emulate numerous clinical features associated with human disease" (Belser, 2011) . This research was not done in the safest BSL-4 laboratories but BSL-3 laboratories.

H5N1 infections in humans are very rare. There have only been around 600 cases, mostly in Asian countries, and they occur in exclusively individuals who are in prolonged close contact with infected birds, in most cases poultry. The mortality rate of H5N1 is estimated at around 53%, which is 455 out of 861 confirmed cases (WHO, 2020). Although one could expect this mortality rate to be reduced as the virus moves through the population, it is clear that the H5N1 virus has the pandemic potential of smallpox – the deadliest disease in history, which killed over 500 million people between 1878 and 1978, with an average mortality rate of 30%.

This example shows how dangerous genetically modified pathogens can be. If a genetically engineered pathogen were accidentally released into the environment, it could throw the entire world into another pandemic, but this time much more deadly with potentially much worse economic and social consequences. The prospect of widespread loss of human life and economic damage requires very careful risk assessment: potential benefits must be weighed carefully against the potential release and its consequences.

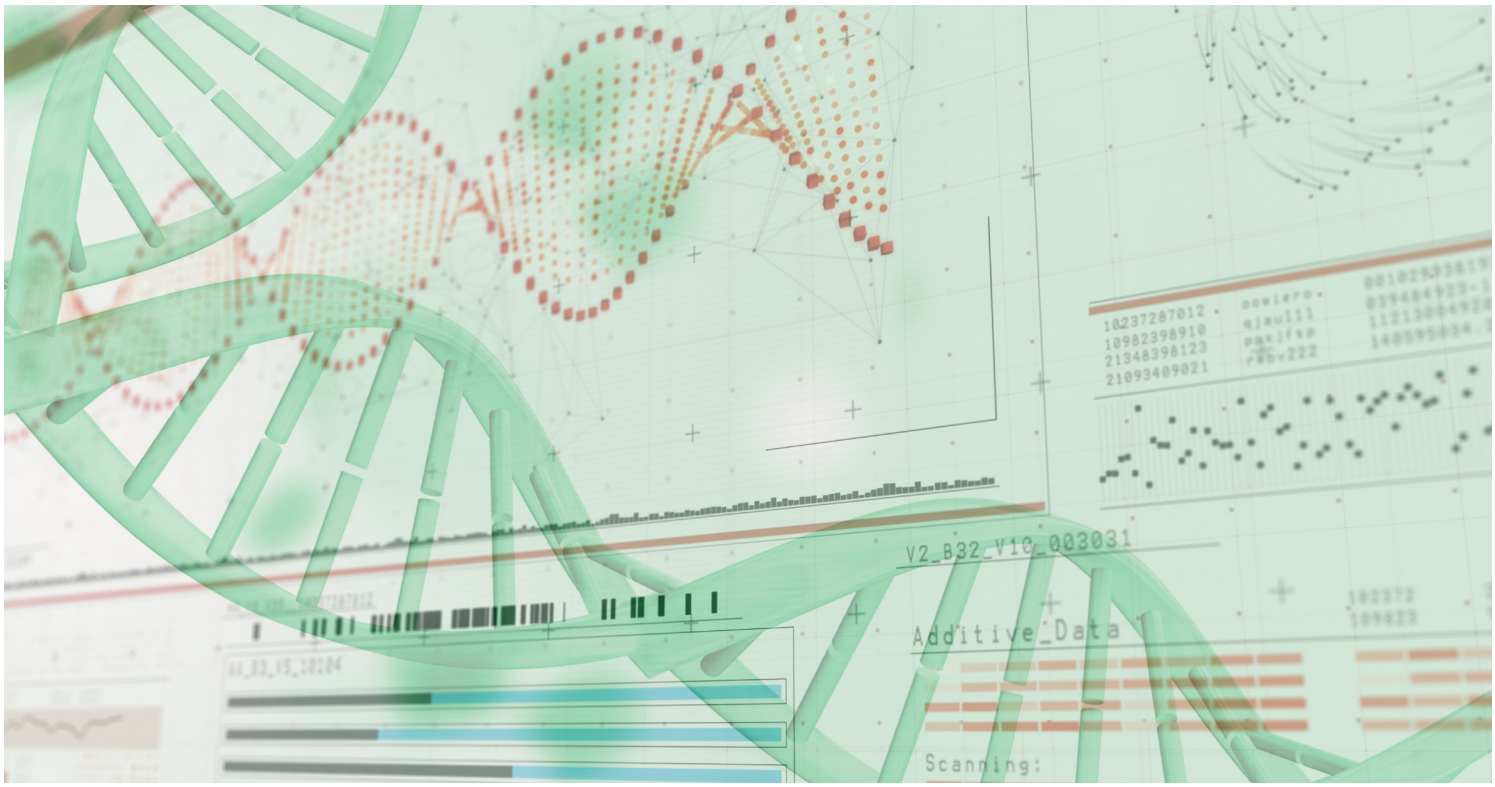
Genetic engineering techniques aren't the only methods that pose a danger with respect to known pathogens. In 2017, a group of Canadian scientists without specialist knowledge, in a small lab and at the cost of around $100,000, used large-scale synthetic biology to recreate the extinct horse pox virus (Noyce et al., 2018).

Horse pox belongs to the same family of viruses as the variola virus causing smallpox. This research demonstrated that virus-like variola could be created in a lab with relatively limited resources and technical knowledge. The fact that the virus was declared eradicated in 1980 means that there have been no active vaccination programs since most Western countries discontinued vaccinations against smallpox in the late 1960s. Although a vaccine exists, there are no large stocks available, so it would have to be manufactured and distributed in case of a pandemic.

The prospect of widespread loss of human life and economic damage requires very careful risk assessment: potential benefits must be weighed carefully against the potential release and its consequences.

The last smallpox outbreak in Europe was in 1972 in Yugoslavia: WHO, Western and Eastern bloc countries rushed their vaccine stock to provide for vaccination of the entire population of Yugoslavia. The endeavor took months, even with vaccine stocks available.

These examples highlight the risks associated with the application of gene-editing technologies to pandemic pathogens. Since the outcomes of experimentation with these microbes cannot be predicted with any degree of certainty, it is vital that the risks of exposure of the population to these microbes be minimized.



Biosafety and Biosecurity Issues: The Failure to Contain

Effective containment of genetically modified organisms, especially microbes, is a necessary component of any biosafety strategy. The rapid development of gene-editing and modification technologies has also significantly expanded their use. To maintain appropriate safety standards, regular and rigorous oversight is required by regulatory agencies and transparency when it comes to mistakes and failures of this system. Although there is a public perception that the research involving gene-editing is conducted under strict biosafety measures by highly trained staff, under scrutiny by regulators and inspections, this is not always the case. There is a well-documented history of safety and security failures in laboratories handling genetically modified organisms, including deadly pathogens.

Reporting by USA Today revealed that hundreds of labs across the United States working with the most dangerous pathogens known to man have repeatedly failed to achieve the goal of fully containing these pathogens and reported numerous incidents of contamination and accidental release (USA Today, 2015a, 2015b, 2016, 2017). Even more troubling, according to the USA Today investigation, the universities and federal research facilities operating these labs have done their utmost to deny access to the information about these incidents (which they were required to do as a condition of their federal funding). Meanwhile, the Center for Disease Control and Prevention (CDC) has been very slow to respond to FOIA requests from the media.

These biosafety issues are not unique to operating with microorganisms. A recent review of recorded contamination incidents associated with GM crops found 396 incidents from 1997-2013. It includes nine cases of contamination from unauthorized GM lines, i.e., those at the research and development stage with no authorization for commercial cultivation anywhere in the world (Price, 2014).

This highlights that the current system of oversight of biosafety is woefully inadequate to handle the potential risk of accidental release of GM microorganisms that could inflict significant environmental damage or pose a serious risk to human health. ***Therefore, a new legislative and regulatory framework is needed to address these failings and appropriately address the risks posed by these microorganisms.***

Gaps in Cybersecurity Compromise Biosafety

Another increasingly important aspect of biosafety is the cybersecurity of research labs: malevolent actors could penetrate the systems of a secure lab and steal information on GM microbes, edit the digitally stored genetic information to corrupt, sabotage, or exploit the research, or even create a biological incident remotely due to increased automation of these labs. Ironically, the increased reliance on automation and remote operation is meant to increase biosafety by reducing the risk of exposure for human personnel.

"In August 2017, a team of researchers at the University of Washington used DNA to take over a computer system. To do this, the scientists coded strands of DNA with malware that read as 0s and 1s, the language used by computers. When those strands were sequenced, the malware was activated, allowing the scientists to take over the computer analyzing the DNA. While in this case DNA was used to attack software maliciously, it's easy to imagine how DNA sequence data merely stored on a computer could be hacked and weaponized against the physical world, or misused in other ways" (The Guardian, 2017; Wired, 2017; FifthDomain, 2018).

In light of recent cybersecurity incidents in labs researching COVID-19 vaccine and attacks against the US secure cyberinfrastructure, this scenario is plausible and has to be taken into account when assessing the security risks of using gene-editing to produce new or modified microorganisms (BBC, 2020a; BBC, 2020b; VOANews, 2021).

Genetically Engineered Microbes Can Rapidly Spread and Permanently Alter the Gene Pool

In their discussion of genetically engineered trees, researchers highlighted the risk of pollen movement as an important component of risk analysis: "Any consideration of the potential risks of deploying GMOs in the wild must include an assessment of how far and how fast introduced elements are transferred to the surrounding conspecific (and sometimes congeneric) populations" (Smouse et al., 2007). The movement of genetically modified microbes, however, dwarfs this consideration.

The recent COVID-19 pandemic has demonstrated that in our globalized world, microbes can spread quickly across national borders and continents. Microorganisms are inherently hard to detect and can easily be proliferated across physical boundaries. That means that once released into the environment; GM microbes can be expected to spread largely unhindered unless drastic action is taken to eradicate their entire population immediately upon release.

Although many genetically engineered microorganisms incorporate traits that make them less competitive in the environment than their non-GM counterparts, this is not always the case. Besides, this is extremely hard to evaluate beforehand because the survival of microorganisms is driven by two main factors:

1) Interaction with the environment
2) Adaptation through mutation and natural selection

The interaction with the environment is hard to simulate in laboratory conditions, where, typically, the only competitor to the GM microorganism is their non-GM counterpart. It is possible that the released GM microorganism can derive an advantage from its interaction with any of the millions of different species present in the natural environment to out-compete its non-GM counterpart and replace it.

New Traits in GM Bacteria May Transfer to Other Strains and Ecosystems

One crucial aspect of the interaction of bacteria with their environment is the process of genetic exchange. Bacterial evolution is boosted by their ability to transfer genes between different species and genera (Davidson, 1999). This horizontal gene transfer in bacteria takes place by transformation, transduction, or conjugation, and it is mediated by plasmids. It is a relatively common evolutionary tool that provided the necessary genetic diversity for the bacteria to adapt to various conditions and environments (Hall et al., 2017).

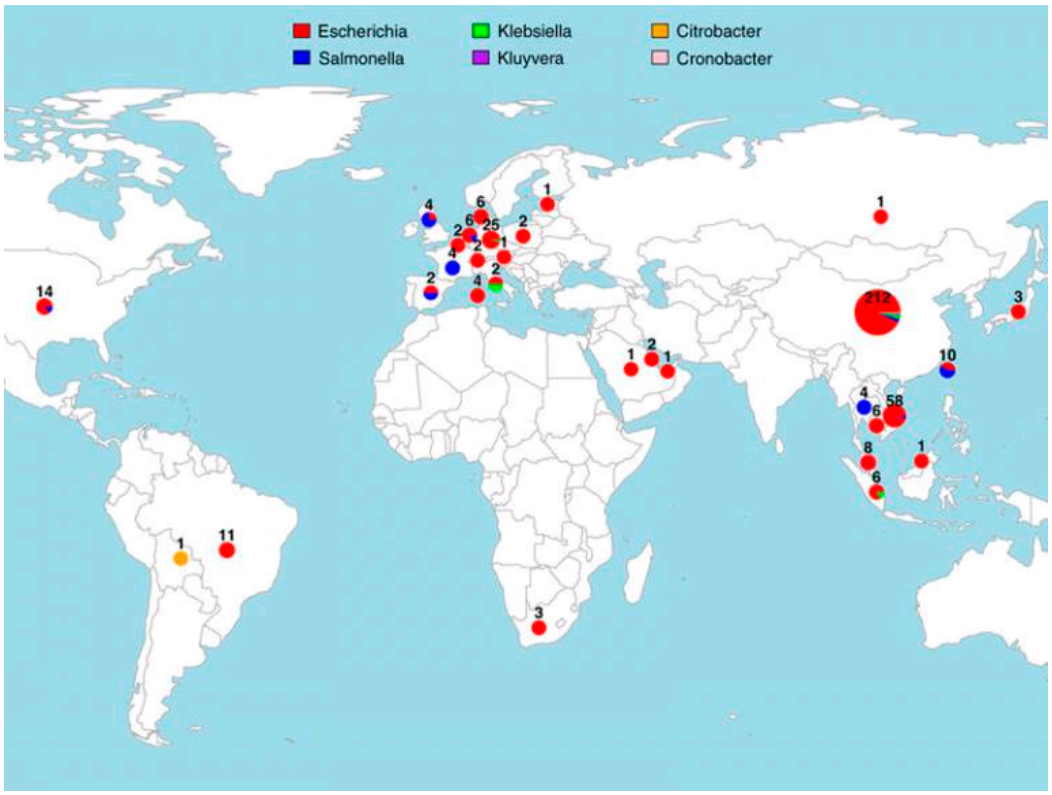


Figure 4. Spread of colistin-resistant bacterial gene *MCR-1* worldwide and across different bacterial species in 2017, detected in human patients. The origin has been traced to a single outbreak on a pig farm in China in 2006. (Reproduced from https://www.ucl.ac.uk/news/2018/mar/drug-resistant-gene-goes-pig-farms-patients-worldwide).

However, its consequences include the proliferation of undesirable plasmids, like antibiotic resistance or virulence genes in pathogens (Heuer et al., 2011; Cooper et al., 2017; Evans et al., 2020; Stevenson et al., 2017; Maheshwari et al., 2017). In addition, the spread of unwanted genetic traits can be faster than anticipated and can easily affect humans (Nature, 2017).

A recent study of genomic composition and pairwise sequence identity for over 10,000 reference plasmids found that more than 60% of plasmids are in groups with host ranges beyond the species barrier – meaning that they can transfer genes across different species of bacteria (Redondo-Salvo et al., 2020). The authors conclude that "although taxonomic boundaries constrain plasmid transmission, these are permeable enough to sustain large gene exchange networks throughout an entire bacterial order."

This represents a significant paradigm shift from the previous consensus that the bacterial gene transfer is mainly limited to DNA from similar bacteria. However, it was recognized that low-frequency events that could not be detected in the lab could have significant environmental consequences (Thomas, 2005). The process of horizontal gene transfer is not completely understood. In particular, the factors that promote the process still remain largely unknown.

However, a recent study found that microplastics promotes horizontal gene transfer in aquatic ecosystems (Arias-Andres, 2018). Considering that microplastic pollution is widespread, this is a troubling finding (Free et al., 2014; Yang et al., 2015; Sharma and Chatterjee, 2017). All of this highlights the potential long-term risks of proliferation of unwanted genes through bacterial populations. Engineered traits introduced into one strain for one purpose may travel to other strains, other ecosystems, and even the human microbiome with unpredictable and potentially long-term consequences.

GM Microbes Can Mutate, Adapt and Function Differently Than Designed

TThe other potential risk of introducing GM microorganisms into the environment is their inherent ability to adapt to the environment through selective mutation over numerous generations. The short generation time of microorganisms allows relatively rapid genetic evolution and potentially incorporates or develops new traits.

If GM microorganisms are, through changes in their genome, taken out of the equilibrium and given a

stimulus to evolve, this process is likely to be highly unpredictable due to the lack of reliability of gene-editing technology and the inherent difficulty of predicting the nature and intensity of interaction of the new microorganism with the environment.

Legislative and Regulatory Limitations

Most international GMO legislation and regulation incorporates at its core some form of risk assessment. Its goal is to introduce risk management to the use of GMO – to balance the potential benefits and risks and make an informed decision whether one outweighs the other. However, in practice, this legislative and regulatory framework's enforcement is widely open to interpretation by the individuals in the regulatory apparatus. In most countries, these tended to skew heavily in favor of GMOs.

Compared to vaccines and pharmaceuticals, which before the current pandemic was required to undergo extensive testing before approval (10.5 years on average for vaccines and 11.5 years on average for pharmaceuticals), and then are monitored for side-effects or other issues, commercial GM microorganisms are not required to provide a similar degree of evidence of their environmental or health impact before their release. Unless the GM organism contains foreign genetic material, like that from another bacterium, the regulators consider it equivalent to the naturally occurring variant. They do not even consider their potential impact.

The regulatory record for GMOs worldwide has been skewed heavily towards approval in developed countries: for instance, in Australia, out of 167 applications to the Office of the Gene Technology Regulator in 2001-2019, 155 were approved, 12 withdrawn, and none rejected.

European Union

"In the European Union, at the Union level, GMO regulation is handled by the European Food Safety Authority (EFSA). EFSA was established to provide an independent scientific risk assessment on the health and safety of GMOs by interpreting the meaning of general goals and issuing recommendations.

EFSA evaluates each GMO by acquiring data on the composition, toxicity, allergenicity, and nutritional value. A selection is made on selected proteins in the GMO for study and comparison. Other than compositional analysis, EFSA also recommends the use of animal feeding studies in certain circumstances to detect toxicologically or nutritionally relevant differences" (Krimsky, 2019).

Although the EU approval process is far more rigorous than that used in the US, critics point out that it is still inadequate to protect against many of the adverse outcomes of the genetic engineering process. A recent article, for example, highlights some of the regulatory challenges encountered in the EU (Hilbeck et al., 2020):

"While EU legislation assumes that processes of genetic engineering can potentially cause different, more unpredictable and unintended adverse effects than the application of conventional breeding methods. EFSA instead assumes, without EU legislative authorization, that the molecular-level changes assumed to be controllably and precisely engineered into a GMO may be individually and separately identified by chemical analyses and assessed based on those chemical data. But biological activity, toxicological potency and ecological interactions cannot be identified or characterized solely by reference to the results of crude (or even sophisticated) chemical analyses alone."

U.S. Regulatory Framework

The U.S. approach to the risk assessment of GMOs is entirely different: the regulatory authority is divided between three agencies – the Food and Drug Administration (FDA), the US Department of Agriculture (USDA), and the Environmental Protection Agency (EPA).

Current GMO legislation in the U.S. is practically non-existent. There is no federal legislation addressing GMOs as a category. No legislation requires GMOs to be treated with particular care or undergo a separate evaluation of potential environmental impact. The regulatory framework

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for GMOs treats them the same as non-GMOs, which can have significant loopholes because the environmental impact is not something that EPA, FDA, or USDA regularly require when registering new plants or products.

Therefore, if one was to genetically modify an existing microorganism, introducing new features or capabilities that give it an evolutionary advantage over the naturally occurring organism, the potential environmental impact of displacing the naturally occurring organism is not considered by the regulator unless that new organism creates toxins or incorporates genetic material from an organism that is considered potentially dangerous.

Although the new organism could interact differently with other organisms in the environment, potentially even inflicting widespread environmental damage with its genetic enhancements, the potential risks are typically not considered by the regulator during the approval process if this new organism is derived from innocuous parents.

The U.S. Food and Drug Administration (FDA) relies on food manufacturers to conduct the risk assessment for GMOs. It offers a "voluntary consultation" process, where food producers can consult with FDA scientists regarding tests on the composition (toxicology, allergenicity, and nutritional levels) of the GMOs.

FDA has not established standardized tests for this consultation process: they simply recommend the acquisition of data comparing the composition of the GM organism to the originating non-GM organism.

"The FDA assumes that foods developed by the addition of foreign genes are generally regarded as safe (GRAS) (substantially equivalent) unless proven otherwise, whereas, in Europe, the designation of GRAS has to be demonstrated after testing is complete. If data on the compositional analysis of a GMO fail to demonstrate safety or substantial equivalence, then animal testing is suggested. First, a ninety-day study and then long-term studies (if needed) for evaluating chronic effects" (Krimsky, 2019).

The concept of substantial equivalence, which the FDA used to justify its hands-off approach, has been widely criticized. "The concept of substantial equivalence has never been properly defined; the degree of difference between a natural food and its GM alternative before its 'substance' ceases to be acceptably 'equivalent' is not defined anywhere. Substantial equivalence is a pseudo-scientific concept because it is a commercial and political judgment masquerading as if it were scientifically created primarily to provide an excuse for not requiring biochemical or toxicological tests" (Millstone, 1999).

The Environmental Protection Agency (EPA) regulates pesticides and other toxic materials. GMOs fall under its jurisdiction if modified to produce any of the substances regulated by it: like Bt toxin-producing crops or any other foreign protein or toxin. The producer is required to show

that the toxin is environmentally safe, not likely allergenic, and conduct a food safety analysis.

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Services (APHIS) regulates "plant pests": organisms that cause disease, injury, or damage to plants or plant products, including viruses, bacteria, fungi, and parasitic plants. GMOs are regulated under their authority if they contain genetic material from any plant pest or were created through gene transfer with Agrobacterium tumefaciens, which is classified as a plant pest.

USDA regulates through either a permit or a notification process. A permit is required for field trials of GM crops that produce pharmaceutical or industrial chemicals, requiring either an Environmental Assessment or an Environmental Impact Statement.

The central piece of regulation of GM microbes would be the Toxic Controlled Substances Act (TSCA), which allows EPA to regulate GM microbes that are not regulated by other agencies, and most GM microbes fall under the TSCA. EPA requires manufacturers of intergeneric GM microbes (that contain foreign genetic material) to submit a Microbial Commercial Activity Notice (MCAN) for review at least 90 days prior to the commercialization of the product. For non-intergeneric GM microbes, a premanufacturing notice (PMN) is required.

They are treated the same as a new chemical substance or significant new use of the existing chemical substance, which is a lower level of scrutiny than MCAN. Field trials of GM microbes require a TSCA Experimental Release Application (TERA) submitted at least 60 days prior to the field test.

This 60-day period is extremely short and not nearly long enough to properly evaluate the scientific information provided with the application, let alone collect additional information or data. Considering the myriad of potential issues with genetically modified microbes described above like gene-editing errors, mutations, gene transfers, etc. It would be extremely difficult for the regulators to simply review the data that would have to be provided by the manufacturer to address these, let alone leave enough time to fill in any potential gaps in the submitted information.

This imposes such severe limitations on the risk analysis process to the point of rendering it meaningless. Lack of comprehensive risk analysis can lead to authorization to release an environmentally dangerous GM microbe that could impact human and animal health and cause considerable economic and environmental damage. This is why the regulators need to be provided with sufficient time and a broad mandate to collect all the necessary information when evaluating GM microbes.

GM microbes' regulatory treatment as toxic chemicals is based on a broad legal interpretation of the EPA's regulatory mandate, which has never been seriously challenged in court. This ambiguous regulatory authority is based on the inclusion of microorganisms, especially living microorganisms, like bacteria and algae, in the legal definition of "chemical substances", leaving the EPA's regulatory mandate open to a legal challenge.

Since the EPA's regulatory authority has not been clearly established by the existing legislation, it can be argued that new legislation is needed that would establish this authority. This also raises the question of regulatory scrutiny: whether the EPA, in the absence of clear regulatory authority, has not been enforcing the regulation as strictly as it would have if it had this regulatory authority.

Limitations of the U.S. Regulatory Framework

The current regulatory framework in the U.S. has been shown to lag significantly behind

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the technological developments and the latest research. It largely ignores the unpredictable side effects of genetic engineering, including the gene-editing process, which have been shown to occur commonly and often result in change or loss of function.

It also fails to account for gene interactions and potential synergistic effects. Still, it treats each gene as a separate, non-interactive entity that functions as if completely isolated from the rest of the genome. This leaves the U.S. regulatory framework incapable of holistically evaluating the effects of genetic modification and allows for huge gaps in the regulatory process.

Several government agencies, professional associations, and individuals have criticized the existing legislative and regulatory framework in the U.S. A recent DHS report highlighted the failure of the U.S. legislative and regulatory framework to keep up with the technological advances in the field of gene-editing, saying that "this disconnect must be closed" (DHS, 2020).

The Federation of American Scientists says this on the existing shortcomings in the regulatory role of USDA concerning even the high-risk GMOs—those crops that are engineered to produce pharmaceutical drugs:

"...a number of gaps remain. The current USDA regulatory system does not ensure an in-depth environmental impact assessment before pharma crops are planted. Instead, USDA's gene-confinement measures are designed to "minimize" rather than prevent the contamination of non-GM crops. In practice, USDA is too understaffed to exercise sufficient oversight and largely leaves biotech companies to regulate themselves. Moreover, USDA keeps the locations of all test fields secret from neighboring farmers and the public, does not disclose the identity of the drug or chemical being produced, and condones biotech companies' practice of planting pharma crops anonymously, without identification." (FAS, 2020)

Another significant regulatory gap exists in the Toxic Controlled Substances Act (TSCA), which regulates most GM microbes. TSCA only regulates substances manufactured "for commercial purposes," defined broadly by the EPA as "the purpose of obtaining an immediate or eventual commercial advantage" (Mandel and Marchant, 2014).

This means that GM microorganisms not developed for commercial purposes can easily escape regulatory scrutiny. This is where it is easy to see how the existing regulatory framework has been made obsolete by the technological developments: it is clear that the existing framework assumes significant economic cost associated with the enterprise of genetic modification. This would make the non-commercial development of GM organisms rare or non-existent.

However, this is no longer the case, and gene-editing technology has been made much cheaper and more accessible in the past decade, with the expectation that this trend will continue in the near future. Given that the rapid development of gene-editing technology has significantly expanded the number of actors capable of using it, this raises a serious concern of unregulated development of potentially dangerous GM microorganisms outside any regulatory oversight, including the employment of the necessary biosafety measures and standards.

This can create a significant risk of GM microorganisms' accidental release into the environment without the knowledge of government agencies or any other regulatory or law enforcement authority. It could easily prevent timely containment and remediation, leading to potentially catastrophic consequences. If the development of GM microorganisms can escape any regulatory scrutiny, it would be extremely hard to prevent the intentional release of such organisms, as their origin could not be traced without some record by the regulatory agencies of their development.

Although any such release would be illegal, it is hard to see how the release could be detected quickly with the current state of biomonitoring technology, creating significant potential national security concerns, in addition to environmental and health issues.

In 2016, the US Department of Agriculture (USDA) announced that it would not regulate or

monitor the cultivation or sale and consumption of a mushroom that was genetically edited using CRISPR technology (Nature, 2016). This genetically edited mushroom also does not qualify for EPA review since it is not designed to produce a regulated substance, and it was not evaluated in the voluntary consult by the FDA.

Therefore, this genetically modified organism completely circumvented the existing regulatory framework to enter the food market and human consumption solely on the assumption that the gene-editing process was conducted cleanly and with absolute precision, without any evidence to support this assumption.

The mushroom was edited to delete one of the six genes that encode polyphenol oxidase (PPO), an enzyme that causes browning in many fruit and vegetables. But research published after the mushroom was given a pass by the USDA showed that the process used to knock out a gene failed about 1/3 of the time, with residual protein production detected in these cases (Smits et al., 2019).

While some of these genes continued to produce the original protein, others produced terminally truncated proteins or protein isoforms with missing internal sequences. The protein function of some of these was only partially preserved, producing inhibition-like effects of significantly reduced activity. This can have serious consequences for the cell's normal function, including the accumulation of chemicals to potentially toxic levels (Tomlin et al., 2017). The authors concluded that: "Our results imply that systematic characterization of residual protein expression or function in CRISPR–Cas9-generated KO lines are necessary for phenotype interpretation." (Smits et al., 2019)

This was one of more than 30 GMOs to evade regulatory scrutiny during the 2010s. The USDA decided that no oversight was needed as these GMOs do not contain genetic material from plant pests such as viruses or bacteria (Waltz, 2016). It prompted calls to analyze the genetic makeup of resulting organisms more closely and apply stricter regulatory standards due to the inherent lack of reliability of gene-editing technology (Kim, 2016):

"We wish to point out that gene disruption by transient transfection of cells with plasmids encoding Cas9 and gRNAs can result in cells or organisms that contain small portions of foreign DNA (up to hundreds of base pairs) that are derived from the introduced plasmids. We showed that human cells transfected with Cas9 and gRNA plasmids often contain small insertions (58–280 bp) at off-target sites2. One hypothesis is that introduced plasmids are fragmented in cells, and the resulting small DNA fragments are recombined into nuclease cleavage sites.

Alternatively, plasmid DNA might be used as a template by a cellular DNA polymerase that

functions in DNA double-strand break repair. Unlike small insertions at on-target sites, any insertions at off-target sites can only reliably be detected using whole-genome sequencing. This is because small insertions of up to several hundred base pairs cannot be detected by PCR or Southern blot analyses."

Even when the manufacturer of GM microorganisms is required to submit the MCAN to EPA for a 90-day review, this comes with several large loopholes. Section 5 of TSCA does not impose an affirmative duty on the developer to generate any safety information, let alone sufficient information needed for a comprehensive assessment of health and environmental impact (Mandel and Marchant, 2014).

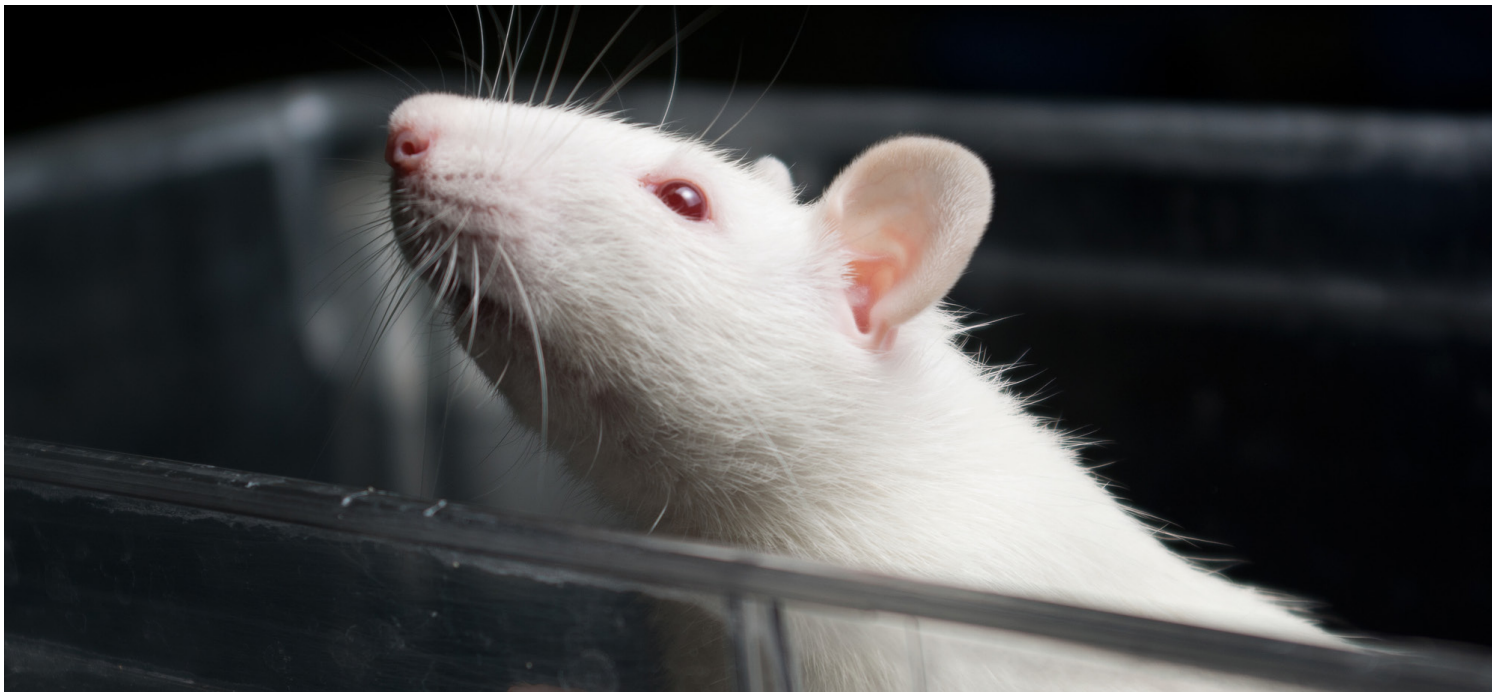
It only required the submission of known and reasonably available data. This can severely limit the EPA's ability to make an accurate risk assessment, especially considering that the EPA usually conducts the evaluation using existing risk assessment models. These models might not be appropriate for some GM microbes if the genetic modification results in a significant change of the function.

Besides, the environmental risks are much harder to evaluate than human health risks due to the system's higher complexity and much more limited existing dataset to evaluate against. Because the regulatory framework places the burden of proof on the EPA, there are serious doubts that effective and comprehensive risk evaluation can be conducted within the existing legislative and regulatory framework.

The lack of affirmative duty of the developer to generate safety information is also the complete opposite of the regulatory obligation imposed on the drug manufacturers, which are required to provide sufficient safety information to make a risk assessment of a new drug.

The only remedy for this situation has been voluntary consent decrees where the manufacturer agrees to conduct additional testing and safety evaluation and impose the use of protective equipment or restrict the product's use. However, the EPA is still limited in this by the manufacturer's available data and the voluntary consent of the manufacturer, especially considering the potential environmental impact. Besides, even when the relevant data exists, it can be of limited value for risk assessment due to the inherent shortcomings of the testing methodologies used to acquire it.

Animal studies for whole foods have well-identified limitations for measuring human health risks (OECD, 1996): dietary imbalances in the test animals can produce false positives. Simplified



models of the genome understate the risk of unanticipated events from transplanting foreign genes because they don't consider the potential for gene interactions, treating genes as isolated units.

Therefore, a more comprehensive analysis that includes the analysis of the molecules and metabolites is required when comparing GMOs to their non-GMO counterparts. Omics analysis – genome sequencing, transcriptome, proteome, and metabolome, would provide a better chance to detect the unintended effects in GMOs:

"One of the major challenges is how to analyze the overall metabolite composition of GM plants compared to conventional cultures, and one possible solution is offered by metabolomics. The ultimate aim of metabolomics is to identify and quantify all small molecules in an organism; however, a single method enabling complete metabolome analysis does not exist" (Rischer and Oksman-Caldentey, 2006). No regulatory agency currently requires such an analysis, and there are no standards for undertaking it.

In these cases, the regulators assume that the resulting GMOs have not been mutated or damaged during the gene-editing process. The resulting genetic makeup is the result of a consistently repeatable and reproducible process of gene editing.

The current gene-editing technology does not produce reliably repeatable and reproducible results and can often create unexpected changes to the genetic makeup of the organism. Therefore, the assumption that the new organism created this way is equivalent to the non-modified organism cannot be accepted at face value anymore.

The same principle would apply to any microbe that was genetically edited similarly – to edit or remove a portion of its DNA rather than introduce new foreign genetic material. As shown above, the current gene-editing technology does not produce reliably repeatable and reproducible results and can often create unexpected changes to the genetic makeup of the organism. Therefore, the assumption that the new organism created this way is equivalent to the non-modified organism cannot be accepted at face value anymore.

Unlike medicines, which can be recalled, relabeled, or adjusted for dosage after the release of GM microorganisms, they can only be contained using extreme measures as soil sterilization or "scorched earth" and only for a limited period after the release. These methods themselves inflict significant environmental damage and are costly to implement.

It has been shown that absolute containment in open field testing cannot be achieved reliably and consistently. After breaking containment, microorganisms can spread worldwide without any reasonable capability to monitor and stop their spread. Given the potential economic cost and environmental damage associated with the release of GM microorganisms, it is reasonable to expect that the level of scrutiny applied to them should be greater than that of the other products carrying a potential risk to human health and environment: medicine, chemical products, motor, and transportation vehicles, etc.

The new legislative and regulatory framework has to take into account that relatively small changes in the genetic makeup of an organism can result in significant changes in the behavior of that organism and its interaction with the environment. Therefore, GM microorganisms' risk assessment has to be completely different from that of microorganisms that have been a feature of our environment for centuries. To have effective and productive regulation of GMOs, it is also necessary

to ensure that the enforcement is conducted in the spirit and with the intent imbued in the legislative and regulatory framework.

Synthetic Biology

Synthetic Biology is a multidisciplinary field investigating the possibilities of creating new biological entities, devices or systems, or a redesign of naturally occurring entities. The creation of entirely new biological entities that are different from any existing entity raises unique concerns: the existing entities typically occupy an environmental niche. They exist in a long-term environmental equilibrium with the rest of the ecosystem. The introduction of an entirely new species may have unforeseen consequences, going as far as to completely upset the ecological balance and create a cascade effect that permanently changes the environmental conditions.

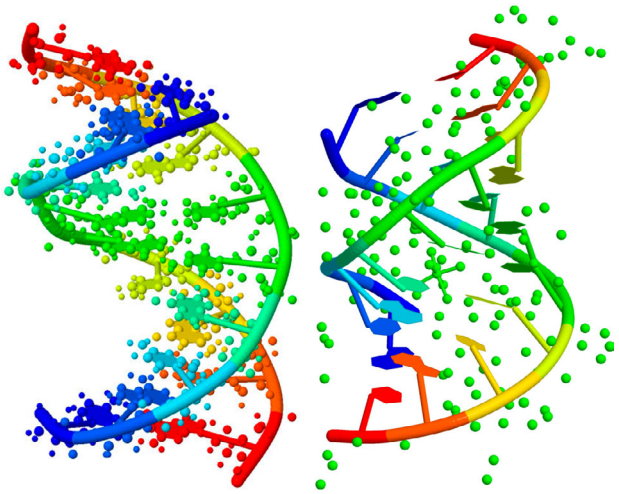


Figure 5. Synthetic DNA: Synthetic DNA duplex dodecamer (left); 1.16Å X-ray structure of the synthetic DNA fragment with the incorporated 2'-O-[(2-Guanidinium) ethyl]-5-methyluridine residues (right).

One parallel that can be drawn is introducing invasive species in isolated ecosystems on Earth, like Australia, or isolated islands in the Pacific Ocean. A recent study looked into the causes of recent extinction (953 extinctions in the last 500 years):

"We compared how frequently alien and native species have been implicated as drivers of recent extinctions in a comprehensive global database, the 2017 International Union for Conservation of Nature (IUCN) Red List of Threatened Species.

The current legislative and regulatory framework attempts to guard and protect against the spread of invasive species, including microorganisms like bacteria, algae and fungi. Therefore, the risk of the introduction of synthetic microbes into the environment cannot be understated.

Alien species were considered a contributing cause of 25% of plant extinctions and 33% of animal extinctions. In contrast, native species were implicated in less than 5% and 3% of plant and animal extinctions, respectively" (Blackburn et al., 2019).

It can be argued that the introduction of a completely new synthetic microbe could be compared to the historical introduction of potentially invasive species into the isolated ecosystems when considering the extent of potential environmental damage. History has taught us that this has regularly caused significant changes to those ecosystems that could not be reversed, or it took considerable effort and cost to reverse these changes.

The current legislative and regulatory framework attempts to guard and protect against the spread of invasive species, including microorganisms like bacteria, algae and fungi. Therefore, the risk of the introduction of synthetic microbes into the environment cannot be understated.

However, the current legislative and regulatory framework does not recognize synthetic organisms as a separate category. The EPA regulation requires a higher level of regulatory scrutiny to be applied to intergeneric GM microorganisms – those containing

foreign genetic material (genetic material from another organism), through submission of Microbial Commercial Activity Notice (MCAN). However, synthetic genes do not originate from another organism. It could be argued that synthetic GM microorganism might not be regarded as intergeneric under the EPA regulatory definition and could avoid a higher level of regulatory scrutiny.

The preamble to the 1986 Coordinated Framework for Regulation of Biotechnology partially addresses this, stating that:

"Organisms are considered dissimilar for the purposes of this policy if they are from different genera. In the case of chemically synthesized genes, the agency will follow a similar principle. The genetic sequence of the synthesized gene may be identical to a sequence known to occur in an organism in the same genus as the recipient microorganism. If so, the resulting microorganism will be considered intrageneric. Conversely, the sequence of the synthesized gene may be different or not known to be identical to a sequence in the genus of the recipient microorganism. In this case, the resulting product will be considered inter-generic" (Mandel and Marchant, 2014).

However, the regulatory guidelines specifically require MCAN for intergeneric microorganisms, defined as "a microorganism that is formed by the deliberate combination of genetic material originally isolated from organisms of different taxonomic genera" (40 C.F.R. § 725.3(2)(v), (2013). This allows synthetic GM microorganisms to avoid strict regulatory scrutiny under the current regulatory framework, which would be at odds with their higher potential environmental and health risks.

In 2010, the Presidential Commission for the Study of Bioethical Issues, prompted by the publication of the world's first self-replicating bacterium with a synthetic genome, concluded that "there was no justification for a moratorium or the development of new federal regulations." (Presidential Commission for the Study of Bioethical Issues, 2010). This assessment has been rendered obsolete by the rapid development of gene-editing technology, the reliability issues that have been recently associated with it, and the expanding knowledge about potential environmental consequences of a release of synthetic genetic material into the environment and its proliferation in the native bacterial population.

Synthetic microbes require special consideration because some of their intended uses, like bioremediation, require their release into the environment. While it is expected that most of these organisms would be at an evolutionary disadvantage to naturally occurring organisms due to their relative genetic simplicity, they can still be expected to mutate and interact with other microbes in the environment. This can lead to the unexpected exchange of genetic material and the proliferation of the artificial genetic code (Balmer, 2008; Presidential Commission for the Study of Bioethical Issues, 2010).

Although organisms can theoretically be designed to be killed off by applying a particular stimulus, such as a chemical or antibiotics, the potential for a mutation and other considerations could make this highly unreliable for biocontainment. Recent studies have shown the propensity of synthetic microbes to undergo mutations, including large-scale DNA deletions and gene loss that led to the loss of function (Sleight, 2010; Hosseini, 2018).

Naturally occurring organisms developed mechanisms to combat this: clustering of essential metabolic genes, a greater-than-expected distance of synthetically lethal metabolic gene pairs, and the clustering of non-essential metabolic genes. However, synthetic microbes tend to be designed without these redundancies but with a minimal genome – without non-essential genes that provide genetic robustness to the naturally occurring microbes.

This significantly increases the chance of mutation that would alter a synthetic microbe to change its function or lose it completely, rendering it not just useless to the intended purpose but also potentially dangerous to the environment. Once they are released into the environment, the evolution of such organisms would be extremely difficult to predict. All of these represent significant risk factors: each one of these would require serious regulatory consideration. The fact that these risks are compounded suggests that strict new laws are essential.

Genome Editing Technology as a Tool for Global Terrorism

Traditionally, biological weapon development has been practiced exclusively by major countries due to significant up-front investment required for sophisticated equipment and facilities needed; high operating costs of these facilities, extreme hazards of working with bioweapons, and limited availability of dedicated laboratory equipment because larger countries tend to regulate and monitor its use. The research staff capable of conducting this type of research has been relatively hard to find due to the high level of scientific and technical expertise required. However, modern technological developments have been changing this situation.

Former Director of National Intelligence James R. Clapper highlighted genetic engineering as one of the "technological challenges that we're going to have in the future" (Clapper, 2016b). Recent developments in gene-editing technology have significantly expanded the potential applications of this technology and, at the same time, made it more accessible.

This significantly lowered the threshold of required scientific and technical expertise while greatly expanding the number of people in the scientific community capable of conducting this type of research. As a result, the requirements for creating a lab capable of producing biological weapons have been significantly reduced both in terms of human expertise and equipment, expanding the potential number of malicious agents capable of undertaking such an endeavor. This has caused the technology of gene editing to be increasingly viewed as a national security threat (Esvelt, 2017).

The Director of National Intelligence James R. Clapper before the Senate Armed Services Committee, 9 February 2016 said the following about the research in genome editing: "Given the broad distribution, low cost, and accelerated pace of development of this dual-use technology, its deliberate or unintentional misuse might lead to far-reaching economic and national security implications" (Clapper, 2016a).

The main risk from introducing GM microbes into the environment stems from the changes to the genetic makeup of the microbe's natural population that could lead to change in function, causing environmental disruption or outright damage. Gene drives are a method of propagating genetic changes through the natural population much faster than they would normally occur by introducing GM organisms. They can achieve the same outcome as the introduction of GM microbes in a much shorter period. There have been warnings from the scientific communities about the need to regulate the use and application of gene drives due to "environmental and security challenges", which can also be applied to the environmental release of GM microbes:

Targeted wild organisms. Scientists have minimal experience engineering biological systems for evolutionary robustness. Drive-induced traits and altered population dynamics must be carefully evaluated with explicit attention to stability.

Non-targeted wild organisms. In theory, precision drives could limit alterations to targeted populations, but these methods' reliability in preventing spread to non-target or related populations will require assessment. To what extent and over what period of time might cross-breeding or lateral gene transfer allow a drive to move beyond target populations? Might it subsequently evolve to regain drive capabilities in populations not originally targeted? There may also be unintended ecological side effects.

Crops and livestock. A technology capable of editing mosquito populations to block disease transmission could also be used to alter populations of agricultural plants or livestock by actor's intent on doing harm.

Source: *Science*, 2014

Security concerns have also been raised in other developed countries in the world, like France. A report from the National Biosecurity Advisory Council (Conseil national consultatif pour la biosécurité) states:

"...the improvement of genome construction techniques through synthetic biology poses the question of the ability to recreate de novo

microorganisms which already exist in Nature, such as viruses whose virulence and contagiousness could constitute real risks for the population's health security. In this respect, the development of new techniques in the field of the synthesis of DNA and the multiplication of private companies who master those tools to create synthetic genes pose genuine security and potential proliferation questions". (InfoGM, 2017)

A recent report from the Department of Homeland Security stated:

The following three recommendations address how the Department of Homeland Security should respond to ensure CRISPR is not used to harm the United States.

Recommendation #1

Actively get ahead of advances in CRISPR and gene therapy delivery systems. CRISPR is an unprecedented technological advancement in molecular biology. It poses many benefits but also many threats. In the coming years, threats to the Homeland will develop from CRISPR genome manipulations. [DHS needs to] predict witting and unwitting threats of CRISPR that might harm the health, food resources, and/or national interests of the United States.

Recommendation #2

At some point, it will become essential to determine whether CRISPR has been used, regardless of whether it was an accidental or intentional deployment of the technology. As the basic science of CRISPR is rapidly becoming a tool for genome modification, the DHS should also be concerned with developing means to detect its use.

Recommendation #3

DHS should be monitoring and/or developing means to prevent the action of CRISPR technology or their delivery systems to prevent unwanted CRISPR modifications. For example, in the case of an accidental or intentional release of a gene drive that might harm U.S. citizens, food supply, vegetation, or wildlife, it may become necessary to understand mechanisms to inhibit the action of CRISPR technology.

Source: DHS, 2020

While the 20th century was the age of nuclear weapons, the 21st has become the age of biological threat. The widespread economic damage and loss of life caused by the COVID-19 demonstrate the dangers our world faces from microorganisms – either genetically modified or naturally occurring. If a malicious agent were to attack the U.S., there are several potential vectors of biological attack using genetically engineered bioweapons:

1) Target the population with an engineered infectious pathogen;
2) Target the food supply with an engineered pest or bacteria;
3) Target the economy with engineered pest or bacteria.

All of these take into account several factors:

1) The malicious agent will maximize the damage to the U.S. or the Western countries while minimizing the risk to their own country or population.
2) The bioweapon release does not have to be wide, and the bioweapon does not have to spread quickly or through the entire U.S.
3) Most of the economic damage will be indirect: from countermeasures, remediation effort, and quarantine measures.
4) The main goal is not to inflict maximum damage but to undermine the public's confidence in the government and its agents.

Since a direct biological attack would be tantamount to a declaration of war, it would be necessary to disguise it as an accidental release or to hide it in a regularly approved release of genetically modified organism into the environment – either in an open field trial or in an actual release. This way, the immediate blame for the damage could be put on the U.S. government. In the immediate aftermath of the COVID-19 outbreak, China suffered a significant diplomatic backlash over its handling of the outbreak. If a particularly dangerous GM microbe was released in the U.S., it is expected that the U.S. reputation would suffer even more than China's.

In addition to gene-editing of existing organisms, the rapid development of synthetic biology has

been recognized to pose unique national security challenges. In 2018, the National Academies of Sciences, Engineering, and Medicine (NASEM) released a report warning that the emergence of synthetic biology expands the landscape of potential defense concerns related to the use of gene-editing technology (NASEM, 2018).

This report highlighted that these concerns extend to "the potential applications of synthetic biology (also described as synthetic biology–enabled capabilities or uses of synthetic biology)." This includes "the manipulation of biological functions, systems, or microorganisms resulting in the production of a disease-causing agent or toxin." Since the same tools and methods used in synthetic biology are used to create new GM organisms,

A malicious agent could also exploit this technology to release genetically engineered pest that could replace the naturally occurring species and potentially wreak havoc on the US food supply, transmit the infectious disease to humans or animals, or cause environmental damage.

the same concerns would apply to GM microbes created using gene-editing technology.

Three potential dangers of the use of gene-editing technology from a national security aspect are highlighted in the 2018 NASEM report as those warranting the most concern: (1) re-creating a known pathogenic virus, (2) making existing bacteria more dangerous through gene-editing, (3) and making dangerous biochemicals (using genetically modified microorganisms to produce harmful chemicals in humans). They also acknowledge concerns about enhancing the dangers of known viruses and modifying human microbiome.

The report recommended that while the U.S. Department of Defense (DoD) should continue to pursue ongoing chemical and biological defense strategies, they also need to develop new broader approaches to account for wider capabilities enabled by synthetic biology.

Other developments are not yet considered within the realm of existing technology but could pose a significant risk in the future. Synthetic biology is expected to:

i. Expand the range of what is possible.

ii. Decrease the amount of time required to engineer dangerous organisms and expand the range of actors who could accomplish such an endeavor.

iii. Create high-potency molecules produced through simple genetic pathways with modest resources and technical knowledge.

iv. Expand the possibility of changing human physiology in novel ways – different than the effects of the currently known pathogens.

v. Create the potential for some other malicious application that is not plausible or not conceivable now.

A malicious agent could also exploit this technology to release genetically engineered pest that could replace the naturally occurring species and potentially wreak havoc on the US food supply, transmit th e infectious disease to humans or animals, or cause environmental damage.

All of this highlights the need for increased vigilance and regulatory scrutiny in the future. The potential national security risks from misuse and abuse of genetic engineering technologies are too great to ignore.

DARPA Programs and Practices

The U.S. Defense Advanced Research Projects Agency (DARPA) has many projects related to genetic engineering under development. These include a variety of potential applications like:

- Protection from accidental or intentional misuse of genome editing technologies.
- Protection against the infectious disease threat.
- Environmental monitoring for airborne pathogens and biosurveillance.

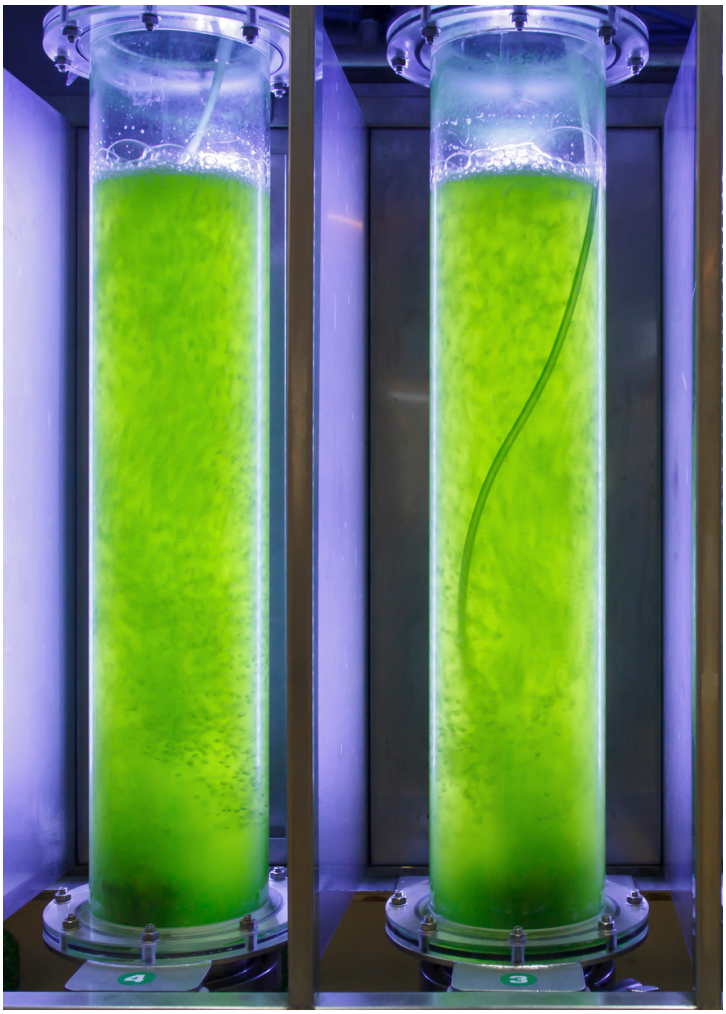
According to DARPA, a common characteristic of all of these projects is that they are conducted exclusively in a secure and contained laboratory or greenhouse environment. DARPA specifically prohibits open field tests and open release on its projects (DARPA, 2020).

Therefore, the U.S. Department of Defense has already put into practice safety measures required to reduce the potential risks to the population and the environment. It would be reasonable to apply the same standard of safety and security used by the DoD to public institutions and private entities doing research on GM microbes or using them for commercial purposes.

Conclusion

Genetically modified organisms have become a feature of our modern world since their introduction more than 25 years ago. The most visible of these in public policy debates have been plants. However, modern gene-editing technologies have expanded the potential use of genetic modification, and, in particular, it expanded the variety of genetically modified organisms. One of the broad categories of organisms that are frequently genetically modified for medical, industrial, or other purposes are microorganisms – viruses, bacteria, algae, etc.

The recent COVID-19 pandemic has brought into focus the ever-present danger of microbes like pandemic pathogens. The human loss has been high – over 2.5 million lives – and the economic and social damage has been substantial and worldwide. The rapid global spread, persistence during the warm weather months, and our societies' inability to contain it have shown that the public has underestimated the danger of pandemic pathogens to our globalized world.



The legislative and regulatory framework of genetically modified and synthetic microorganisms has been exposed as wholly inadequate.

This has also demonstrated how easily microorganisms can spread across national borders and continents in the age of mass travel, with the global proliferation of potentially harmful genetic material. Their containment or eradication methods typically incur significant cost and/or environmental damage, making it imperative to prevent rather than manage their unwanted release.

In light of all this, the legislative and regulatory framework of genetically modified and synthetic microorganisms has been exposed as wholly inadequate. Recent developments in genetic engineering have made it more accessible than ever while revealing clear shortcomings: frequent errors and deletions occur, both off-target and on and around target site of edited DNA – dubbed "chromosomal mayhem" in an article in Nature in 2017.

These introduce unpredictable and unwanted genetic changes, requiring more stringent scrutiny of genetically edited microorganisms. Compared to products with far less potential for harm to human health or the environment, GM microorganisms undergo significantly less testing or regulatory scrutiny. Some government organizations engaged in genetic engineerings, like DARPA, already state that they use higher safety standards: the work is performed in laboratory containment, and open field tests and organism releases are not conducted under its auspices.

One must also consider the national security aspect of this technology: the potential dangers of genetic engineering have been recognized by the Department of Homeland Security and the National Security Council. There is considerable risk associated with the accidental or intentional, malicious release of microorganisms and engineered pathogens into the environment. The proliferation of cyber technology might even allow a malicious foreign agent to remotely access the laboratory and sabotage it, effecting a microorganism release.

Therefore, a clear need for a new legislative and regulatory framework would effectively address the current state of gene-editing technology, the risks to human health and the environment associated with its use in microbes, and the potential national security implications.

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