
Down with the Sickness

Biological Weapons in the Past, Present, and Future

Michael Redlich^{a,b}



HARVARD Kennedy School

BELFER CENTER

for Science and International Affairs

Disclaimer

The views expressed in this academic research paper are those of the author and do not reflect the official policy or position of the US government or the Department of Defense.

Table of Contents

Executive Summary 3

Introduction 4

History until 1972 9

Biological Weapons Convention14

History since 1972.....16

United States biological defense program..... 24

Artificial Intelligence 26

Recommendations..... 29

Appendix 134

Appendix 2.....35

Works Cited38

^a Belfer Center for Science and International Affairs, Harvard Kennedy School, Cambridge MA

^b Northern Virginia Community College, Annandale VA

Correspondence: mredlich@nvcc.edu

Executive Summary

In this paper I perform a survey of biological weapons. I begin with an overview of the subtypes of biological weapons and investigate the technical considerations associated with their production and deployment. I trace the history of their usage from the 14th century B.C.E. to the present, with particular emphasis on the Biological Weapons Convention of 1972 and biological weapon production since then. Consideration is given to the motivations of states that have chosen to pursue biological weapons. I examine, too, the United States' biodefense program. In the final section I discuss possible future usage of biological weapons and propose several policy stances to reduce that threat.

Introduction

What is a biological weapon?

A biological weapon is an offensive tool that leverages either a biological agent or its byproduct to cause harm. Canonically one can divide these agents into five categories.

Toxins are poisons naturally produced by living organisms; typically these are proteins. A classic example is the botulinum toxin – a protein produced by the bacterium *Clostridium botulinum* and the causative agent of botulism. Harvesting toxins is a relatively straightforward operation – one must cultivate the relevant bioagent and then extract the toxin. In the case of ricin, for example, one extracts the toxin from the seeds of the castor oil plant, a common perennial.

As a potential weapon, toxins may be attractive as they are often lethal in lower doses than chemical warfare agents. The mechanism of action varies between toxins; the botulinum toxin acts by blocking the release of a neurotransmitter associated with muscle action – leading to paralysis and ultimately respiratory failure¹. Ricin, by contrast, operates by entering the cell and preventing proteins from being created – leading to cell death, which in sufficient quantities will cause organ failure². Most toxins can be treated with activated charcoal if administered quickly. The charcoal acts as a general treatment by binding to the toxin in the digestive system, preventing absorption into the body. Alternatively, if absorption has already occurred, treatment can involve a toxin-specific anti-toxin. Notably, however, toxins are not contagious meaning that associated disease is unlikely to spread beyond an initial attack.

Viruses are bioagents that are comprised of a protein shell enclosing an RNA/DNA genome which encodes the proteins necessary to produce a new virus. However, viruses require cellular machinery to reproduce. *Variola major*, for example, is the virus that causes smallpox. Viruses attach to the cell membrane and gain entry. Once inside, the viral RNA/DNA is released from its protective protein shell and simultaneously begins to replicate its genome and, leveraging the host cell's ribosomes, produces new viral proteins, which are then assembled into fresh copies of the virus. The virus then exits, commonly by bursting the cell³. While viruses vary in the specifics, each causes disease by interrupting cell function – either by redirecting cellular resources for viral production or killing the cell during the lytic cycle (Figure 1)⁴. As they cannot reproduce on their own, to gather viruses en masse they must be grown in host cells which in turn are cultured in bioreactors.

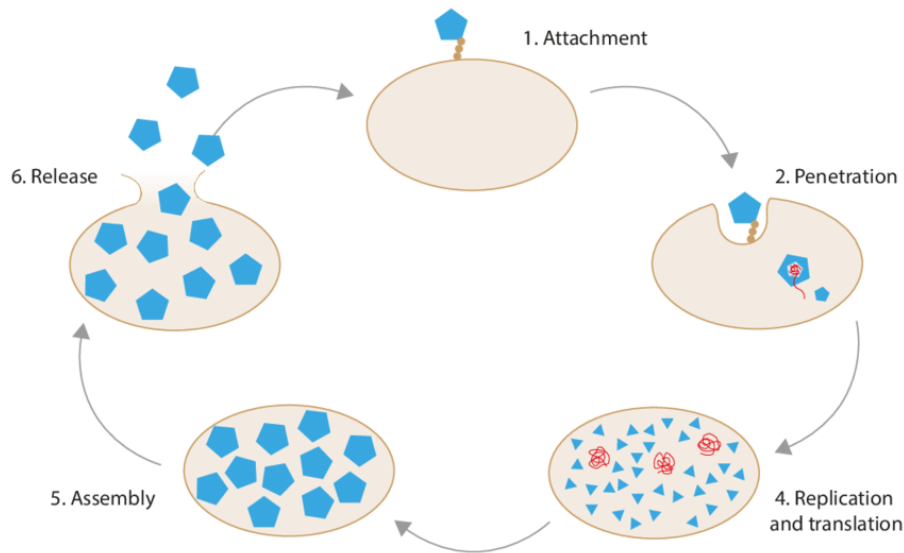


Figure 1. The lytic cycle of virus reproduction. Viruses inject their genetic material into a cell where it can be reproduced. Figure from Munke (2020)⁵.

Among biological weapons, viruses are generally the most difficult to weaponize. They require cultivation in an associated cell; in the case of human viruses, that would require human cell lines which are notoriously fragile compared to *Escherichia coli* – a common model organism in microbiology which has also been modified for industrial use by pharmaceutical companies. Because the cells are being infected by viruses, a prospective bioweaponer must be careful to modulate/control the virus spread in culture lest it kill off their host cells too swiftly. Further, most viruses mutate rapidly⁶; frequent and constant quality control is essential to ensure that the desired characteristics are maintained throughout the replication process. Once harvested, the virus must be kept at extremely low temperatures to maintain its viability, with thawing occurring just before deployment.

While viruses are more difficult to weaponize, they are also more difficult to treat than other biological agents. There is no universal first line intervention for viruses as there are with toxins (activated charcoal) or bacteria (antibiotics); because viruses are not alive, they have fewer vulnerabilities. Some viruses can be treated with antiviral drugs which serve to prevent the virus from multiplying by denying entry to the cell, halting the production of viral proteins, disabling assembly, preventing escape from the cell or stimulating the immune system to attack infected cells before the virus can fully take hold.

Bacteria are the most common vector for biological weapons – including *Bacillus anthracis* (Anthrax), *Yersinia pestis* (Plague), and *Francisella tularensis* (Tularemia). Bacteria are cultivated in bioreactors; a small sample of seed bacteria is placed in a vial with the appropriate nutrients. As the bacteria grows it is introduced to larger containers until the desired load is reached. In this stage, bacteria can be exposed to antibiotics to develop treatment-resistant strains, or simply genetically altered to incorporate known resistance genes. To enable weaponization, bacteria must be packed with preservatives and centrifuged to gain requisite concentration.

Bacteria can cause illness by secreting toxins which damage or kill native cells. Further, the body's natural immune response to bacteria infection can itself cause damage or death – as in the case of the “cytokine storm” reaction to the H1N1 flu which leads to fluid buildup in the lungs⁷. Bacterial infections can be treated with antibiotics, such as penicillin. Antibiotics work either by directly killing the bacteria (e.g., by bursting

the cell wall), or by inhibiting bacterial reproduction⁸. Due to frequent use of antibiotics, resistant strains of bacteria appear naturally, outpacing the discovery of novel antibiotics. With knowledge of the specific bacteria causing an infection, more targeted treatments can be provided.

Parasites include protozoa or worms that enter the body and cause disease due to mechanical damage, secretion of toxic agents, and inflammation⁹. Examples of protozoan parasites include *Plasmodium falciparum* (the causative agent of Malaria) and *Trypanosoma cruzi* (the causative agent of Chagas disease). Typically, protozoan parasites are transmitted via insects – e.g., Malaria via mosquitoes, so weaponization of these bioagents would involve the mass breeding of the agent and infection of a cadre of hosts¹⁰. For other protozoan infections, as well as worms, transmission occurs via ingestion. Here, weaponization would involve tainting the food or water supply of the targeted area with the parasite. Medical intervention varies depending on the agent used. Like toxins, however, parasites are typically not particularly contagious, limiting the potential spread of the disease.

Pests are animals that themselves cause damage, without an additional biological mechanism. Classic examples would include *Ceratitis capitata* (medfly) or locusts. They cause disruption simply by consuming agriculture, leading to crop failures. Weaponization would involve the mass breeding and release of the selected organism. Protection against pest damage typically involves the spraying of pesticides. For some pests, it is possible to release sterilized males who displace the natural males, driving down the reproduction rates of the species and potentially ending the infestation¹¹.

Production and delivery

A key benefit to a biological weapon is the apparent ease of construction. Biological agents of interest, with rare exception, are abundant and can be obtained discreetly. However, there are barriers.

While some of the pathogens discussed in this paper can be handled in a Biosafety level (BSL) 2 laboratory, the majority require handling in a BSL-3 or BSL-4 laboratory. Besides the requisite personnel training, BSL-3 laboratories require specialized biosafety cabinets and negative pressure rooms such that air does not carry pathogens out of the laboratory into uncontrolled areas. BSL-4 laboratories (Figure 2), which are used to study the most dangerous agents, have extensive protocols associated with them – including full-body pressurized suits, airlock entry, and decontamination showers – to prevent the spread of pathogens^{12,13}. As a result, the facility necessary to engage with the bioagents would be costly and even a cursory inspection would reveal the difference between an innocuous pharmaceutical production plant and a facility for handling biohazards. Alternatively, without installing expensive and conspicuous ventilation equipment, the laboratory could simply operate at significant risks to its employees – a pyrrhic strategy.

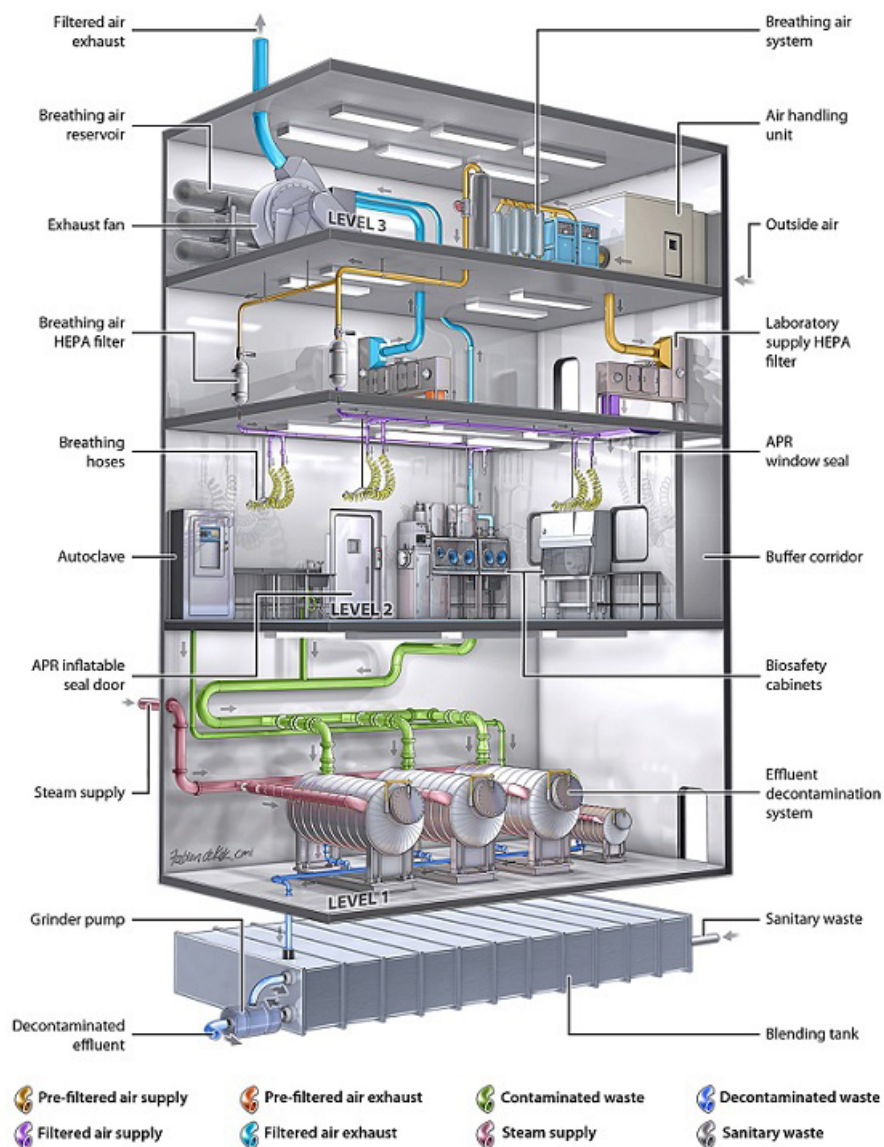


Figure 2. A schematic of a BSL-4 laboratory. Note the extensive atmosphere and ventilation system. Unseen in this diagram are airlock entryways and decontamination showers. Figure taken from the National Institute of Allergy and Infectious Diseases.¹⁴

A second difficulty is the need to engage in research to produce weaponizable bioagents. Even for *Bacillus anthracis* – perhaps the most “popular” biological weapon agent – a significant effort would need to be undertaken to determine optimal growth conditions and maintain the pathogenicity of the strain, if beginning work from scratch. While a relatively low hurdle for state actors, it is a barrier for terror groups. It becomes even more scientifically involved to implement genetic alteration should one wish to improve the virulence of the agent. This means that the development of a biological weapon involves substantial schedule and performance risk – as compared to “off the shelf” alternative weapons.

A third difficulty involves the delivery of the weapon. By far the most effective method is an aerosol – allowing the pathogen to be carried by air and inhaled by its victims. This requires the engineering of a

sprayer system that will distribute the weaponized aerosol to the intended target areas without reducing the pathogen concentration below infective dose or infecting the weaponeer. To disseminate via plane (a la crop dusters) would be likely possible only with completely uncontested aerial superiority. On the other hand, as a tactical battlefield weapon it would suffer from a relatively long incubation period; though it varies by pathogen, incapacitation and/or death would likely take days at a minimum, eroding any semblance of tactical advantage. Ultimately, this factor favors enclosed, uncontested, high-density targets.

Relevant metrics for biological weapons

In order to evaluate a given pathogen's suitability as a bioweapon we consider several metrics.

Aerosolizability refers to the ability of the pathogen to be made airborne in sufficient concentrations for sufficient periods in order to cause infection. The bioagents will either be prepared as a fine powder or be suspended in a fluid and sprayed, producing liquid particles of varying sizes (likely on the order of micrometers to tens of micrometers in diameter). In either case, these particles then remain airborne for some time due to atmospheric turbulence. The smaller the particle, the longer it will remain in air before settling, on average¹⁵. We can quantify the aerosol quality of a bioweapon by its half-life – the time it takes for half of the original particle mass to settle on a surface.

While most bacteria will be small enough to be effectively aerosolized, many will be so heavily susceptible to environmental degradation as to render them far less effective. For instance, *Mycobacterium tuberculosis* (the causative agent of tuberculosis) has a thick cell wall allowing it to remain viable for long periods, whereas *Morbillivirus hominis* (the measles virus) is much more sensitive to temperature, humidity, and ultraviolet radiation exposure¹⁶. A candidate biological weapon agent will ideally survive for a long time in the environment.

Infectivity can be quantified as the minimum number of individual agents necessary to generate an infection. In practice, this varies greatly depending on the pathogen, host, and the circumstance of infection. In principle, a single agent could generate an infection, but this is unlikely. On one end of the spectrum, *Varicellovirus humanalpha3* (the causative agent of Chickenpox) is known to be highly infective, whereas *Salmonella enterica* (typhoid) typically requires a relative abundance of pathogen to kickstart an infection¹⁷. A candidate biological weapon agent will ideally be able to infect with a relatively low number of agents.

Pathogenicity refers to the likelihood of causing disease; even when an infection takes place, the host could be entirely non-symptomatic, thereby avoiding disease altogether. Pathogenicity can be measured quantitatively as the fraction of infections that cause disease. On the highly pathogenic side is *Rabies lyssavirus* (rabies) – with extremely rare exception¹⁸, every infection will result in disease. Towards the other extreme is poliovirus, where some 70% of infections are asymptomatic¹⁹. A candidate biological weapon agent will likely desire a relatively high pathogenicity, although if asymptomatic carriers can spread the disease, that too may be desirable.

Virulence refers to the damage caused by the disease. Quantitatively, one can measure this as the number of cases of serious illness divided by the total number of disease cases. A disease with a near 100% death rate, like rabies²⁰, would be considered highly virulent. Meanwhile, a rhinovirus (the “common cold”) would typically be considered to be a low-virulence pathogen. A candidate biological weapon will be of high virulence – the goal is either to cause death or at least severe incapacitation.

A pathogen's basic reproductive number (R_0) is a quantitative measure of the expected number of additional infections a single infected person will produce within a susceptible (e.g., non-immune) population. A value

of R_0 greater than 1 will produce an outbreak (i.e., if each diseased person infects more than 1 other person on average). This is related to the infectivity, as described above, but encompasses additionally the behavior of the infected – e.g., a disease that stimulates coughing and sneezing may be just as infective as a disease that does not, but the former will have a higher R_0 . In practice, this figure is complicated to estimate²¹. For our purposes, we will think of this as the “contagiousness” the disease.

For a highly contagious disease, it will be difficult to control the outbreak, requiring virtually universal immunity to prevent further spread. For example, it is estimated that the R_0 value of smallpox is ~3.5-6, suggesting that an outbreak would very rapidly lead to an epidemic²². By contrast, Ebola has an R_0 value ~1.5, making it easier for a public health response to prevent widespread disease²³. A candidate biological weapon will likely have a high R_0 in order to outpace the ability of the target to quarantine, vaccinate, and otherwise stem the infections. The greater the R_0 , the less of an initial infected cadre is necessary to kickstart an outbreak. However, this risks an uncontrolled spread beyond the designs of the bioweaponer.

The incubation period of a pathogen is the time interval between exposure and the emergence of disease. Influenza has among the shortest incubation periods of any disease at ~1-4 days²⁴. A more typical incubation period for a disease is in the range of ~1-3 weeks, with some substantially longer²⁵. The lower bound of the incubation period is set by the time it takes for the pathogen to replicate inside of the host. As such, a biological weapon is unlikely to provide a tactical advantage in the extremely short term.

Finally, it is relevant to ask how treatable the disease is. This is a purely qualitative measure of the ability of medical officials to intervene to avoid the worst outcomes of the disease – taking into account all of the previously discussed metrics, among others – including the availability of a vaccine, ability to manage symptoms, and familiarity of the disease among medical staff. Marburg virus, for instance, is extremely virulent and has no approved vaccine, making it a strong candidate for a potential biological weapon²⁶.

History until 1972

Miasma Theory

The Germ Theory of Disease, which identifies microorganisms (e.g., parasites, bacteria, viruses) as the vectors of disease, only took hold in the middle of the 19th century. Before that time, the Miasma Theory of Disease²⁷ – introduced by Hippocrates – held that illness was caused by pollution, or bad air, often emitted from items such as rotting carcasses. While incorrect, Miasma Theory does suggest several important characteristics of disease, namely (1) that disease is something that can be levied upon a person by an external force, (2) that it can spread between persons, and (3) that it is associated with decaying flesh and sewage. This theoretical framework coupled with an empirical understanding of disease was sufficient for the ancients to engage in rudimentary biological warfare.

Hittites

In the late 14th century B.C., a plague (likely tularemia) spread across Anatolia in what was then the Hittite empire which was in conflict with its on-again off-again rival, the Arzawans. The Arzawans took advantage by attacking at a time when the Hittite's were weakened by plague. Hittite texts describe a procedure whereby rams, a carrier of the plague, were sent along with a handler to the Arzawan villages – notably, not the military

encampments – with intent to spread the disease to their enemy²⁸. From the accounts of the war, this was a successful maneuver, preventing the Arzawans from mounting an effective campaign. The Arzawans, for their part, appear to have reacted in kind, suggesting that they had sufficient cognizance of disease to understand what had occurred and why. While by no means necessarily the first instance of biological warfare, this marks the earliest well-attested instance of it²⁹.

Middle Ages

During their invasion of Europe in the mid-14th century A.D., the invading Mongol army laid siege to the port city of Caffa on Crimea, then occupied by Genoese traders. After several years, the increasingly desperate Mongol army catapulted corpses of Bubonic Plague victims into the city³⁰. The intention was to weaken the ability and/or the will of the civilians to maintain an active defense. The siege failed; the Mongols were unable to capture the city. However, the Genoese inhabitants thus infected introduced the disease to southern Europe upon their return home, initiating the Black Death in Europe.

Smallpox blankets

To Sundries got to Replace in kind those which were taken from people in the Hospital to Convey the Smallpox to the Indians Viz':				
2 Blankets	@20/	£2“	0“	0
1 Silk Handkerchef.	10/			
& 1 linnen do:	3/6	0"	13"	6

Figure 3. Invoice for items procured from Fort Pitt for the purposes of spreading Smallpox among the Indigenous population. Recreated from Fenn E./Journal of American History, February 2000³¹

Biological warfare techniques did see some refinement. During the 17th century French and Indian War in North America, at least some attempt was made to deliberately spread smallpox to the opposing Native American forces by means of infected items taken from a military hospital at Fort Pitt (Figure 3). While this is an isolated incident rather than a deliberate, coordinated policy, it is likely that this strategy was employed repeatedly throughout the conquest of North American by white settlers³². Here too the intended target did not necessarily consist of members of an opposing military force. Instead the intent was to weaken a civilian population.

Germ Theory

In the 1670s, Antonie van Leeuwenhoek introduced the study of microbiology by using newly invented microscopes to examine life at a small scale – “animalcules”. While earlier thinkers had proposed the existence of microbes, van Leeuwekhoe’s discovery of microscopic discovery opened the door to the experimental research necessary to understand the underlying dynamics of disease. Still, it would be another two centuries before the Germ Theory of Disease would become the dominant paradigm. It was thanks to the work of figures like John Snow, Florence Nightingale, Louis Pasteur, Ignaz Semmelweis, and Robert Koch that

the medical community was able to begin driving down mortality rates by employing sanitation efforts and engaging in basic epidemiology informed by Germ Theory.

World War One

The First World War would be the earliest major war in which more deaths were due to combat rather than disease³³. This was driven not by the new weapons of war, but by the infusion of medical logistics into military forces, influenced by the great strides having been made in the civilian world. Though famous for the debut of chemical weapons, here too we find the first modern attempt at biological warfare. Dr. Anton Dilger, born to German immigrant parents in America and then raised in Germany, worked for the German government during World War One as a saboteur³⁴. In his home in Maryland, he incubated large stores of Anthrax and Glanders with the intent of infecting horses being sent over to Europe; though not yet a combatant in the war, the United States was a key supplier to the Allied powers. Though thousands of animals were injected, it is unclear how effective the plot was as no widespread outbreak of either disease was recorded³⁵. Whether or not it made a difference on the battlefield, this represents the earliest concerted effort by a nation state to employ biological warfare informed by Germ Theory.

Geneva Protocol

Traumatized by the horrors of chemical attacks during the First World War, the post-war world powers convened in 1925 to draft the Geneva Protocol³⁶ which prohibits the use of chemical weapons as well as “the use of bacteriological methods of warfare”. However, the agreement only applied to use against other nation states, not to situations where chemical or biological weapons would be used domestically (e.g., Saddam Hussein’s later reprisals against the Kurdish people). Further, many nations entered into the treaty with the stipulation that they would not abide by these restrictions if an adversary used such weapons first (i.e., effectively a “no first strike” statement). All in all, this represented a fairly weak agreement that, without contravention of the Protocol, allowed for the creation of biological weapon stockpiles and even their deployment.

World War Two

The nation to first employ biological warfare at large scale was Imperial Japan. In the mid-1930s Japan was locked in war with China; the might of the Imperial Japanese military was pitched against the vastness of the Chinese nation. Looking for ways to innovate their methods of offensive warfare, Unit 731 was set up to perform biological weapons research. Among other strategies, they poisoned village wells and deliberately released plague-infected fleas³⁷. While the campaign succeeded in killing thousands of Chinese civilians, it is unclear if it had any impact on Japan’s military efforts – and indeed as it turned out, the data gathered after over a decade of human experimentation proved virtually worthless to the allied biological weapons programs after the war³⁸. Ultimately, the methods used were too crude and untargeted to be of scientific value; little could be learned.

Meanwhile, the United Kingdom engaged in their own program at Porton Down. Originally set up as a facility to study chemical weapons during World War I³⁹ – and indeed it continued to perform that mission during World War II – the microbiology department was set up in 1940 in response to suspicions of Japanese and German biological weapon development. Simultaneously, scientists began to see that advances in microbiology made weaponization possible, but that there existed virtually no empirical understanding of the practical

limits. An initial set of experiments involved the detonation of bombs containing anthrax spores and sheep held at various distances to determine dispersion patterns and effective doses. The results demonstrated the potential efficacy of an anthrax bomb – in particular when delivered via cluster munitions. However, delivery systems appeared to be the bottleneck in producing a weapon. With the threat of biological weapons established, the urgent question was how to respond to a potentially imminent German attack. It was decided that the best defense was deterrence; cow feed seeded with anthrax spores was prepared in mass. If Germany were to use biological weapons, Britain would begin a campaign dropping this feed on agricultural areas in Germany in an attempt to cripple their food supply⁴⁰.

Starting in 1942, the United States began ramping up its own biological warfare capabilities at Fort Detrick, Maryland with a testing range at Dugway Proving Ground, Utah. Spurred on by British concerns and the work being performed at Porton Down, the United States began to undertake industrial production of pathogens – in particular, anthrax spores⁴¹.

Cold War (to 1972)

Despite the German surrender in May 1945, the British biological weapons program continued to surge. In 1946 tests expanded to a greater variety of animals. Beginning in 1948 the sea trials began. These trials took place off the coast of British territories in the Caribbean. Animals were caged in dinghies and left in fixed spots. A pathogen was released upwind, and the animals were later collected and monitored for effects. In addition to anthrax, tularemia and brucella were tested – and in addition to sheep, guinea pigs and monkeys were used as subjects⁴². Ultimately, these trials demonstrated that biological weapons were practically feasible, and more toxic per pound than chemical weapons. Rather than exploding munitions, the aerosol spray technique was established to great effect. However, the rate of loss of viability of these pathogens in open air did provide some realistic limits on the use of these weapons. Subsequent research projects studied the distribution pattern of inert particles (“simulants”) from sprays mounted on aircraft – determined to be the most effective vector for the delivery of biological weapons⁴⁰. Realizing the tremendous threat of biological weapons, the British program largely diverted to defensive research and development for the rest of the cold war period and beyond.

During the same period, the American biological weapon program rapidly developed. Initial work at Fort Detrick involved botulinum toxin and anthrax – but that was soon expanded to tularemia, brucellosis, plague, smallpox, Q fever, and Venezuelan equine encephalitis. The work necessitated the creation of a safety program from which modern biosafety standards descend – for example, negative pressure rooms and incineration systems⁴³. The One-Million-Liter Test Sphere (also known as the “Eight Ball”) was constructed at Fort Detrick to serve as a chamber for biological testing, including on humans – specifically conscientious objectors who had been drafted (Figure 4)⁴⁴.

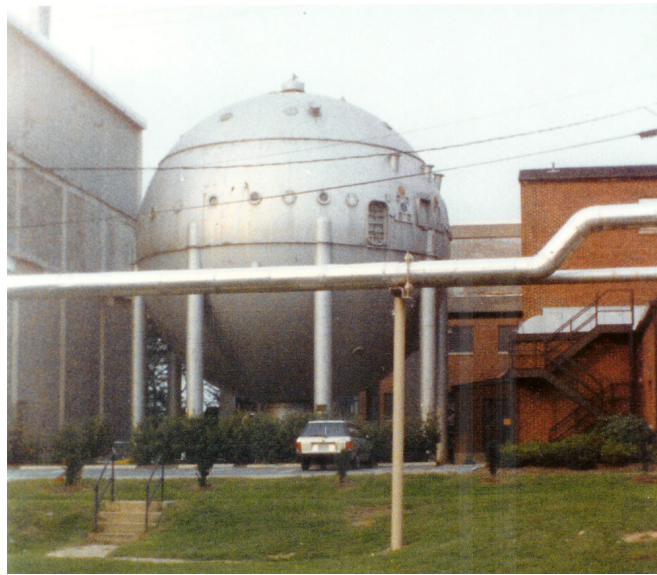


Figure 4. The “Million Liter Test Sphere” at Fort Detrick served as a testing area for the US biological weapons program. Subjects included enlisted soldiers and conscientious objectors – primarily Seventh Day Adventists.⁴⁵

The early post-WWII work largely involved the initial study of a variety of agents. Although the United States had virtually no specific biological weapons expertise, it was able to leverage an enormous corps of academic and industrial biologists, chemists, and agronomists. Breakthroughs included the development of fermentation techniques to scale up the production of biological agents – helpfully also contributing to the industrial production of penicillin. Despite the advances, very little was produced in the way of deployable weapons; the bombs that were produced were found unsatisfactory due to logistical difficulties. Sprayers were developed, as well as one warhead – but neither was produced in quantity⁴³. In addition to organizational challenges, delivery mechanisms appeared to represent the technological bottleneck for biological weapon deployment. While the People’s Republic of China and North Korea accused the US of employing biological weapons during the Korean war – including smallpox and plague – these charges are largely thought to be groundless by modern scholarship⁴⁶.

The Soviet biological weapons program, although initiated in the 1920s, only really advanced after World War II, in part due to fears of the western biological weapons programs. In this immediate post-cold war period, the Soviet program was distributed across various institutes including medical centers and laboratories in the Agriculture Ministry⁴⁷. During the Khrushchev era, work had progressed sufficiently to enable the construction of dedicated production plants. These came online in the 1960s, eventually retaining up to 3,000 employees each. While nominally providing products to the Soviet medical establishment, these new plants also served as reserve capacity for the rapid production of biological weapons should the need arise, with military curators attached to each plant to ensure readiness. In this way, the Soviets had folded biological weapon production into their civilian industrial network⁴⁸.

American cessation of biological weapons research/production

In 1969, President Nixon outlined the end of the American offensive biological weapons program in his “Statement on Chemical and Biological Defense Policies and Programs”. In this brief speech, he argues that biological weapons are wildly unpredictable, and that their use could “*produce global epidemics and impair*

*the health of future generations*⁴⁹. With no conceivable scenario in which these weapons would be used there was both a financial and moral incentive to discontinue the offensive program, while leaving the defensive program intact. In making his statement, Nixon aligned the US with the UK-initiated then-draft Biological Weapons Convention⁵⁰. US biological weapons stores would be eliminated over the next half decade, with the US ratifying the Biological Weapons Convention in 1975.

Biological Weapons Convention

Aims

In 1968, the British government produced a working paper⁵¹ that identified several concerns with the 1925 Geneva Protocol – including that:

- (1) Many nations had not signed the Protocol
- (2) Signatories reserved the right to use biological and chemical weapons against non-signatories, violators of the protocol, and their allies
- (3) Signatories retained the right to manufacture biological and chemical weapons
- (4) The Protocol was insufficiently comprehensive with respect to its definition of “bacteriological”, leaving open the use of similar agents (e.g., viruses) in warfare
- (5) The Protocol only forbade usage of such weapons in war time but did not forbid use in hostilities short of war

To combat these concerns, the British government recommended the creation of separate agreements for biological and chemical weapons, citing an asymmetry between the two. Chemical weaponry had been used previously in war at a large scale; further, the British perceived some acceptable usage of chemical weapons (e.g., riot control), that would complicate negotiations. These reasons suggested that an agreement on chemical weapons would be more difficult to secure than one on biological weapons.

Simultaneously, it was recognized that verification of adherence to a biological weapons ban would be virtually impossible as biological weapon development would leverage the existing biomedical industry – e.g., viruses and bacteria that could be weaponized already exist in medical labs and could be rapidly shifted into military production lines. However, it was believed – or perhaps hoped – that a collective “abhorrence” of the specter of biological warfare would be sufficient to motivate compliance with any agreement banning development of biological weapons.

Contents

In Article I, signatories of the Biological Weapons Convention agreed:

“never in any circumstances to develop, produce, stockpile or otherwise acquire or retain (1) microbial or other biological agents, or toxins whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective or other peaceful purposes; (2) weapons, equipment or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict”.

In Article II, signatories agree to destroy or redirect for civilian use all “biological agents, toxins, weapons, equipment, and means of delivery” within nine months of the Convention coming into force. Article III prohibits signatories from transferring such material to another state or organization intent on manufacturing biological weapons, while in Article IV signatories agree to take measures necessary to prohibit the future development of biological weapons within their territory.

Interestingly, Article VI contains the only reference to enforcement, stating that any party to the Convention may inform the United National Security Council if they believe another party is in breach of their obligations. The Security Council may then initiate an investigation. However, no sanction is specified in the Convention in the event of the discovery of a breach.

Collectively, the Convention terms met the requirements set out by the British working paper some four years earlier while continuing to recognize that verification of compliance remained a fundamental issue.

In addition, Article V of the Convention allows any signatory to request a consultative meeting to discuss potential concerns with respect to the Convention. This has been invoked twice – by Cuba in 1997, and by Russia in 2022. The 1997 concern related to the overflight by Cuba of a US Department of State plane that was engaged in an anti-narcotic mission in Colombia. The Cuban government asserts that the plane released an unknown substance during that overflight that gave rise to a plague of *Thrips palmi* – an insect that damages crops – 2 months later^{52,53,54}. The United States Department of State strongly denied the claims, instead asserting that the pilot released smoke to make their location known to another aircraft⁵⁵. Although most members of the review committee determined that the Cuban position was without sufficient evidence, the final report of this consultative meeting notes that no “definitive conclusion” could be reached on the matter, and the case was closed⁵⁶.

In a 2022 meeting of the United Nations Security Council, the Russian representative Vasily Nebenzya alleged that the United States Department of Defense operated laboratories in Ukraine for the purposes of conducting biological weapons research⁵⁷. A formal consultative meeting was held, and no consensus was reached⁵⁸. The Russian concern eventually escalated to a formal complaint to the United Nations Security Council under Article VI of the Convention, but the resolution failed, receiving support only from Russia and China⁵⁹.

Approximately every five years the Implementation Support Unit⁶⁰ hosts a meeting at the United Nations to review the Convention in accordance with Article XII. In addition to these review meetings, ad hoc meetings have been held to review potential verification measures and to propose Protocols to strengthen the convention⁶¹. The most recent of these resulted in a 2001 proposal⁶² to strengthen the Biological Weapons Convention. This proposal would have required an annual declaration of biological materials by each of the signatories with a technical team conducting randomly selected transparency visits to the identified sites and provided a robust framework for conducting investigations into potential breaches, although it did not come into force. It failed in part due to US opposition, motivated by a desire to keep secret aspects of the US biological defense program that could be revealed by international inspection⁶³. Conceivably, details regarding the focus of defense work could provide bad actors with insights into perceived weaknesses²².

Per a decision taken in the second review conference in 1986, signatories submit annual Confidence Building Measure forms to provide information on biological research centers/programs, disease outbreaks, vaccine production, and past biological weapons research – whether offensive or defensive⁶⁴. These reports are accessible by the other signatories of the Convention and act as the closest mechanism to a verification method – albeit self-reported. These filings act as a repository of declared programs, centers, and production facilities that can be cross-referenced in the determination of potentially anomalous activities.

Signatories

With the exception of Chad, Comoros, Djibouti, Eritrea, Israel, and Kiribati, all UN member states have signed the Biological Weapons Convention – although Egypt, Haiti, Somalia, and Syria have yet to ratify it. Of these, only Israel and Syria have ever been seriously accused of harboring biological weapons.

History since 1972

Soviet Union/Russia

Despite signing the Biological Weapons Convention, Soviet work on biological weapons not only continued, but intensified. In 1974 Biopreparat was created to centralize the biological weapons work within the Soviet Union – including ownership of the aforementioned dual-use production plants. At the center of Biopreparat was the Institute of Applied Biochemistry (VNIIBiokhimmashprekt). This institute designed and produced the equipment necessary to supply the factories with the capability to churn out biological weapons at an industrial scale. The institute would also serve as a key link between the biological weapons program and the Ministry of Defense. Biopreparat also included a series of experimental bases where weaponization and testing could occur. At one such facility at Stepnogorsk the teams developed an Anthrax assembly line – exposing seed stock to the right temperatures and nutrients to grow rapidly before being concentrated via centrifuges. The concentrate was combined with additives to keep the bacteria stable before being pumped out into a separate area to be loaded into munitions⁶⁵.

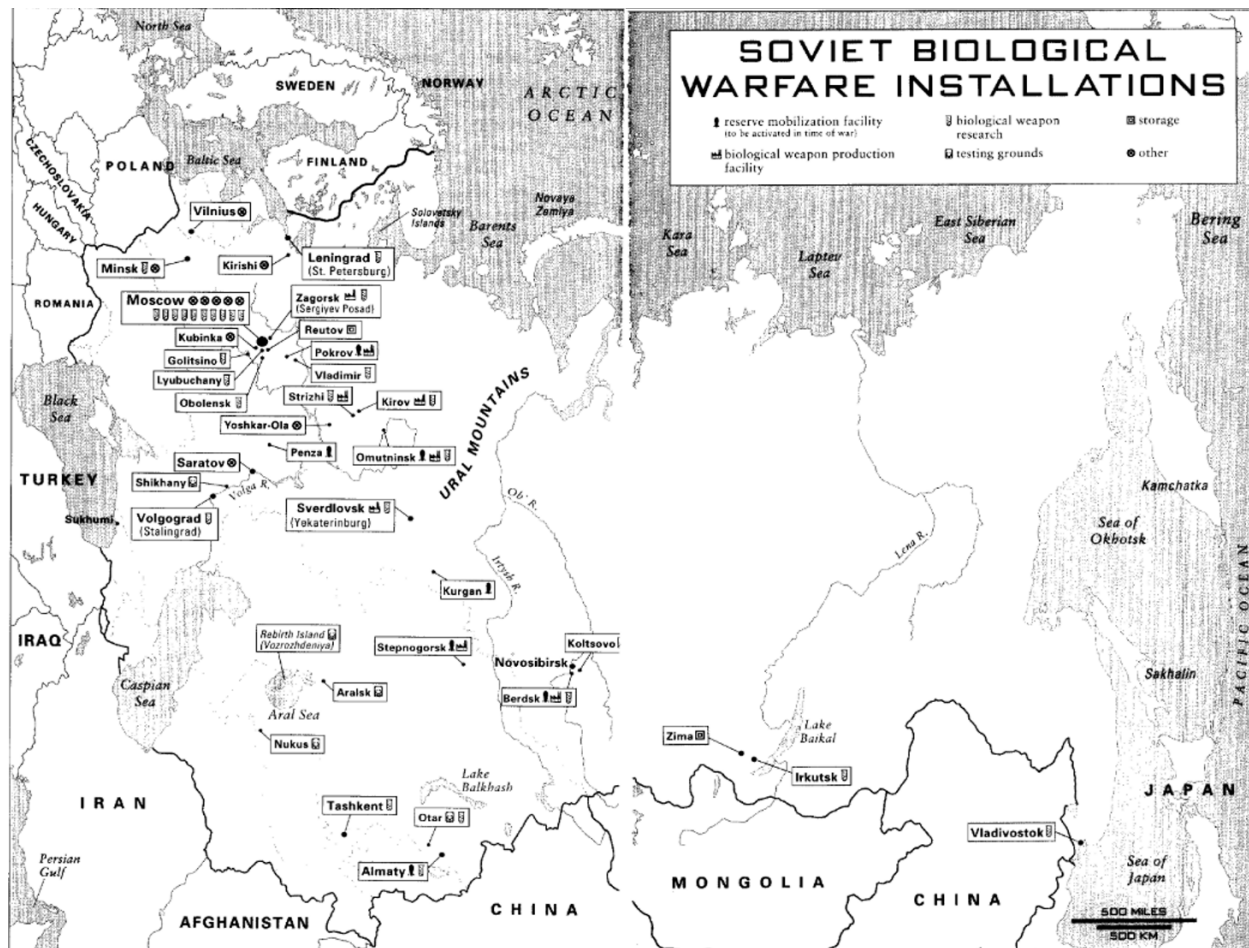


Figure 5. Map of institutions associated with the Soviet biological weapons program - by far the most extensive such program ever developed. Produced from Biohazard (Alibek 1999)⁶⁵

At its peak, Biopreparat employed some ~40,000 personnel with a vast array of institutions scattered across the Soviet Union (Figure 5). Suspicions began to arise in the west in 1979 with the accidental release of Anthrax in Sverdlovsk resulting in the deaths of dozens of civilians. The full extent of the program was only revealed with the defection of Vladimir Pasechnik, a leading Biopreparat scientist, to the UK in 1989. After the fall of the Soviet Union, and with western help the weapons aspect of Biopreparat was decommissioned with assets stripped away to be sold to private pharmaceutical firms⁴⁸. The motivations for the maintenance of the Soviet biological weapons program are complex and remain somewhat hidden. It is likely that the Soviet military rejected oversight as a matter of principle, and that American biological R&D funding – particularly in the 1980s – convinced Soviet analysts that the American offensive program had continued unabated⁶⁶.

The United States Department of State asserts that Russia has continued to fund a biological weapons program – suggesting that the decommissioning of the Soviet program was incomplete, and that Russia is continuing to operate in this domain. They point to a defense research institute not previously declared in Russia's annual Confidence Building Measures report⁶⁷. The view that a biological weapons program continued in Russia beyond the early 1990s is shared by former Soviet bioweaponer, Ken Alibek from whom most of the public knowledge of the Soviet biological weapons program stems⁶⁵.

People's Republic of China

The People's Republic of China (PRC) ratified the Biological Weapons Convention in 1984⁶⁸. While the PRC has indicated that they never possessed an offensive biological weapons program, some evidence suggests that such a program existed and may continue to exist. Ken Alibek writes that two separate cases of hemorrhagic fever were found in an area of China that also contained what appeared to be a biological facility sometime in the 1980s; Soviet analysts believed that these outbreaks were accidents related to a weaponization program⁶⁵. In the 1990s, American officials publicly stated suspicions that the PRC maintained a biological weapons program, a claim quickly denied by the PRC^{69,70}. In 1997, then Secretary of State Madeleine Albright noted reports that the PRC was selling dual-use technology to Iran that could be used for the manufacture of biological weapons⁷¹. However, the PRC's engagement with the Iranian biological weapons program remains unclear⁷². The US State Department, in their annual report on the Adherence to and Compliance with Arms Control, Nonproliferation, and Disarmament Agreements and Commitments, has continued to flag concerns with the PRC's compliance with the Biological Weapons Convention.

In particular, there is no evidence that the PRC shut down whatever existed of an offensive biological weapons program after acceding to the Biological Weapons Convention – suggesting that stockpiles of hazardous agent may have continued to exist in contravention of the convention. Further, the reports allude to evidence that the PRC is engaging in dual-use research within the Peoples Liberation Army (PLA) laboratories that may have biological weapon applications⁶⁷. With life sciences identified as a leading research and development priority for the PRC, it is expected to see an enormous buildup of related centers and industries. As such, increased biological research activity does not in itself hint at a biological weapons program. With a great capacity for dual-use capabilities however, a PRC biological weapons program could – like the Soviet program – hide within civilian channels. Tellingly, there are examples of PLA affiliated biological laboratories ceasing publication on topics while continuing to operate, suggesting that their work has entered classified space⁷³.

In the wake of the COVID-19 pandemic, a hypothesis emerged that suggested that SARS-CoV-2 escaped from a Chinese laboratory, likely the Wuhan Institute of Virology. The idea is that either researchers isolated SARS-CoV-2 from a wild sample, or genetically modified coronaviruses leading to this particular strain. Either way, an accident may have occurred that led to the rapid spread of the disease. However, the immediate evidence did not seem to support this “lab leak” hypothesis, instead favoring the “natural origin” hypothesis – that the virus was transmitted from animals to humans⁷⁴. With more comprehensive study over the next few years, evidence continued to mount in favor of the natural origin hypothesis – including the identification of SARS-CoV-2 in animal samples taken in January 2020⁷⁵. In 2025 the new US administration reversed the Intelligence Community's previous assessment and released a “low confidence” report in favor of the lab leak hypothesis, although it provided no new evidence⁷⁶. Regardless, it is important to note that the lab leak hypothesis is distinct from a claim that the virus was deliberately manufactured as a biological weapon.

Any potential PRC-based work on biological weapons, past or present, might be motivated by the experience of being subject to biological weapons attacks during World War II, as well as perceived attacks by American forces during the Korean war.

Iran

The United States Department of State, in 2003, judged that Iran “ha[d] an offensive biological weapons program in violation of the BWC”, suggesting that it was initiated at the behest of eventual President of Iran, Akbar Rafsanjani in 1988⁷⁷. However, by 2011 Department of State analysis had significantly changed, with

analysis suggesting only that Iran “engaged in activities with potential dual-use BW [Biological Weapon] applications” and that it remains “unclear whether any of these activities were prohibited by the BWC [Biological Weapons Convention]”⁷⁸, a judgement that remains consistent in the 2024 report⁶⁷. Iran does have a strong domestic biotechnology sector capable of vaccine development and pesticide work – both of which have opportunities for dual-use biological weapons work⁷⁹. On balance, there is little open-source evidence that an Iranian biological weapons program continues to exist currently. Iran has supplied proxy groups – including Hezbollah, Hamas, the Houthis, and Shi’ite militias – in the Middle East with weapons by way of its Quds Force. Despite the myriad conflicts in the region since Arab Spring, there is no robust evidence that these proxies – or any other group – has deployed biological weapons.

Iraq

At the end of the Iran-Iraq war Iraq initiated a biological weapons program under Dr Rihab Taha, producing large quantities of botulism toxin and anthrax. After the first Gulf War, Iraq at least partially complied with the weapons inspection process carried out by the United Nations Special Commission. The Commission investigated sites suspected of being involved in the production of biological weapons – including Al Hakam. Notionally an animal feed and bio-pesticide plant, the factory was discovered to be Iraq’s premiere biological agent production facility, producing botulism toxin and anthrax in mass quantities. Initial suspicions arose due to the volume of growth media consumed – far more than would be expected for chicken feed – and the size of the fermenters – far smaller than would be expected. Notably, either to cut costs or avoid detection, the plant used ventilation systems inadequate to guarantee protection to its operators⁸⁰. Inspectors discovered munitions filled with these biological agents, demonstrating that full weaponization had taken place⁸¹.

In 1995, after the director of Iraq’s Military Industrialization Corporation – General Hussein Kamel Hassan – defected to Jordan, the Iraqi government provided disclosure about its offensive biological weapons program, specifically including the weaponization of biological agents. Prior to the dismissal of the United Nations weapons inspection teams in 1998, Iraq had not produced satisfactory documentation to suggest that it had destroyed existing stockpiles of biological weapons and dismantled the program altogether. In 2002, amid mounting concerns from the United States, Iraq once again allowed inspections – this time from the United Nations Monitoring, Verification and Inspection Commission. In May of 2003, the Commission reported that it found no evidence of the continuation or resumption of a biological weapons program⁸². However, the United States judged that Iraq continued to develop biological weapons – among others – and had moved their production into mobile facilities⁷⁷ leading to Colin Powell’s address to the United Nations in February of 2003 where he laid out the rationale for the subsequent invasion⁸³.

Post-invasion, it was discovered that no such weapons program existed in Iraq; the mobile laboratory platforms turned out to be trailers used to fill artillery balloons with hydrogen⁸⁴. The Iraq Survey Group, established by the United States after toppling Saddam Hussein, concluded that Iraq largely destroyed its biological weapons stores in the years following the First Gulf War, and decommissioned their remaining biological weapons facilities following the 1995 defection of General Hussein Kamel Hassan. Iraq, the Group concluded, retained only a capability to restart a biological weapons program – though they never did or even had an intention of doing so⁸⁵.

Israel

Israel is relatively unique as a non-signatory to the Biological Weapons Convention. In the same vein as its potential nuclear program⁸⁶, Israel makes a strong effort to hide the breadth of its offensive capabilities. In 1948, Israel was locked into an existential war with its immediate Arab neighbors. With then President Ben-Gurion's authorization, a campaign was begun by the Science Corps (HEMED) of the Israel Defense Forces (and formerly the Haganah – a paramilitary force) to introduce typhoid into the water supply of the opposing armies, particularly the Egyptian. The purpose was to cause disarray in the enemy ranks and to discourage displaced people from returning to villages captured by Israeli forces. Ultimately, no widespread outbreaks occurred, and the operation did little to affect the battlefield⁸⁷. Regardless, it is a rare example of post-World War II usage of biological warfare.

HEMED evolved into the Israel Institute for Biological Research⁸⁸, which continues to this day to operate as a highly secure military laboratory. It is widely thought that Israel continued to maintain a small biological weapons program through the early 90s⁸⁹ until the Gulf War revealed the extent of Saddam Hussein's arsenal – prompting more rapid expansion of biodefense capabilities⁹⁰. With a robust biodefense capability and large biotech industry, it is certain that Israel has the means to mount an offensive biological weapons program. Further, the research performed at the Israel Institute of Biological Research is consistent with research towards offensive biological weapons⁹¹. However, there is little evidence of active stockpiling biological agents or weaponization. It is more likely that Israel retains a breakout capability⁹².

The initial motivations for biological weapons are clear – Israel faced existential threats from its neighbors on its founding and armed itself accordingly. More recently, there is likely less call for Israel deployment of biological weapons. Israel's adversaries are close by – any epidemic caused by such weapons would be highly likely to spread to Israel, especially if it were deployed in Palestinian territory (i.e., Gaza or the West Bank). There is no evidence that Israel has used biological weapons during the Israel-Hamas war.

North Korea

In their annual report on the Adherence to and Compliance with Arms Control, Nonproliferation, and Disarmament Agreements and Commitments, the US Department of State asserts that North Korea has fielded offensive biological weapons capabilities since the 1960s. North Korea ratified the Biological Weapons Convention in 1987 and has submitted only one Confidence Building Measure form to date – in 1990⁹³. A North Korean defector, Ju-Hwal Choi, described in 1997 an ongoing biological weapons program headed by the Germ Research Institute capable of producing “infectious biological warfare agents...and possible crude biological weapons”. A 2012 South Korean defense white paper suggests that North Korea “likely has the capability to produce...anthrax, smallpox, pest, [*F*] *rancisella tularensis*, and hemorrhagic fever virus”⁹⁴.



Figure 6. A spray dryer shown during Kim Jung-Un's visit to a pesticide factory. This equipment is used to dry bacteria spores into powder - whether fertilizer or anthrax.⁹⁵

Meanwhile, a 2015 North Korea news report on Kim Jong Un's tour of the Pyongyang Biotechnical Institute - a pesticide facility - provided insight into North Korea's capability to produce biological weapons. Photos of the tour show off modern machinery readily capable of producing *Bacillus anthracis*, the bacteria that causes anthrax, for weaponization (Figure 6). This is common among known biological weapons programs; the process for growing *Bacillus thuringiensis* - a biopesticide - is virtually identical to that for growing *Bacillus anthracis*, the causative agent of anthrax⁹⁵.

However, there is little evidence that North Korea has operationalized biological weapons; the open-source evidence is consistent with a scenario in which North Korea reserves the capacity to produce biological agents en masse but is not actively doing so. North Korea's motivations for a biological weapons program are likely relatively straightforward. Unable to compete with conventional American and South Korean military forces, North Korea has invested heavily in deterrence up to and including its successful nuclear weapons program. This makes invasion highly costly and helps to ensure regime stability⁹⁶.

South Africa

Caught in an expensive, long-term war in Angola and Namibia, and facing a deteriorating domestic situation in the aftermath of the Soweto riots and other unrest, the South African government undertook a total security strategy which aimed to enable the military and police forces with capabilities necessary to preserve the state at all costs. Notwithstanding their 1975 ratification of the Biological Weapons Convention, the apartheid-era South African government initiated a biological weapons program in the early 1980s, called Project Coast.

The South African Defence Forces, in a program directed by Wouter Basson, set up a series of front companies that could operate with relative freedom in the commercial sector. This way favored over traditional procurement as the military procurement agency – ARMSCOR (Armaments Corporation of South Africa) – faced greater sanction, notably the UN sponsored arms embargo. Following a technology transition, however, final weaponization of chemical and biological weapons was to occur within military labs⁹⁷.

In particular, a company called Roodeplaat Research Laboratories was established to conduct research and produce proof-of-concept biological weapons. However, the full program went well beyond just the front companies as they collaborated extensively with universities and other companies both in South Africa and abroad, to possibly include foreign government assistance. The program leadership sought to develop both battlefield weapons for the purpose of inflicting mass casualties, as well as weapons for individual assassination. These could serve either to create a decisive advantage in the ongoing wars or to quell potential uprisings domestically, or in the Bantustans. Interestingly, a unique emphasis of Project Coast was the development of infertility drugs, to be given without knowledge, in order to reduce the birth rate of the black population – an act that could amount to genocide of the nation's own citizens⁹⁸.

The evidence that chemical weapons were employed, as well as the conventional means that the South African Defence Forces employed, suggests a willingness to use biological weapons in order to preserve the integrity of the South African state. As part of the transition to democracy in 1994 Project Coast was supposedly shut down, although it may have continued to operate beyond the reaches of the new leadership for some time⁹⁹.

Ultimately, the creation of front companies and the associated lack of oversight provided opportunities for personal enrichment via corruption, which in turn incentivized the creation of bigger and more expensive projects. South Africa's program was relatively unique in this regard, with other nations leveraging existing civilian biological industries for development and production rather than creating entirely novel firms, and it can be considered an organizational failure¹⁰⁰.

Syria

Despite occasional claims by American officials to the contrary¹⁰¹, there is little reason to suspect that Syria has operated an offensive biological weapons program based on open-source reporting¹⁰². This contrasts with Syria's chemical weapons program which has been more robustly exposed by analysts¹⁰³ - in particular the 2013 Ghouta attack¹⁰⁴, the 2017 Khan Shaykhun attack¹⁰⁵, and the 2018 Douma attack¹⁰⁶. It is reasonable to speculate that chemical weapons were selected in these instances specifically to instill terror in opposition forces¹⁰⁷. It is unclear what Syria would accomplish with biological weapons that it was unable to do so more precisely and economically with chemical weapons; a biological attack would potentially threaten spreading to Syrian and allied forces. However, it does suggest a set of circumstances that might incentivize the use of biological weapons.

Compare this with a biological weapons attack against Israel which would not only threaten a potential response in kind but would be significantly less effective against an adversary prepared to face biological agents on the battlefield¹⁰⁸. That said, there is no evidence of any use of biological weapons during the Syrian civil war, and the recent fall of the Assad regime has not – as of the date of writing – revealed any heretofore secret biological weapons programs.

Non-state actors

There have been very few recorded biological attacks carried out in modern history. However, the nature of a biological attack is such that without a group claiming responsibility, identification that an attack was deliberate and attribution to a specific group would be difficult. I will briefly summarize the few well-attested attacks in recent history.

The first documented bioterror attack in modern American history occurred in Oregon in 1984. The Rajneesh cult had constructed a compound in Wasco county, Oregon in the early 1980s. After having planning permissions denied by the county, cult members decided to run for county office. In order to boost chances of victory, members spread salmonella throughout salad bars at local restaurants. Notably, this only came to light after the group was investigated for unrelated crimes¹⁰⁹. The group had purchased the strain of salmonella through a commercial supplier and cultured it in their own laboratory prior to repeated dissemination. 751 people were sickened by the disease, though none died¹¹⁰. The Rajneesh cult failed to win the elections – in part because cult members did not appear to vote¹¹¹.



Figure 7. Aum Shinrikyo spraying aerosolized anthrax from atop their building in Tokyo in 1993.¹¹²

In 1993, the Aum Shinrikyo cult in Tokyo – famous for their 1995 sarin gas attack in the Tokyo metro – released aerosolized *Bacillus anthracis* from the roof of their eight-story headquarters in Tokyo (Figure 7). Residents reported a bad smell; authorities collected samples, and the cult was forced to end their spraying. Only after the 1995 attack were the samples tested – they were found to contain a strain of anthrax prepared for animal vaccination¹¹³. However, there were no reports of anyone contracting Anthrax in Tokyo at this time suggesting that the attack failed entirely. This is likely due to three insufficiencies – (1) insufficient concentration of spores, (2) insufficiently aerosolized media, and (3) the use of a strain of *Bacillus anthracis* that does not produce a capsule – a protein shell to protect the genetic material – which rendered it insufficiently virulent¹¹².

The 2001 anthrax attacks (“Amerithrax”) are the most famous examples of bioterrorism. The events began to unfold less than a month after the September 11th attacks. Robert Stevens – a photojournalist in Florida

– contracted pulmonary anthrax and died. Two of his colleagues also caught anthrax, and cases began popping up elsewhere around the nation, including in New York City and Washington DC. It was discovered by investigators that each case had been preceded by the infected individual handling a letter containing an unknown powder¹¹⁴. A total of 22 people were sickened, and 5 of those perished¹¹⁵. The Federal Bureau of Investigation (FBI) would, in 2008, formally accuse the then recently deceased Dr. Bruce Ivins – a former anthrax researcher at USMRIID – of being solely responsible for the Amerithrax attacks¹¹⁶, although there is credible doubt that he would have been able to manufacture the anthrax used in the attacks¹¹⁷.

While by no means exhaustive, these three events are the most well-known examples of non-state actors engaging in biological attacks. Common among all three, though, is the incredible difficulty in attribution. Neither the Rajneesh cult nor Aum Shinrikyo would have been identified if not for unrelated scrutiny. The Amerithrax case was open for seven years before the FBI claimed to identify the perpetrator, and even that came with significant doubt. While another anthrax attack on the United States – or any developed nation – would likely be identified (though not necessarily attributed) immediately, it is highly plausible that small scale biological attacks occur regularly around the globe, beyond the ability of the local health authorities to detect and counter.

United States biological defense program

Institutions

The United States biological defense program continued after the dismantling of the offensive program. The effort was led by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) headquartered at Fort Detrick, Maryland. However, biological defense has become a whole of government effort, involving agencies across the Departments of Health and Human Services, Defense, Agriculture, and Homeland Security under the banner of the National Interagency Confederation for Biological Research (NICBR)¹¹⁸. Several of these are of particular relevance to this report:

The Biological Defense Research Directorate of the Naval Medical Research Command (NMRC) conducts biological defense research to protect warfighters in case of a biological weapon attack¹¹⁹.

USAMRIID has a mission to “provide medical capabilities to deter and defend against current and emerging biological threat agents”¹²⁰. The Institute maintains research groups for the study of bacteriology and virology, among other disciplines, and operates a Biosafety Level 4 laboratory.

The Centers for Disease Control and Prevention (CDC) has a broad mission to “protect American from health, safety and security threats...[w]hether disease start at home or abroad, are chronic or acute, curable or preventable, human error or deliberate attack...”¹²¹. The CDC would serve as the coordinating agency in the event of a disease outbreak in the United States, much as they did during the recent COVID-19 pandemic. The CDC operates a Biosafety Level 4 laboratory.

The National Biodefense Analysis and Countermeasures Center (NBACC) is a laboratory and analysis center focused on preventing and mitigating potential biological attacks on the United States¹²². In order to protect against potential attacks, the Center conducts “red team” exercises to understand how weaponized agents spread¹²³. This aspect of the mission, combined with the secrecy of the Center, has caused some concern about lack of oversight and potential violation of the Biological Weapons Convention¹²⁴. The NBACC operates a Biosafety Level 4 laboratory.

In addition to these and the other agencies within the NICBR umbrella, biodefense work occurs elsewhere within the Department of Defense, Intelligence Community, and Public Health Service, among other government entities. Further, expertise in industry and academia is leveraged in support of this work.

As with the other nations mentioned in the previous section, components of the United States' biological defense program remain secretive, and beyond the realm of open-source reporting. In 2001, for instance, it came to light that the United States had pursued a trio of projects that some thought ran afoul of the Biological Weapons Convention¹²⁵. Project Jefferson sought to test the existing anthrax vaccine against a strain of *Bacillus anthracis* modified by Russian scientists for greater lethality. Project Clear Vision examined a Soviet bomblet designed to carry biological agents to measure its aerosols spreading capability. Project Bacchus simulated a biological weapons production facility using minimal equipment, such as that which may be available to non-state actors.

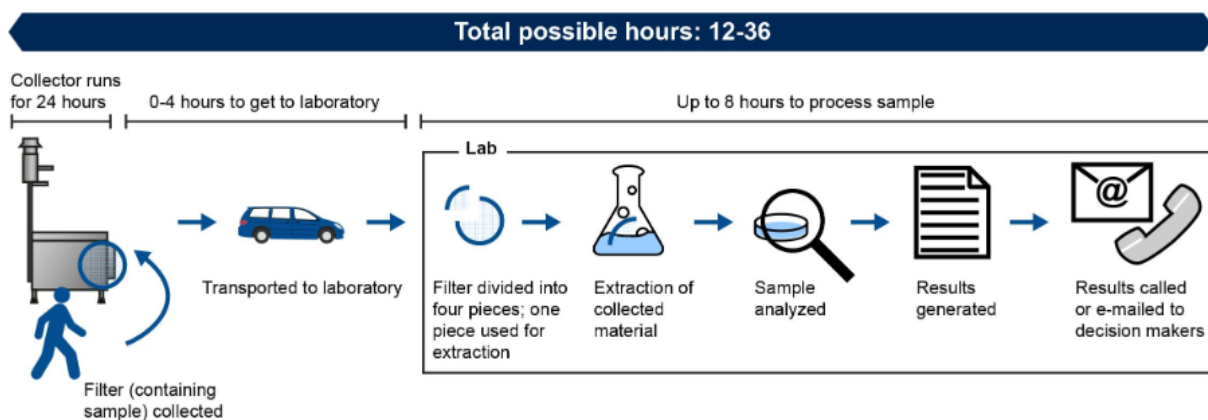


Figure 8. Current BioWatch process. Samples are physically collected and screened at a nearby laboratory with a detection latency of up to 36 hours. In addition to generating a BAR, positive detections are provided to laboratories with more extensive capabilities for definitive characterization. Sourced from Government Accountability Office report GAO-21-292¹²⁶.

Begun in 2003 in response to the Amerithrax attacks, BioWatch is a Department of Homeland Security (DHS) program operating in more than 30 major metropolitan areas across the United States. It consists of a series of air monitoring stations and associated laboratories that provide continual monitoring for aerosolized biological agents of concern^{127,128}. A physical collection station passively gathers biological material in a filter which is then dropped off after 24 hours at an associated laboratory for analysis (Figure 8). A BioWatch Actionable Result (BAR) occurs with the detection of a hazardous biological agent. A BAR kicks off certain local, state, and federal responses – potentially including expanded testing within the relevant jurisdiction, distribution of medical countermeasures to clinics, and even quarantines¹²⁹.

The Countering Weapons of Mass Destruction Office (CWMD) – the DHS program office currently executing BioWatch – pursued an updated system called Biological Detection for the 21st Century (BD21). CWMD perceives the most critical gap to be detection latency – currently up to 36 hours. Their vision for BD21 included an autonomous continuous monitoring system built within the physical sensor system¹³⁰. However, the BD21 acquisition was curtailed after it was decided that it faced serious challenges with respect to technology readiness and scalability – in addition to acquisition concerns¹²⁶. BioWatch continues to be the biosurveillance program of record while CWMD performs a capabilities-based assessment to inform next steps – whether that involves making incremental updates to BioWatch or initiating an acquisition of a new follow-on system¹³¹.

Distinct from BioWatch is the Laboratory Response Network – a CDC led effort to coordinate laboratory analysis of biological agents in case of a crisis scenario¹³². The network exists on three levels¹³³ (Figure 9).

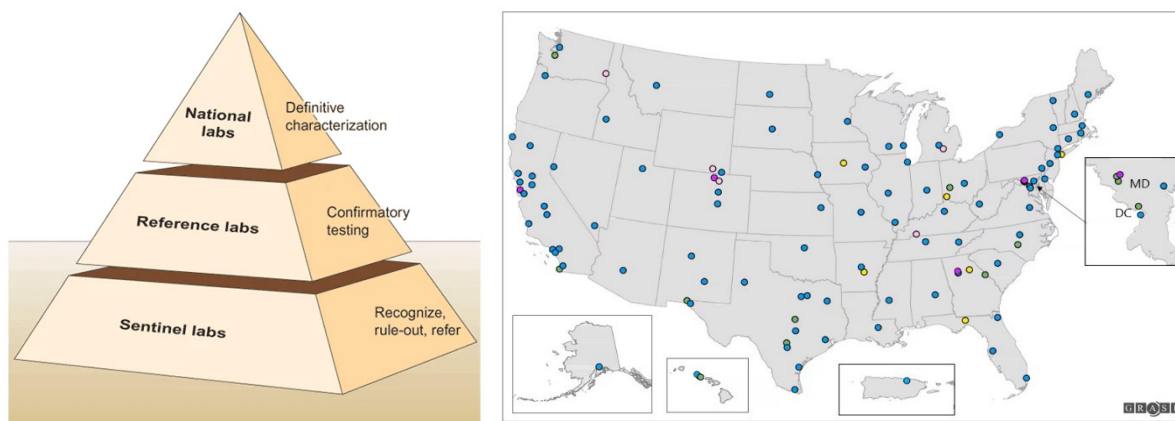


Figure 9. (Left) Schematic of the Laboratory Response Network levels. Taken from Rotz (2004).¹³⁴ (Right) Location of member labs. Colors correspond to laboratory sub-types (e.g., civil, military, veterinary, etc.).¹³⁵

Sentinel labs exist at the forefront of any outbreak (e.g., hospitals, clinics, etc.). Their job is to provide initial detection of an outbreak, rule-out specific agents, and to forward information through the network.

Reference labs are larger, more resourced laboratories at the Biosafety Level 3 tier that provide secondary confirmation using more time-consuming and precise methods, e.g., Polymerase Chain Reaction (PCR).

National labs are laboratories at the Biosafety Level 3 or 4 tier that provide final characterization and analysis of the biological agent in question. For example, such labs exist at the CDC, USAMRIID, and the NMRC¹³⁶.

Many BioWatch affiliated laboratories also exist within the Laboratory Response Network, providing integration between the two programs. Taken together, BioWatch and the Laboratory Response Network establish a pre-coordinated process for the rapid identification and characterization of a potential biological hazard. Critically, both of these programs connect federal response agencies with local health departments, providing the infrastructure for an integrated health response system.

Artificial Intelligence

As a novel and powerful tool, Artificial intelligence (AI) has the potential to upset the balance of incentives, taboos, and costs that have so far delivered a world relatively safe from biological warfare. In addressing the potential risks posed by AI I draw a distinction between two types of risks. “General” AI risks are common across disciplines – as opposed to being biotechnology specific – and typically benefit non-institutional actors. “Specific” AI risks arise from AI providing targeted solutions to specific problems within the biological domain; large state actors are more able to take advantage of these. Four identified risks are summarized below (Table 1).

Risk	Type	Relative Risk Level
Curating relevant biological information	General	Low
Aiding experimental design	General	Medium-low
Discovery of susceptibilities	Specific	Medium
Discovery of operational DNA patterns	Specific	Medium-high

Table 1. Relative risk levels of 4 broad AI risks with regard to biological weapon production.

Curating relevant biological information

Chat-GPT's marquee capability is in collating information otherwise spread across the internet for easy consumption. Non-expert actors, with the use of Chat-GPT or another large language model (LLM) would be able to rapidly glean relevant information about the construction and dissemination of biological weapons. Even with safeguards, "jailbreaking" – engineering a prompt such that the LLM reveals harmful information that it otherwise would attempt to withhold – appears a simple and intuitive strategy¹³⁷. Even discounting the use of virtual private networks, as LLMs can be operated in an offline capacity it would not necessarily be possible to police malign usage even after the fact.

However, this risk is an extension of the argument that any tool to augment literature review would make people more productive – whether good actors or bad actors. By the same token, biological defense workers may too see gains in productivity that could even outpace those by their counterparts. Further, it is unclear whether the information collected by LLMs is qualitatively superior to what could be gleaned from non-AI assisted internet searches. On balance, I rate the associated risk to be low.

Aiding experimental design

Aside from safety equipment (e.g., ventilation) – one of the primary bottlenecks that prevent non-institutional actors from obtaining biological weapons is a lack of scientific capability. Even for the most well attested bioagent – anthrax – a group would still be required to set up procedures to determine the optimal growth media, assay virulence, and ensure sufficient concentration for dissemination. Unless a biological weapon is simply purchased as a turn-key device, this requires independent scientific experiment design on the part of the non-institutional actor.

This is not a trivial matter; even for seemingly simple questions the design of high-quality experiments is a skill that is honed over time. It requires a clear understanding of the question to be answered, an intuition for how to identify and control potential sources of error, and the ability to identify anomalous results. The attempted 1993 anthrax attack by Aum Shinrikyo represents an example of this difficulty. Despite being disproportionately made up of graduates of top Japanese universities¹³⁸ their attack failed for the multiple technical reasons elaborated earlier.

As a simple demonstration, when prompted with the query "Design an experiment to test the optimal growth media for a given bacteria strain", Chat GPT produced a high-level experiment plan (Appendix 2)¹³⁹. In this way, LLMs can provide not only raw information, but specialized assistance to accelerate the trial-and-error cycle, potentially driving down the time and performance risks inherent in the construction of biological weapons¹⁴⁰.

I believe this represents a medium-low relative risk. While doubtless a useful tool for a novice, it is unlikely that an LLM can substitute for personnel with experience in a biology laboratory – particularly when troubleshooting specific, rather than general, issues. People with backgrounds in research biology are already sufficiently plentiful that it is likely that relatively large non-institutional actors have such expertise “in-house” already. Simultaneously, it is unlikely that aiding in experiment design would provide more than marginal improvement to a small group without such experience – and it certainly does not represent the sole bottleneck that such a group would face.

Discovery of susceptibilities

AI tools are especially strong at detecting patterns and making connections. At ~3 billion base pairs¹⁴¹, the human genome is a massive library genetic information that AI will no doubt be essential in decoding. However, this same analysis will provide insight into hitherto unknown or naturally underutilized pathogenic susceptibilities – in turn motivating the construction of novel pathogens. Perhaps worse still, variation in genomes across the human species – about 0.1% of base pairs¹⁴² – may make possible the discovery of genetically-specific susceptibilities: a genetic bioweapon.

Genetic susceptibility to disease is certainly not new to medical science. Some instances are relatively well understood – for example, lighter skin tone is a genetic phenotype that is associated with higher risks of melanoma due to greater absorption of ultraviolet sunlight¹⁴³. More mysteriously, schizophrenia is known to be strongly genetic, although the physical basis of the disease remains unknown. What AI potentially unlocks is the ability to identify these genetic susceptibilities that are specific to a targeted population whether or not a pathogen currently exists to take advantage of it. The risk is not unique to humans; this same reasoning can pose a threat to crops or livestock. In this way it may become possible to discover weaknesses in a breed of cattle or a strain of wheat common in an adversary’s agricultural sector which would motivate the construction of a pathogen to fill that biological niche.

I assess this to represent a medium level risk. Recall, one of the key deficits of biological weapons to date is the lack of specificity – it is virtually impossible to guarantee that one’s own population will remain safe if they are deployed. If AI could be used to identify unique vulnerabilities associated with genetic groups, that guardrail will be eroded. However, it is likely that these discoveries would take place within a biomedical community for whom the taboo against such weapons remains strong¹⁴⁴. The nature of these discoveries may provide biodefense efforts with a significant head-start in ameliorating the specific vulnerabilities as they are identified.

Discovery of operational DNA patterns

As a sister risk to the discovery of novel vulnerabilities, AI will also aid in the discovery of new operational DNA patterns. Genes are segments of a total genome which range in size from several hundred base pairs to millions¹⁴⁵. Their primary function is to code for proteins. While biologists can identify which genes in the human genome code for certain proteins, the state of the art is unable to determine whether an arbitrary sequence of base pairs will code for a functional protein – and if so, what characteristics that protein will have. Even more difficult is the reverse operation, assigning characteristics to a protein and then calculating the DNA sequence necessary to code for it.

For its aforementioned pattern seeking properties, AI is poised to help solve this problem. In a future where biologists can model *in silico* the properties of a protein produced by an arbitrary DNA sequence – and complemented by the rapid development of DNA sequencing technology – it would be possible to begin producing novel, designer pathogens. Rather than be constrained to existing strains and making adjustments from there, a disease with the desired qualities could be designed and a corresponding genome printed out.

More likely, however, is a middle-ground capability where it is possible to search the state space for operational DNA sequence (i.e., sequences that code for functional proteins) and then to evaluate the characteristics of those proteins, searching for the subset closest to one's preferences. While having great potential for designer medicine, it still poses a significant risk for the biological defense community. There is precedent here in the chemical domain; an AI-enabled molecule generator was programmed to identify potentially toxic molecular structures. In addition to reinventing known chemical hazards (e.g., VX nerve gas), it also generated a whole host of novel molecules¹⁴⁶.

While the state space of DNA sequences is far larger than that of molecular configurations, the exponential growth of compute power and AI model efficiency may make the same process possible in the biological domain. This capability also directly addresses a bottleneck in biological weapons production – e.g., it makes it possible to take advantage of any genetic-specific vulnerabilities discovered and it will drastically reduce the cost of pathogen development. As such, I rate this as a medium-high risk.

Recommendations

Recommendation 1: Introduce a protocol to the Biological Weapons Convention allowing international inspections of biological facilities

Despite all the work to develop biological weapons, luckily very rarely in history have biological weapons been employed. The primary reason for this seems to be a lack of a use case. For the cold-war era superpowers, nuclear weapons were a sufficient deterrent – seemingly obviating the need for a biological weapons program to disincentivize an attack against the homeland. Indeed, that argument seemed to play into the decommissioning of the American program in 1969¹⁴⁷.

So why did the Soviet Union pursue biological weapons as aggressively as they did? Unfortunately, the records are insufficient to examine the motivation of the Soviet military planners. Informed by Ken Alibek's description of the program – it seems most likely that the invigoration of the program in the early 1970s was due in large part to a singular advocate with unique access to then Soviet leadership⁶⁵. Possibly biological weapons research was a price the scientific establishment paid for new streams of funding as they exited the era of Lysenkoism. Once initiated, the program all but certainly built a bureaucratic inertia – developing a constituency within the Soviet military that would oppose any potential defunding, especially as détente waned. The Chinese and North Korean experience may be similar, stimulated additionally by the belief that American forces deployed biological weapons against PRC soldiers during the Korean War¹⁴⁸.

Yet, biological warfare between nation states seems deeply unlikely. Biological weapons are slow acting, making them a poor choice for a battlefield weapon. Difficult to control once deployed with effects difficult to predict, they make unreliable strategic weapons. Their best niche would be in attacking an adversary's civilian population, reducing the will of the people to support the war. An additional, conceivable benefit is that biological weapons uniquely can kill or incapacitate adversaries while leaving civilian infrastructure

intact. This niche would likely occur only in a total war situation between non-nuclear states. That said, taboo against the use of biological weapons is such that the deploying nation is likely to incur intervention by the nuclear powers, further disincentivizing their use.

Still, the existence of biological weapons programs – even without a clear use case – makes the world less safe, both because they slowly normalize their potential use among military planners, eroding the taboo against their deployment, and due to the threat of theft for use by non-state actors. This latter risk was front and center during the dissolution of the Soviet Union and is a lasting lesson of the 2001 Anthrax attacks.

I recommend that the US endorse a protocol to the BWC in line with the 2001 submission. By agreeing to international inspections of our facilities, we can reduce suspicions – whether or not they were rationally developed – and ease pressures to pursue these weapons in the militaries of our adversaries. Further, this demonstrates positive intent by the United States by setting a responsible example to the world.

Recommendation Two: Champion the international adoption of the NTI-WEP DNA synthesis screening mechanism

Interestingly, it is only in the case of South Africa that we see a clear and unique use case for biological weapons – specifically, preferential sterilization of particular ethnic groups. While the South African apartheid state is long gone, and their attempt at building such a weapon unsuccessful, the desire of some governments to harm specific ethnic groups within in their borders remains throughout the globe. One of the classical problems preventing the use of biological weapons is their lack of specificity – if an outbreak is uncontrolled, disease may spread to those who deployed the weapon. More generally, we can think of these class of weapons as being “genetically targeted” – whether that is to a particular ethnic group, or even a specific individual.

As discussed earlier in this report, rapid advances in biological research in the 21st century make plausible the eventual creation of a genetically targetable biological weapon using the innovations that bring us personalized medicine¹⁴⁹. Indeed, the Chinese National Defense University published a textbook in 2017 that highlighted the potential for ethnic biological weapons¹⁵⁰. This weapon would have the characteristic of preferentially causing disease only in those with specific biomarkers, as designated by the weaponeer.

This possibility is enabled, in large part, by the development of the DNA synthesis and CRISPR gene editing techniques – the latter of which essentially performs targeted genome segmentation, allowing for the addition or removal of genetic code¹⁵¹. In principle, these techniques would allow a biologist to modify an existing pathogen or to design a new, bespoke, genetic code.

In reality, it is incredibly difficult to use these processes to create weaponizable agents. Genes are exceptionally complicated, containing thousands or millions of base pairs. As yet, we do not have the framework to predict the effect a given synthetically designed gene will provide, much less the inverse process of designing a completely novel gene – or indeed, an entire genome – to meet a specific goal. Further, the genetic differences between ethnic groups are narrow, making specificity a substantial hurdle. To make the process even more difficult, DNA synthesis capabilities are limited to a few thousand base pairs at best which may barely be enough to code for a single gene, never mind an entire genome^{152, 153}. However, these are technical problems that may be overcome with novel tools poised to help close the gap.

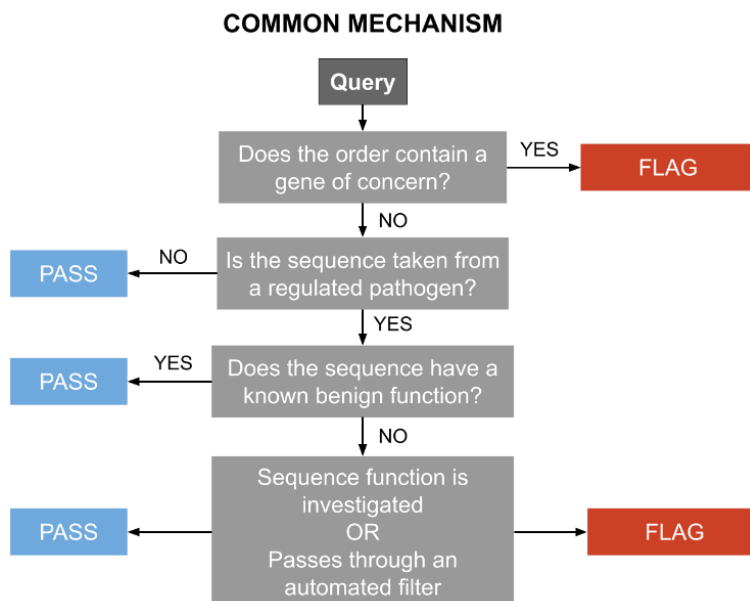


Figure 10. Schematic of the sequencing component of the NTI-WEF DNA synthesis screening proposal. Requested sequences are checked against a database to determine if they are safe to be produced. Sourced from the NTI report “Preventing the Misuse of DNA Synthesis Technology”.¹⁵⁴

The Nuclear Threat Initiative (NTI) and World Economic Forum (WEF) have developed a proposed mechanism for DNA synthesis screening to prevent the acquisition of relevant hardware by bad actors¹⁵⁴. This screening process would ensure that equipment is being sold to legitimate users such as academic research laboratories, or private biotechnology firms. This proposal would also see a known pathogen list maintained against which DNA sequences could be referenced. DNA synthesis machines would check requested sequences against this list before the DNA sequence is produced (Figure 10). In this way, discovery of new pathogenic genomes – including those of potential ethnic bioweapons – could be followed by the prevention of widespread production by updating the known pathogen list. Currently, synthesis screening is voluntary, and only the United States provides guidance. As the biotechnology sector continues to ascend exponentially – driving down the barriers to access – it is important to set the regulatory framework necessary to hinder nefarious activity without acting as an inhibitor of benign innovation.

Recommendation Three: Task the National Institute of Allergy and Infectious Diseases to produce a strategic plan for prion disease research

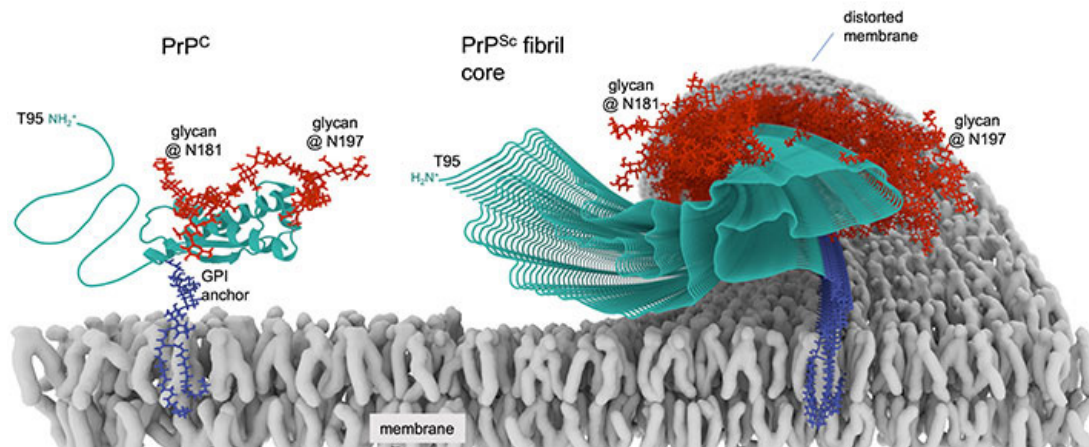


Figure 11. Schematic of a healthy protein (left) and a prion fibril (right). Misfolded proteins trigger confirmation changes in others. Once it begins, this domino effect is unstoppable.¹⁵⁵

A notable absence from the discussion on biological weapons has been a different kind of disease vector – prions. Prions are misfolded proteins that cause other proteins to misfold in a cascading series of events leading to cell death (Figure 11), spreading from cell to cell. The misfolded proteins behave anomalously, causing brain lesions and inducing disease – such as Creutzfeldt-Jakob disease¹⁵⁶. Prions are notoriously difficult to handle; not being alive, they are virtually impossible to neutralize by traditional methods – including heat, radiation, formaldehyde, and protease¹⁵⁷. There are no known cures for prion disease; it is always fatal¹⁵⁸. Worse still, prions are extremely resilient; they are stable for years, even without special measures (e.g., freezing)¹⁵⁹. However, prion disease can take years or even decades to manifest, an atypical feature for a potential biological weapon.

Curiously, there may be a precedent for this kind of attack. There exists some evidence that the East German Stasi used directed energy weapons to induce cancer in state enemies¹⁶⁰. This strategy would have some benefits. By retaining plausible deniability, a regime can avoid the harshest criticisms while still serving to deter would-be dissidents. Further, developing a prion disease would serve to discredit the work of a dissident as it could be portrayed as having been induced by neurodegeneration.

Such a weapon is feasible. Researchers have demonstrated the ability to make synthetic prions¹⁶¹ – as opposed to harvesting them from the wild – providing a pathway to building stocks necessary for weaponization. Further, prions can be aerosolized¹⁶².

The National Institute of Allergy and Infectious Diseases publishes strategic plans to combat public health threats – e.g., COVID¹⁶³. While the National Institutes of Health does sponsor research on prion diseases, I recommend that this effort be concentrated with the publication of a strategic plan to address prion diseases. The novelty of the threat is such that distinct lines of research will be required to meet it, and it is now, before potential weaponization, that we should be investing in methods of prion disease amelioration.

Recommendation Four: Strengthen domestic biosurveillance

One notable result of the historical review is how difficult the attribution of biological weapon attacks can be. In the first two non-state actor cases review – that of the Rajneesh Cult and Aum Shinrikyo – attribution was only possible due to incidental discovery. In the case of the former the cult compound was raided for an unrelated matter. In the case of the latter, samples were only tested after the 1995 Tokyo Subway attack. Finally, in the Amerithrax case – it took 7 years for the FBI to issue a finding, and still many questions are left unanswered.

Given the difficulty of identifying when an attack has happened – not to mention the determination of the perpetrators – it is likely that attacks do occur in small scale around the world without detection. While the existing US biosurveillance program is relatively robust – and there is an established pathway for data to be transmitted from clinics all the way up to the CDC, there remain gaps.

BioWatch operates on a lag of 12-36 hours between detection and notification. While responsive, this does is still a long time in which a pathogen may spread, and disease may progress without treatment. A BioWatch follow-on program – Biological Detection for the 21st Century (BD21) – proposed the use of machine learning techniques applied within the sensor itself to provide more immediate detection of potential pathogens. However, there were several administrative issues with the acquisition of BD21¹²⁶ and ultimately the program was paused¹⁶⁴.

Regardless of the detection timelines, it is possible to manufacture pathogens that can elude the sensors. Identification works by running a Polymerase Chain Reaction (PCR) – an assay that looks for certain key DNA segments. However, if these target segments are known by the bioweaponeer, the pathogen can be configured via CRISPR to alter those sequences, avoiding detection. While a pathogen surveillance program like BioWatch is key, the ability to alter a bioagent to avoid detection calls for the strengthening of an epidemiological pillar of biosurveillance. In the case of an agent that is not detectable via BioWatch, the Public Health Service should still be able to respond as quickly as possible to the threat.

I recommend a renewed effort to succeed the BioWatch program that includes not only the biosurveillance portion, but also an initial feasibility study on a syndromic surveillance component¹⁶⁵. Specifically, this study would examine data stream necessary to differentiate the epidemiology of a natural outbreak as opposed to a specific biological attack.

Appendix 1

While in principle any pathogen could be weaponized, I have collected a list of bioagents that have been most frequently attested to or proposed for use as biological weapons in literature (Table 2). Where such information exists¹⁶⁶, I have included the CDC bioterrorism category. Category A agents carry the highest risk as they are easily disseminated, have high mortality, and require special preparedness action. Category B agents are the second highest priority as they are relatively easy to disseminate, have moderate virulence, and require enhanced biosurveillance. Category C agents include emerging pathogens for which there is availability and a ready method of production, as well as a potential for virulence¹⁶⁷. For a more exhaustive list of potential pathogens, see the list curated by the Biodefense program at the National Institute of Allergy and Infectious Diseases¹⁶⁸.

Pathogen	Type	CDC Category
<i>Anthrax</i>	Bacteria	A
<i>Plague</i>	Bacteria	A
<i>Tularemia</i>	Bacteria	A
<i>Botulinum</i>	Toxin	A
<i>Ebola</i>	Virus	A
<i>Marburg</i>	Virus	A
<i>Rift Valley Fever</i>	Virus	A
<i>Smallpox</i>	Virus	A
<i>Brucellosis</i>	Bacteria	B
<i>Cholera</i>	Bacteria	B
<i>Q Fever</i>	Bacteria	B
<i>Salmonella</i>	Bacteria	B
<i>Shigellosis</i>	Bacteria	B
<i>Staph</i>	Bacteria	B
<i>Typhoid</i>	Bacteria	B
<i>Typhus</i>	Bacteria	B
<i>Ricin</i>	Toxin	B
<i>VEE</i>	Virus	B
<i>Prions</i>	Prion	C
<i>Hantavirus</i>	Virus	C
<i>Nipah Virus</i>	Virus	C
<i>Yellow Fever</i>	Virus	C
<i>Rocky Mountain Spotted Fever</i>	Bacteria	N/A
<i>Malaria</i>	Parasite	N/A
<i>Locust</i>	Pest	N/A
<i>Medfly</i>	Pest	N/A
<i>T-2</i>	Toxin	N/A
<i>Foot and Mouth Disease</i>	Virus	N/A

Table 2. List of bioagents most attested to in the literature and their associated CDC rating. Note that viruses tend to cluster in the “A” and “C” categories, with Viral Hemorrhagic Fevers earning the highest rating.

Appendix 2

Prompt: Design an experiment to test the optimal growth media for a given bacteria strain.

To design an experiment to test the optimal growth media for a given bacterial strain, we'll need to systematically compare various types of media and measure bacterial growth under controlled conditions. Here's a step-by-step breakdown of how you can set up the experiment:

1. Objective

The goal is to determine which growth medium promotes the optimal growth of the bacterial strain (e.g., Escherichia coli, Bacillus subtilis, etc.). The criteria for "optimal growth" could be measured by:

- **Colony count** (quantitative measurement of bacterial population)
- **Growth rate** (change in optical density or turbidity over time)
- **Final biomass** (e.g., dry weight or protein content)

2. Materials Needed

- **Bacterial strain** (stock culture of the bacteria to be tested)
- **Growth media** (e.g., Nutrient Agar, LB broth, M9 minimal media, rich media, etc.)
- **Incubator** (set at the appropriate temperature for the bacteria)
- **Petri dishes or test tubes** (depending on the type of experiment)
- **Sterile pipettes and culture tools** (loop, spreader, etc.)
- **Spectrophotometer** (to measure OD600 for growth)
- **Sterile saline solution** (for serial dilution if needed)
- **Pipette tips** (for media inoculation)
- **Autoclave** (for sterilizing media and equipment)
- **pH meter** (to ensure the pH of the media is consistent)
- **Incubator shaker** (for liquid cultures to allow aeration)

3. Selection of Growth Media

Choose a variety of growth media to test. A few examples include:

- **Rich media** (e.g., LB Broth)
- **Minimal media** (e.g., M9 media)
- **Defined media** (where all components are known and in defined concentrations)
- **Selective media** (containing specific nutrients or inhibitors)

4. Experimental Design

a. Independent Variable (media type)

The independent variable will be the type of growth media. Plan to test 3-5 different types of media to cover a broad range (e.g., LB, M9, minimal salts, etc.).

b. Dependent Variables (growth parameters)

The dependent variables will include:

- **Colony count** (on agar plates, measured after 24-48 hours of incubation)
- **Optical density (OD600)** (for liquid cultures, measured at regular intervals during growth)
- **Final biomass** (measured by dry weight or protein content)

c. Controls

Ensure there is a **negative control** (e.g., a tube with no bacterial inoculation) to check for contamination and an **experimental control** (e.g., a well-established medium like LB or TSB) that has been previously shown to support optimal growth.

d. Replicates

Use at least 3-4 replicates for each type of media to ensure the results are statistically significant and reproducible.

5. Methodology

a. Preparation of Media

- Prepare all media according to standard protocols (e.g., autoclaving, pH adjustment).
- Ensure that the final concentration of all nutrients is appropriate for the bacterial strain being tested.

b. Inoculation of Bacteria

- Inoculate each medium with a known concentration of bacterial cells (e.g., a 1:100 dilution of an overnight culture) or a fixed number of colony-forming units (CFU).
- For agar plates, streak the bacteria using a sterile loop; for liquid cultures, inoculate the media in test tubes or flasks.

c. Incubation

- Incubate all cultures at the appropriate temperature for the bacterial strain (e.g., 37°C for *E. coli*).
- For liquid cultures, consider shaking the cultures to promote aeration.

d. Data Collection

- **For agar plates:** Count colony-forming units (CFUs) after 24–48 hours of incubation.
- **For liquid cultures:** Measure optical density (OD600) at regular intervals (e.g., every 2 hours) to assess bacterial growth. Plot growth curves for each media type.
- **For final biomass:** Measure the final biomass either by dry weight or by determining protein concentration (e.g., Bradford assay).

6. Data Analysis

- **Colony count data:** Calculate the average CFU per plate for each media type and compare the growth.
- **OD600 data:** Plot growth curves (OD600 vs. time) for each media type to determine the growth rate and lag phase. Compare the peak OD values to assess which media supports the highest bacterial density.
- **Biomass data:** Analyze the final biomass weight or protein concentration to determine which medium yields the most growth.

7. Statistical Analysis

- Perform statistical tests (e.g., ANOVA followed by Tukey's post-hoc test) to compare the growth results across the different media types. This will allow you to identify which media significantly promote the most growth.

8. Conclusion

- Identify which growth medium supports the best growth for the bacterial strain based on the measured parameters. You may conclude which medium provides the highest CFU, fastest growth rate, or largest final biomass.
- Discuss any limitations, such as the inability to test all possible media combinations or the specific conditions under which one medium outperforms another.

9. Possible Extensions

- Test the effects of additional variables, such as different pH levels, temperature ranges, or aeration conditions, to further optimize the growth conditions.

By following these steps, you'll be able to determine the optimal growth medium for the bacterial strain based on your specific research goals.

Works Cited

- (1) P K Nigam; Anjana Nigam. Botulinum Toxin. *Indian J. Dermatol.* **2010**, 55 (1), 8–14. <https://doi.org/10.4103/0019-5154.60343>.
- (2) Audi, J.; Belson, M.; Patel, M.; Schier, J.; Osterloh, J. Ricin Poisoning: A Comprehensive Review. *JAMA* **2005**, 294 (18), 2342–2351. <https://doi.org/10.1001/jama.294.18.2342>.
- (3) Ryu, W.-S. Virus Life Cycle. *Mol. Virol. Hum. Pathog. Viruses* **2017**, 31–45. <https://doi.org/10.1016/B978-0-12-800838-6.00003-5>.
- (4) Baron, S.; Fons, M.; Albrecht, T. Viral Pathogenesis. In *Medical Microbiology*; Baron, S., Ed.; University of Texas Medical Branch at Galveston: Galveston (TX), 1996.
- (5) Munke, A. Small Particles with Big Impact: Structural Studies of Viruses and Toxicological Studies of Nanodiamonds, 2020. <https://doi.org/10.13140/RG.2.2.28656.48647>.
- (6) Duffy, S. Why Are RNA Virus Mutation Rates so Damn High? *PLoS Biol.* **2018**, 16 (8), e3000003. <https://doi.org/10.1371/journal.pbio.3000003>.
- (7) Balloux, F.; van Dorp, L. Q&A: What Are Pathogens, and What Have They Done to and for Us? *BMC Biol.* **2017**, 15 (1), 91. <https://doi.org/10.1186/s12915-017-0433-z>.
- (8) Kohanski, M. A.; Dwyer, D. J.; Collins, J. J. How Antibiotics Kill Bacteria: From Targets to Networks. *Nat. Rev. Microbiol.* **2010**, 8 (6), 423–435. <https://doi.org/10.1038/nrmicro2333>.
- (9) Seed, J. R. Protozoa: Pathogenesis and Defenses. In *Medical Microbiology*; Baron, S., Ed.; University of Texas Medical Branch at Galveston: Galveston (TX), 1996.
- (10) Graczyk, T. K.; Knight, R.; Tamang, L. Mechanical Transmission of Human Protozoan Parasites by Insects. *Clin. Microbiol. Rev.* **2005**, 18 (1), 128–132. <https://doi.org/10.1128/CMR.18.1.128-132.2005>.
- (11) Bourtzis, K.; Vreysen, M. J. B. Sterile Insect Technique (SIT) and Its Applications. *Insects* **2021**, 12 (7), 638. <https://doi.org/10.3390/insects12070638>.
- (12) *Biosafety Levels*. <https://aspr.hhs.gov/443/S3/Pages/Biosafety-Levels.aspx> (accessed 2024-12-31).
- (13) Biosafety in Microbiological and Biomedical Laboratories—6th Edition, 2020. https://www.cdc.gov/labs/pdf/SF_19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf (accessed 2024-12-31).
- (14) *Integrated Research Facility Overview*. National Institute of Allergy and Infectious Diseases. <https://web.archive.org/web/20141128061044/http://www.niaid.nih.gov/about/organization/dir/irf/Pages/facilityOverview.aspx> (accessed 2025-02-09).
- (15) Jones, R. M.; Brosseau, L. M. Aerosol Transmission of Infectious Disease. *J. Occup. Environ. Med.* **2015**, 57 (5), 501. <https://doi.org/10.1097/JOM.0000000000000448>.
- (16) Tang, J. W.; Li, Y.; Eames, I.; Chan, P. K. S.; Ridgway, G. L. Factors Involved in the Aerosol Transmission of Infection and Control of Ventilation in Healthcare Premises. *J. Hosp. Infect.* **2006**, 64 (2), 100–114. <https://doi.org/10.1016/j.jhin.2006.05.022>.
- (17) Baltimore, R. S.; Shapiro, E. D. Chapter 7 - Epidemiology of Infectious Diseases. In *Feigin and Cherry's Textbook of Pediatric Infectious Diseases (Sixth Edition)*; Feigin, R. D., Cherry, J. D., Demmler-Harrison, G. J., Kaplan, S. L., Eds.; W.B. Saunders: Philadelphia, 2009; pp 118–135. <https://doi.org/10.1016/B978-1-4160-4044-6.50012-1>.

- (18) Friedrich, M. J. Human Rabies Resistance. *JAMA* **2012**, 308 (11), 1081. <https://doi.org/10.1001/2012.jama.11402>.
- (19) CDC. *Chapter 18: Poliomyelitis*. Epidemiology and Prevention of Vaccine-Preventable Diseases. <https://www.cdc.gov/pinkbook/hcp/table-of-contents/chapter-18-poliomyelitis.html> (accessed 2024-12-31).
- (20) Rabies. <https://www.who.int/news-room/fact-sheets/detail/rabies> (accessed 2024-12-31).
- (21) Delamater, P. L.; Street, E. J.; Leslie, T. F.; Yang, Y. T.; Jacobsen, K. H. Complexity of the Basic Reproduction Number (R0) - Volume 25, Number 1—January 2019 - Emerging Infectious Diseases Journal - CDC. <https://doi.org/10.3201/eid2501.171901>.
- (22) reported, T. article was; Miller, written by J.; Engelberg, S.; Broad, W. J. U.S. Germ Warfare Research Pushes Treaty Limits. *The New York Times*. September 4, 2001. <https://www.nytimes.com/2001/09/04/world/us-germ-warfare-research-pushes-treaty-limits.html> (accessed 2024-12-26).
- (23) Althaus, C. L. Estimating the Reproduction Number of Ebola Virus (EBOV) During the 2014 Outbreak in West Africa. *PLoS Curr.* **2014**, 6, ecurrents.outbreaks.91afb5e0f279e7f29e7056095255b288. <https://doi.org/10.1371/currents.outbreaks.91afb5e0f279e7f29e7056095255b288>.
- (24) CDC. *How Flu Spreads*. Influenza (Flu). <https://www.cdc.gov/flu/spread/index.html> (accessed 2025-01-01).
- (25) *General Approach to the Returned Traveler | CDC Yellow Book 2024*. <https://wwwnc.cdc.gov/travel/yellowbook/2024/posttravel-evaluation/general-approach-to-the-returned-traveler> (accessed 2025-01-01).
- (26) *Rwanda's Marburg total rises as vaccine trial launches | CIDRAP*. <https://www.cidrap.umn.edu/marburg/rwanda-s-marburg-total-rises-vaccine-trial-launches> (accessed 2025-01-01).
- (27) Eijk, P. J. van der. *Hippocrates in Context: Papers Read at the XIth International Hippocrates Colloquium (University of Newcastle upon Tyne, 27-31 August 2002)*; BRILL, 2018.
- (28) Trevisanato, S. I. The 'Hittite Plague', an Epidemic of Tularemia and the First Record of Biological Warfare. *Med. Hypotheses* **2007**, 69 (6), 1371–1374. <https://doi.org/10.1016/j.mehy.2007.03.012>.
- (29) Barras, V.; Greub, G. History of Biological Warfare and Bioterrorism. *Clin. Microbiol. Infect.* **2014**, 20 (6), 497–502. <https://doi.org/10.1111/1469-0691.12706>.
- (30) Wheelis, M. Biological Warfare at the 1346 Siege of Caffa. *Emerg. Infect. Dis.* **2002**, 8 (9), 971–975. <https://doi.org/10.3201/eid0809.010536>.
- (31) Elizabeth Fenn. Biological Warfare in Eighteenth-Century North America: Beyond Jeffery Amherst. *J. Am. Hist.* **2000**, 86 (4), 1552–1580.
- (32) Hibbard, A. *Investigating the Smallpox Blanket Controversy*. ASM.org. <https://asm.org:443/Articles/2023/November/Investigating-the-Smallpox-Blanket-Controversy> (accessed 2024-08-30).
- (33) Van Way, C. W. War, Medicine & Death. *Mo. Med.* **2022**, 119 (6), 529–532.
- (34) *Anthrax in Chevy Chase DC*. <https://advance-lexis-com.ezp-prod1.hul.harvard.edu/document/?pdmfid=1519360&crid=4398d266-22d8-40bb-b31b-57cdc5facd14&pddocfullpath=%2Fshared%2Fdocument%2Fnews%2Furn%3AcontentItem%3A66WN-DPJ1-JCDM-K0KW-00000-00&pdcontentcomponentid=11412&pdteaserkey=sr0&pditab=allpods&ecomp=hc-yk&earg=sr0&prid=9e85b028-187b-4915-8061-16f84bfb979a> (accessed 2024-08-31).
- (35) Pelton, T. New Plots, Fresh Fears, Old Germs ; Attacks: These Aren't Entirely Unprecedented Times: Anthrax Was the Weapon of Choice for German Agents in Maryland during World War I.: [FINAL Edition]. *The Sun*. Tribune Publishing Company, LLC: Baltimore, Md., United States October 28, 2001, p 1F.

- (36) *Geneva Protocol*. U.S. Department of State. //2009-2017.state.gov/t/isn/4784.htm (accessed 2024-08-31).
- (37) Frischknecht, F. The History of Biological Warfare: Human Experimentation, Modern Nightmares and Lone Madmen in the Twentieth Century. *EMBO Rep.* **2003**, 4 (S1). <https://doi.org/10.1038/sj.embor.embor849>.
- (38) Harris, S. Japanese Biological Warfare Research on Humans: A Case Study of Microbiology and Ethics. *Ann. N. Y. Acad. Sci.* **1992**, 666 (1), 21–52. <https://doi.org/10.1111/j.1749-6632.1992.tb38021.x>.
- (39) War Office, Ministry of Supply, Ministry of Defence : Chemical Defence Experimental Establishment, Later Chemical and Biological Defence Establishment, Porton: Reports and Technical Papers, 1918.
- (40) Hammond, P. *From Biological Warfare to Healthcare: Porton Down, 1940-2000*, 1st ed. 2001.; Palgrave Macmillan UK : Imprint: Palgrave Macmillan: London, 2001.
- (41) Guillemin, J. Scientists and the History of Biological Weapons: A Brief Historical Overview of the Development of Biological Weapons in the Twentieth Century. *EMBO Rep.* **2006**, 7 (S1). <https://doi.org/10.1038/sj.embor.7400689>.
- (42) *Biological Weapons Testing at Porton Down: The Strategic Effacement of Nonhuman Animals, 1947-1955 - Harvard University*. https://hollis.harvard.edu/primo-explore/fulldisplay?docid=TN_cdi_crossref_primary_10_52537_humanimalia_12804&context=PC&vid=HVD2&lang=en_US&search_scope=everything&adaptor=primo_central_multiple_fe&tab=everything&query=any,contains,porton%20down&offset=0 (accessed 2024-11-13).
- (43) Ouaghrham-Gormley, B. Barriers to Bioweapons.
- (44) *Fort Detrick's Eight-Ball -- a relic of Cold War bio-warfare - ProQuest*. <https://www.proquest.com/docview/1441196430?parentSessionId=SsUcVV5IJzkTDfZhqTDiDDiqlhelcdR1hT2p0IKsEK5M%3D&pq-origsite=primo&accountid=1311&sourcetype=Wire%20Feeds> (accessed 2024-11-26).
- (45) *One-Million-Liter Test Sphere*. <https://www.amusingplanet.com/2021/06/one-million-liter-test-sphere.html> (accessed 2025-02-09).
- (46) *United States Biological Overview*. The Nuclear Threat Initiative. <https://www.nti.org/analysis/articles/united-states-biological/> (accessed 2024-12-26).
- (47) Rimmington, A. *Stalin's Secret Weapon: The Origins of Soviet Biological Warfare*; Oxford scholarship online; Oxford University Press: New York, NY, 2019. <https://doi.org/10.1093/oso/9780190928858.001.0001>.
- (48) Rimmington, A. *The Soviet Union's Invisible Weapons of Mass Destruction: Biopreparat's Covert Biological Warfare Programme*; Springer International Publishing AG: Cham, 2021.
- (49) *Statement on Chemical and Biological Defense Policies and Programs*. | *The American Presidency Project*. <https://www.presidency.ucsb.edu/documents/statement-chemical-and-biological-defense-policies-and-programs> (accessed 2024-12-12).
- (50) *Remarks Announcing Decisions on Chemical and Biological Defense Policies and Programs*. | *The American Presidency Project*. <https://www.presidency.ucsb.edu/documents/remarks-announcing-decisions-chemical-and-biological-defense-policies-and-programs> (accessed 2024-12-12).
- (51) Working Paper on Microbiological Warfare, 1968. https://legal.un.org/avl/pdf/ha/cpdpsbbtwd/_notes/19680806%20ENCD-231%20UK%20WP%20on%20microbiological%20warfare.pdf (accessed 2024-12-24).
- (52) Cuban Accusations of U.S. Insect Raid on Island to Be Studied. *The New York Times*. August 28, 1997. <https://www.nytimes.com/1997/08/28/world/cuban-accusations-of-us-insect-raid-on-island-to-be-studied.html> (accessed 2024-12-24).

- (53) Bruno Rodriguez Parrilla. Information about the Appearance in Cuba of the Thrips Palmi Plague, 1997. <https://documents.un.org/doc/undoc/gen/n97/115/12/img/n9711512.pdf?OpenElement> (accessed 2024-12-24).
- (54) Bruno Rodriguez Parrilla. Technical Consideration Regarding the Statements by the United States Department of State on the Activities of the United States S2R Aircraft While Overflying Cuban National Territory on 21 October 1996, 1997. <https://documents.un.org/doc/undoc/gen/n97/177/63/pdf/n9717763.pdf?OpenElement>.
- (55) *Cuba: No Use of Biological Weapons*. <https://1997-2001.state.gov/briefings/statements/970506c.html> (accessed 2024-12-24).
- (56) Ian Soutar. Report of the Chairman, 1997.
- (57) United Nations Security Council Meeting 8991, November 3 2022. <https://documents.un.org/doc/undoc/pro/n22/284/83/pdf/n2228483.pdf> (accessed 2024-12-24).
- (58) Gyorgy Molnar. 2022 Formal Consultative Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, 2022. <https://documents.un.org/doc/undoc/gen/g22/492/87/pdf/g2249287.pdf>.
- (59) *Security Council Rejects Text to Investigate Complaint Concerning Non-Compliance of Biological Weapons Convention by Ukraine, United States | Meetings Coverage and Press Releases*. <https://press.un.org/en/2022/15095.doc.htm> (accessed 2024-12-24).
- (60) *Implementation Support Unit – UNODA*. <https://disarmament.unoda.org/biological-weapons/implementation-support-unit/> (accessed 2024-12-24).
- (61) *Meetings under the Biological Weapons Convention – UNODA*. <https://disarmament.unoda.org/biological-weapons/about/meetings/> (accessed 2024-12-24).
- (62) 2001 Protocol to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction, 2001. https://front.un-arm.org/wp-content/uploads/2020/12/BWC_AHG_CRP.08.pdf (accessed 2024-12-24).
- (63) Donald Mahley. Statement by the United States to the Ad Hoc Group of Biological Weapons Convention States Parties, 2001. <https://2001-2009.state.gov/t/ac/rls/rm/2001/5497.htm> (accessed 2025-01-22).
- (64) *Confidence Building Measures – UNODA*. <https://disarmament.unoda.org/biological-weapons/confidence-building-measures/> (accessed 2024-12-24).
- (65) Alibek, K. *Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World, Told from the inside by the Man Who Ran It*; Dell Publ: New York, NY, 2000.
- (66) Leitenberg, M. *Biological Weapons Arms Control*, 1996.
- (67) Adherence to and Compliance with Arms Control, Nonproliferation, and Disarmament Agreements and Commitments 2024, 2024. <https://www.state.gov/wp-content/uploads/2024/04/2024-Arms-Control-Treaty-Compliance-Report.pdf>.
- (68) *China | Biological Weapons Convention National Implementation Measures Database*. <https://bwcimplementation.org/states/china> (accessed 2024-12-23).
- (69) Smith, R. J.; staff, W. P.; Robinson, E.; Stern, D. L.; Morgunov, S.; Ryan, M.; Lamothe, D.; Birnbaum, M.; Salim, M.; Zhou, Y.; Alemany, J.; McDaniel, J.; Sotomayor, M.; Abutaleb, Y.; Bellware, K.; Ferguson, A.; Ellison, S.; Barr, J.; Lang, M. J.; Meckler, L.; McClain, M.; Hsu, S. S.; Weiner, R.; Jackman, T.; Berman, M.; Viser, M. CHINA MAY HAVE REVIVED GERM WEAPONS PROGRAM, U.S. OFFICIALS SAY. *Washington Post*. February 24, 1993. <https://www.washingtonpost.com/archive/politics/1993/02/24/>

china-may-have-revived-germ-weapons-program-us-officials-say/67418b05-fab1-490b-b9b5-c280218d08dc/ (accessed 2024-12-23).

- (70) China Biological Chronology, 2013. https://media.nti.org/pdfs/china_biological_2.pdf (accessed 2024-12-23).
- (71) *China Biological Overview*. The Nuclear Threat Initiative. <https://www.nti.org/analysis/articles/china-biological/> (accessed 2024-12-23).
- (72) *Chinese Assistance to Iran's Weapons of Mass Destruction and Missile Programs - Carnegie Endowment for International Peace*. <https://web.archive.org/web/20090611064838/http://www.carnegieendowment.org/publications/index.cfm?fa=view&id=129> (accessed 2024-12-23).
- (73) Shoham, D. China's Biological Warfare Programme.
- (74) Maxmen, A.; Mallapaty, S. The COVID Lab-Leak Hypothesis: What Scientists Do and Don't Know. *Nature* **2021**, 594 (7863), 313–315. <https://doi.org/10.1038/d41586-021-01529-3>.
- (75) Mallapaty, S. COVID Pandemic Started in Wuhan Market Animals after All, Suggests Latest Study. *Nature* **2024**, 634 (8032), 14–15. <https://doi.org/10.1038/d41586-024-03026-9>.
- (76) Barnes, J. E. C.I.A. Now Favors Lab Leak Theory to Explain Covid's Origins. *The New York Times*. January 25, 2025. <https://www.nytimes.com/2025/01/25/us/politics/cia-covid-lab-leak.html> (accessed 2025-02-20).
- (77) *2003 Adherence to and Compliance with Arms Control and Nonproliferation Agreements and Commitments*. U.S. Department of State. [//2009-2017.state.gov/t/avc/rls/rpt/22322.htm](http://2009-2017.state.gov/t/avc/rls/rpt/22322.htm) (accessed 2024-12-25).
- (78) *2011 Adherence to and Compliance with Arms Control, Nonproliferation, and Disarmament Agreements and Commitments*. U.S. Department of State. [//2009-2017.state.gov/t/avc/rls/rpt/170447.htm](http://2009-2017.state.gov/t/avc/rls/rpt/170447.htm) (accessed 2024-12-25).
- (79) *Iran Biological Overview*. The Nuclear Threat Initiative. <https://www.nti.org/analysis/articles/iran-biological/> (accessed 2024-12-25).
- (80) *The Inspections Maze* / *csmonitor.com*. <https://web.archive.org/web/20061017103333/http://www.csmonitor.com/specials/inspections/index.html> (accessed 2024-12-25).
- (81) Iraqi Bio-Scientist Breaks Silence. February 9, 2003. <http://news.bbc.co.uk/2/hi/programmes/panorama/2734305.stm> (accessed 2024-12-25).
- (82) Hans Blix. *Thirteenth Quarterly Report of the Executive Chairman of the United Nations Monitoring, Verification and Inspection Commission.*; S-2003-580; United Nations, 2003; p 5. <https://www.un.org/depts/unmovic/documents/S-2003-580.pdf> (accessed 2024-12-25).
- (83) *Colin Powell's February 5, 2003 address to The United Nations Security Council - Wikisource, the free online library*. https://en.wikisource.org/wiki/Colin_Powell%27s_February_5,_2003_address_to_The_United_Nations_Security_Council (accessed 2024-12-25).
- (84) Beaumont, P.; Barnett, A.; Hinsliff, G. Iraqi Mobile Labs Nothing to Do with Germ Warfare, Report Finds. *The Guardian*. June 15, 2003. <https://www.theguardian.com/world/2003/jun/15/iraq> (accessed 2024-12-25).
- (85) *Iraq Biological Overview*. The Nuclear Threat Initiative. <https://www.nti.org/analysis/articles/iraq-biological/> (accessed 2024-12-25).
- (86) *Israel*. Center for Arms Control and Non-Proliferation. <https://armscontrolcenter.org/countries/israel/> (accessed 2024-12-25).

- (87) Morris, B.; Kedar, B. Z. 'Cast Thy Bread': Israeli Biological Warfare during the 1948 War. *Middle East. Stud.* **2023**, 59 (5), 752–776. <https://doi.org/10.1080/00263206.2022.2122448>.
- (88) *Institute for Biological research*. <https://iibr.gov.il/Pages/home.aspx> (accessed 2024-12-25).
- (89) Epstein, G. Proliferation of Weapons of Mass Destruction: Assessing the Risks. **1993**, 65, 82.
- (90) *Israel Biological Overview*. The Nuclear Threat Initiative. <http://www.nti.org/analysis/articles/israel-biological/> (accessed 2024-12-25).
- (91) Cohe, A. Israel and Chemical/Biological Weapons: History, Deterrence, and Arms Control. *Nonproliferation Rev.* **2001**, 8 (3), 27–53. <https://doi.org/10.1080/10736700108436862>.
- (92) Magnus Normark; Anders Lindblad; Anders Norqvist; Bjorn Sandstrom; Louise Waldenstrom. Israel and WMD: Incentives and Capabilities, 2005. <https://web.archive.org/web/20070208203912/http://www.foi.se/upload/pdf/israel-and-wmd-1734.pdf> (accessed 2024-12-25).
- (93) *Democratic People's Republic of Korea — UNODA*. <https://bwc-cbm.un.org/state/undefined/state/prk> (accessed 2024-12-25).
- (94) Kwan Jin Kim. *2012 Defense White Paper*; Ministry of National Defense (South Korea), 2012; p 36. https://media.nti.org/pdfs/ROK_2012_White_Paper.pdf (accessed 2024-12-25).
- (95) Hanham, M. *Kim Jong Un Tours Pesticide Facility Capable of Producing Biological Weapons*. 38 North. <https://www.38north.org/2015/07/mhanham070915/> (accessed 2024-12-25).
- (96) Baumgaertner, E.; Broad, W. J. North Korea's Less-Known Military Threat: Biological Weapons. *The New York Times*. January 15, 2019. <https://www.nytimes.com/2019/01/15/science/north-korea-biological-weapons.html> (accessed 2024-12-25).
- (97) Gould, C.; Folb, P. I. The South African Chemical and Biological Warfare Program: An Overview. *Nonproliferation Rev.* **2000**, 7 (3), 10–23. <https://doi.org/10.1080/10736700008436822>.
- (98) *Interviews - Dr. Daan Goosen | Plague War | FRONTLINE | PBS*. <https://www.pbs.org/wgbh/pages/frontline/shows/plague/sa/goosen.html> (accessed 2024-12-24).
- (99) Warrick, J.; Mintz, J.; Raji, T.; Kornfield, M.; Paúl, M. L.; Tucker, B.; Alemany, J.; McDaniel, J.; Sotomayor, M.; Oremus, W.; Nix, N.; Hamid, S.; Cornejo, D. Lethal Legacy: Bioweapons for Sale. *Washington Post*. April 20, 2003. <https://www.washingtonpost.com/archive/politics/2003/04/20/lethal-legacy-bioweapons-for-sale/4f1c6c0c-3733-4804-ba17-8f0051fda91d/> (accessed 2024-12-24).
- (100) Stephen Burgess, H. P. The Rollback of South Africa's Chemical and Biological Warfare Program.
- (101) *2005 Adherence to and Compliance With Arms Control, Nonproliferation, and Disarmament Agreements and Commitments*. U.S. Department of State. //2009-2017.state.gov/t/avc/rls/rpt/51977.htm (accessed 2024-12-26).
- (102) *Syria Biological Overview*. The Nuclear Threat Initiative. <http://www.nti.org/analysis/articles/syria-biological/> (accessed 2024-12-24).
- (103) *Timeline of Syrian Chemical Weapons Activity, 2012-2022 | Arms Control Association*. <https://www.armscontrol.org/factsheets/timeline-syrian-chemical-weapons-activity-2012-2022> (accessed 2024-12-26).
- (104) Bouckaert, P. Attacks on Ghouta. *Hum. Rights Watch* **2013**.
- (105) Syria Chemical "Attack": What We Know. *BBC News*. April 5, 2017. <https://www.bbc.com/news/world-middle-east-39500947> (accessed 2024-12-26).

- (106) Syria War: What We Know about Douma “Chemical Attack.” April 10, 2018. <https://www.bbc.com/news/world-middle-east-43697084> (accessed 2024-12-26).
- (107) Hall, N. Emerging Trends in Chemical Weapons Usage in the Middle East. **2024**.
- (108) Diab, M. Z. Syria’s Chemical and Biological Weapons: Assessing Capabilities and Motivations. *Nonproliferation Rev.* **1997**, 5 (1), 104–111. <https://doi.org/10.1080/10736709708436698>.
- (109) Joseph McDade; David Franz. Bioterrorism as a Public Health Threat. *Emerg. Infect. Dis.* **1998**, 4 (3), 493–494.
- (110) Thomas Torok; Robert Tauxe; Robert Wise; John Livengood; Robert Sokolow; Steven Mauvais; Kristin Birkness; Michael Skeels; John Horan; Laurence Foster. A Large Community Outbreak of Salmonellosis Caused by Intentional Contamination of Restaurant Salad Bars. *J. Am. Med. Assoc.* **1997**, 278 (5), 389–395.
- (111) *Guru followers no-show in election - UPI Archives*. UPI. <https://www.upi.com/Archives/1984/11/07/Guru-followers-no-show-in-election/2177468651600/> (accessed 2024-12-27).
- (112) Takahashi, H.; Keim, P.; Kaufmann, A. F.; Keys, C.; Smith, K. L.; Taniguchi, K.; Inouye, S.; Kurata, T. Bacillus Anthracis Bioterrorism Incident, Kameido, Tokyo, 1993. *Emerg. Infect. Dis.* **2004**, 10 (1), 117–120. <https://doi.org/10.3201/eid1001.030238>.
- (113) Keim, P.; Smith, K. L.; Keys, C.; Takahashi, H.; Kurata, T.; Kaufmann, A. Molecular Investigation of the Aum Shinrikyo Anthrax Release in Kameido, Japan. *J. Clin. Microbiol.* **2001**, 39 (12), 4566–4567. <https://doi.org/10.1128/JCM.39.12.4566-4567.2001>.
- (114) *Anthrax In America: A Chronology and Analysis of the Fall 2001 Attacks*; Center for Counterproliferation Research, 2002. <https://wmdcenter.ndu.edu/Portals/97/Documents/Publications/Articles/Anthrax-in-America.pdf> (accessed 2024-12-27).
- (115) *Amerithrax or Anthrax Investigation*. Federal Bureau of Investigation. <https://www.fbi.gov/history/famous-cases/amerithrax-or-anthrax-investigation> (accessed 2024-12-27).
- (116) *Closing the Anthrax Investigation*. FBI. <https://www.fbi.gov/news/stories/2008/august/amerithrax080608a> (accessed 2024-12-27).
- (117) Spertzel, R. Bruce Ivins Wasn’t the Anthrax Culprit. *Wall Street Journal*. August 5, 2008. <https://www.wsj.com/articles/SB121789293570011775> (accessed 2024-12-27).
- (118) *National Interagency Confederation for Biological Research (NICBR)*. <https://nicbr.health.mil/> (accessed 2024-12-26).
- (119) *Biological Defense Research*. Navy Medicine. <https://www.med.navy.mil/Naval-Medical-Research-Command/Research-Areas/Biological-Defense-Research/> (accessed 2024-12-26).
- (120) *USAMRIID: Mission & Vision*. https://usamriid.health.mil/index.cfm/about/mission_vision (accessed 2024-12-26).
- (121) *CDC. Mission and Org Charts*. About CDC. <https://www.cdc.gov/about/divisions-offices/index.html> (accessed 2024-12-26).
- (122) *National Biodefense Analysis and Countermeasures Center | Homeland Security*. <https://www.dhs.gov/science-and-technology/national-biodefense-analysis-and-countermeasures-center> (accessed 2024-12-26).
- (123) National Biodefense Analysis and Countermeasures Center Fact Sheet. <https://doi.org/10.1002/0471686786.ebd0174>.

- (124) Warrick, J.; Rein, L.; Kornfield, M.; Zakrzewski, C.; Balz, D.; Alemany, J.; McDaniel, J.; Sotomayor, M.; Craig, T.; Goldhill, D.; Bonesteel, M.; Bieler, D.; Faiola, A. The Secretive Fight Against Bioterror. *Washington Post*. July 30, 2006. <https://www.washingtonpost.com/archive/politics/2006/07/30/the-secretive-fight-against-bioterror-span-classbankheadthe-government-is-building-a-highly-classified-facility-to-research-biological-weapons-but-its-closed-door-approach-has-raised-concerns-span/17933fac-8e79-4692-b672-c3096411ab40/> (accessed 2024-12-26).
- (125) Jonathan Tucker. *Arms Control Association: Arms Control Today*. Arms Control Association. https://web.archive.org/web/20080517115818/http://www.armscontrol.org/act/2004_10/Tucker.asp (accessed 2024-12-26).
- (126) *DHS Exploring New Methods to Replace BioWatch and Could Benefit from Additional Guidance*; Government Accountability Office, 2021. <https://www.gao.gov/assets/720/714434.pdf> (accessed 2024-12-26).
- (127) BioWatch Facts Sheet. <https://www.dhs.gov/sites/default/files/publications/BioWatch%20Factsheet.pdf> (accessed 2024-12-26).
- (128) *Detecting Bioterrorism | Homeland Security*. <https://www.dhs.gov/archive/detecting-bioterrorism> (accessed 2024-12-26).
- (129) National Academies of Sciences, E.; Studies, D. on E. and L.; Sciences, B. on L. Invited Paper: BioWatch Program Overview: A Local Public Health Perspective for Enhancing Bioterrorism Preparedness and Response. In *Strategies for Effective Improvements to the BioWatch System: Proceedings of a Workshop*; National Academies Press (US), 2018.
- (130) Kathryn Mitchell; Gary Rasicot. *2021 DHS Biosurveillance Systems*; Department of Homeland Security, 2021.
- (131) Dimitri Kusnezov; Gary Rasicot. *2022 DHS Biosurveillance Systems*; Department of Homeland Security, 2023. <https://www.dhs.gov/sites/default/files/2023-03/S%26T%20and%20CWMD%20-%20DHS%20Biosurveillance%20Systems.pdf>.
- (132) CDC. *About The Laboratory Response Network*. The Laboratory Response Network Partners in Preparedness. <https://www.cdc.gov/laboratory-response-network/php/about/index.html> (accessed 2024-12-26).
- (133) *Association of Public Health Laboratories - Laboratory Response Network*. APHL. <http://www.aphl.org> (accessed 2024-12-26).
- (134) Rotz, L. D.; Hughes, J. M. Advances in Detecting and Responding to Threats from Bioterrorism and Emerging Infectious Disease. *Nat. Med.* **2004**, 10 (12), S130–S136. <https://doi.org/10.1038/nm1152>.
- (135) CDC. *Laboratory Response Network in the U.S.* The Laboratory Response Network Partners in Preparedness. <https://www.cdc.gov/laboratory-response-network/php/our-work/index.html> (accessed 2025-02-09).
- (136) Lizotte, S. *How Does USAMRIID Shut Down Impact Nation's Bioterrorism Laboratory Response Network?* Global Biodefense. <https://globalbiodefense.com/2019/08/13/how-does-usamriid-shut-down-impact-nations-bioterrorism-laboratory-response-network/> (accessed 2024-12-26).
- (137) Soice, E. H.; Rocha, R.; Cordova, K.; Specter, M.; Esvelt, K. M. Can Large Language Models Democratize Access to Dual-Use Biotechnology? *arXiv* June 6, 2023. <https://doi.org/10.48550/arXiv.2306.03809>.
- (138) Onishi, N. After 8-Year Trial in Japan, Cultist Is Sentenced to Death. *The New York Times*. February 28, 2004. <https://www.nytimes.com/2004/02/28/world/after-8-year-trial-in-japan-cultist-is-sentenced-to-death.html> (accessed 2025-03-12).
- (139) *ChatGPT*. <https://chatgpt.com> (accessed 2025-03-12).

- (140) Drexel, B.; Withers, C. *AI and the Evolution of Biological National Security Risks*. CNAS. <https://www.cnas.org/publications/reports/ai-and-the-evolution-of-biological-national-security-risks> (accessed 2025-03-12).
- (141) Bates, S. *Base Pair*. National Human Genome Research Institute. <https://www.genome.gov/genetics-glossary/Base-Pair> (accessed 2025-03-13).
- (142) Health (US), N. I. of; Study, B. S. C. Understanding Human Genetic Variation. In *NIH Curriculum Supplement Series [Internet]*; National Institutes of Health (US), 2007.
- (143) *Melanoma Skin Cancer Statistics*. <https://www.cancer.org/cancer/types/melanoma-skin-cancer/about/key-statistics.html> (accessed 2025-03-13).
- (144) *Responsible AI x Biodesign*. Responsible AI x Biodesign. <https://responsiblebiodesign.ai/> (accessed 2025-03-14).
- (145) *What is a gene?: MedlinePlus Genetics*. <https://medlineplus.gov/genetics/understanding/basics/gene/> (accessed 2025-03-15).
- (146) Urbina, F.; Lentzos, F.; Invernizzi, C.; Ekins, S. Dual Use of Artificial Intelligence-Powered Drug Discovery. *Nat. Mach. Intell.* **2022**, 4 (3), 189–191. <https://doi.org/10.1038/s42256-022-00465-9>.
- (147) Tucker, J. B.; Mahan, E. R. *President Nixon's Decision to Renounce the U.S. Offensive Biological Weapons Program*.
- (148) Leitenberg, M. China's False Allegations of the Use of Biological Weapons by the United States during the Korean War, 2016. <https://www.wilsoncenter.org/publication/chinas-false-allegations-the-use-biological-weapons-the-united-states-during-the-korean> (accessed 2025-01-02).
- (149) *Personalized Medicine*. <https://www.genome.gov/genetics-glossary/Personalized-Medicine> (accessed 2025-02-12).
- (150) *Weaponizing Biotech: How China's Military Is Preparing for a "New Domain of Warfare."* Defense One. <https://www.defenseone.com/ideas/2019/08/chinas-military-pursuing-biotech/159167/> (accessed 2025-01-21).
- (151) *What is CRISPR? A bioengineer explains*. <https://news.stanford.edu/stories/2024/06/stanford-explainer-crispr-gene-editing-and-beyond> (accessed 2025-01-03).
- (152) Jason Gammack. *Breaking the Boundaries of Synthetic DNA | Ansa Biotechnologies*. Ansa Biotechnologies. <https://ansabio.com/blog/breaking-the-boundaries-of-synthetic-dna/> (accessed 2025-02-12).
- (153) *Benchtop DNA Synthesis Devices: Capabilities, Biosecurity Implications, and Governance*. The Nuclear Threat Initiative. <https://www.nti.org/analysis/articles/benchtop-dna-synthesis-devices-capabilities-biosecurity-implications-and-governance/> (accessed 2025-02-12).
- (154) Jaime Yassif; Sarah Carter; Nicole Wheeler. *Preventing the Misuse of DNA Synthesis Technology*. The Nuclear Threat Initiative. <https://www.nti.org/about/programs-projects/project/preventing-the-misuse-of-dna-synthesis-technology/> (accessed 2025-01-22).
- (155) Bill Lubinger. *First atomic-level imaging of lethal prions provides sharpened focus for potential treatments*. The Daily. <https://thedaily.case.edu/first-atomic-level-imaging-of-lethal-prions-provide-sharpened-focus-for-potential-treatments/> (accessed 2025-02-09).
- (156) *Prion Diseases*. Johns Hopkins Medicine. <https://www.hopkinsmedicine.org/health/conditions-and-diseases/prion-diseases> (accessed 2025-01-22).
- (157) Qin, K.; O'Donnell, M.; Zhao, R. Y. Doppel: More Rival than Double to Prion. *Neuroscience* **2006**, 141 (1), 1–8. <https://doi.org/10.1016/j.neuroscience.2006.04.057>.

- (158) CDC. *About Prion Diseases*. Prion Diseases. <https://www.cdc.gov/prions/about/index.html> (accessed 2025-01-22).
- (159) Frazer, J. *Prions Are Forever*. Scientific American. <https://www.scientificamerican.com/blog/artful-amoeba/prions-are-forever/> (accessed 2025-02-12).
- (160) BBC News | Europe | Dissidents say Stasi gave them cancer. <http://news.bbc.co.uk/2/hi/europe/352461.stm> (accessed 2025-01-22).
- (161) Legname, G.; Baskakov, I. V.; Nguyen, H.-O. B.; Riesner, D.; Cohen, F. E.; DeArmond, S. J.; Prusiner, S. B. Synthetic Mammalian Prions. *Science* **2004**, 305 (5684), 673–676. <https://doi.org/10.1126/science.1100195>.
- (162) Stitz, L.; Aguzzi, A. Aerosols. *Prion* **2011**, 5 (3), 138–141. <https://doi.org/10.4161/pri.5.3.16851>.
- (163) *Budget & Planning | NIAID: National Institute of Allergy and Infectious Diseases*. <https://www.niaid.nih.gov/about/budget-planning> (accessed 2025-02-12).
- (164) Biesecker, C. *DHS Puts BioWatch Replacement Program on Hold*. Defense Daily. <https://www.defensedaily.com/dhs-puts-biowatch-replacement-program-on-hold/homeland-security/> (accessed 2025-02-20).
- (165) Volk, K. M.; Gering, T. J. Predicting Biosecurity Threats: Deployment and Detection of Biological Weapons. In *Emerging Threats of Synthetic Biology and Biotechnology*; Trump, B. D., Florin, M.-V., Perkins, E., Linkov, I., Eds.; NATO Science for Peace and Security Series C: Environmental Security; Springer Netherlands: Dordrecht, 2021; pp 195–207. https://doi.org/10.1007/978-94-024-2086-9_13.
- (166) *Table: CDC High-Priority Biological Agents and Toxins**. Merck Manual Consumer Version. <https://www.merckmanuals.com/home/multimedia/table/cdc-high-priority-biological-agents-and-toxins> (accessed 2025-02-23).
- (167) CDC Bioterrorism Agents. https://biosecurity.fas.org/resource/documents/CDC_Bioterrorism_Agents.pdf (accessed 2025-02-23).
- (168) *NIAID Biodefense Pathogens | NIAID: National Institute of Allergy and Infectious Diseases*. <https://www.niaid.nih.gov/research/niaid-biodefense-pathogens> (accessed 2025-02-23).