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Insights in biosafety & biosecurity novel developments, current challenges, and future perspectives 2022/2023

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Insights in biosafety & biosecurity 2022/2023: Novel developments, current challenges, and future perspectives

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Editorial: Insights in biosafety and biosecurity 2022/2023: novel developments, current challenges, and future perspectives

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Editorial on the Research Topic
Insights in biosafety and biosecurity 2022/2023: novel developments, current challenges, and future perspectives

Biological research is an essential element for scientific advancements that underpin improvements in the quality and health of humans, agricultural animals and crops, domestic animals, and the environment. While biological research provides enormous benefits to society, there is a concomitant need for researchers to enhance their biosafety and biosecurity knowledge and practices as they continue to work with pathogens and toxins. Periodic assessment and reassessment of our biosafety and biosecurity framework and practices helps to ensure that they effectively address existing and emerging safety and security concerns while continuing to support scientific progress and innovation.

To address this need, in 2021, Frontiers developed a Research Topic entitled "*Insights in Biosafety and Biosecurity 2021: Novel Developments, Current Challenges, and Future Perspectives,*" which was co-edited by Pillai and Raybould (2023). Unfortunately, Dr. Raybould passed away on 5 October 2022 (Dritsas et al., 2023) and Dr. Morse was asked to co-edit the second volume of this Research Topic. The co-editors wish to acknowledge Dr. Raybould's leadership and contributions to this important Research Topic.

For the second volume of this Research Topic, there were 16 submissions of which 9 were accepted and published (Policy and Practice Reviews, N = 4; Perspectives, N = 2; Original Research, N = 2; Hypothesis and Theory, N = 1). The authors of the published submissions were from multiple countries: Germany, United States, Poland, Netherlands, China, Singapore, Thailand, United Kingdom, Georgia, and Canada.

Engineering controls are one of the key measures to ensure the safety and health of the laboratorians. Kurth et al. discussed a previously unrecognized contradiction in the design of BSL-4 laboratories. For decades, it was suggested that both directional airflow and pressure differentials were essential safety measures to prevent the release of pathogens into the environment and to avoid cross-contamination between laboratory rooms. Despite the lack of an evidence-based risk analysis demonstrating increased

safety by directional airflow and pressure differentials in BSL-4 laboratories, they were codified in various national regulations. The authors provided a detailed risk assessment by calculating pathogen mitigation in maximum contamination scenarios. Their results indicated that both directional airflow or a differential pressure gradient in airtight rooms within a secondary BSL-4 containment did not increase biosafety and were not necessary. Instead, they suggested that a reduction of pressure zones from the outside into secondary containment may provide sufficient environmental protection.

High-containment laboratories (HCLs) conduct critical research on high-consequence pathogens and provide diagnostic services for the diseases they cause. Modernization of HCLs has led to an increasingly cyber-connected laboratory infrastructure. Crawford et al. discussed the cybersecurity concerns specific to these HCLs to raise awareness among laboratory decision-makers and offer potential risk mitigation strategies.

Rutjes et al. observed that during the Covid-19 pandemic, the surge in demand for diagnostic tests had a substantial impact on biosafety and biosecurity. To prepare for the next pandemic, particularly in low- and middle-income countries, the authors have provided lessons learned, tools, and recommendations to improve biosafety and biosecurity practices to protect the front-line workers.

Ou and Guo provide an overview of current research on the application of synthetic biology in biomedicine and analyze the safety risks associated with this field. Based on their analysis, they propose fundamental principles for addressing these issues and offer practical recommendations for ethical governance, promoting the development and implementation of relevant policies, improving legal safeguards, and enhancing biocontainment.

Holub and Agena discussed biofoundries, which are highly automated facilities for processing biological specimens, and that have a major role in accelerating innovation and product development by bringing public and private stakeholders together to share resources and develop collaborations on national and international levels. The authors present an argument for expanding the scope for biofoundries to include roles in biosurveillance and biosecurity.

Sabra et al. analyzed the potential bioterrorism threat from *Bacillus anthracis* resulting from advances in synthetic biology, genome editing, information availability, and other emerging and enabling technologies. They concluded that rapid advances and availability of technologies has led to an ever-growing number and types of actors who could potentially weaponize *B. anthracis*.

Zimny proposed a reform of the European Union (EU) regulatory system for New Genomic Techniques (NGT) products to avoid placing EU researchers and investors at a disadvantage when compared to countries such as Argentina, Brazil, Canada, United Kingdom, and the United States.

Many countries have established and implemented regulations and policies for the accountability and control of high consequence pathogens and toxins that can have a significant impact on the economy as well as the health of agricultural animals and plants. In two contributions, (Pillai et al.; Pillai et al.). described a process using Multi-criteria Decision Analysis and Decision Support Framework logic tree approaches for evaluating: i) agricultural animal pathogens, and ii) plant pathogens to either support their inclusion on or exclusion from the list of agents for oversight and control. In contrast to lists of human pathogens where the impact on public health and safety were the primary factors for inclusion, non-biological criteria, i.e., economic consequences and impact on international trade agreements, were of paramount importance in these studies.

The need for enhanced biosafety and biosecurity practices continue to grow as we continue to discover new pathogens with high transmissibility, which can cause major outbreaks or the next epidemic or pandemic. This necessitates that scientists around the world conduct appropriate risk assessments and implement risk mitigation procedures to ensure the safety and health of the laboratorians, their family members, and the surrounding community. As we continue to enhance our biosafety and biosecurity practices, global support, collaboration, contribution, engagement and sharing of best practices are vital for success.

We would like to thank the authors for their contributions to this Research Topic on the importance of biosafety and biosecurity to ensure the safe, responsible, and secure conduct of biological science and research.

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Maintaining differential pressure gradients does not increase safety inside modern BSL-4 laboratories

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This article discusses a previously unrecognized contradiction in the design of biosafety level-4 (BSL-4) suit laboratories, also known as maximum or high containment laboratories. For decades, it is suggested that both directional airflow and pressure differentials are essential safety measures to prevent the release of pathogens into the environment and to avoid cross-contamination between laboratory rooms. Despite the absence of an existing evidence-based risk analyses demonstrating increased safety by directional airflow and pressure differentials in BSL-4 laboratories, they were anchored in various national regulations. Currently, the construction and operation of BSL-4 laboratories are subject to rigorous quality and technical requirements including airtight containment. Over time, BSL-4 laboratories evolved to enormously complex technical infrastructures. With the aim to counterbalance this development towards technical simplification while still maintaining maximum safety, we provide a detailed risk analysis by calculating pathogen mitigation in maximum contamination scenarios. The results presented and discussed herein, indicate that both directional airflow or a differential pressure gradient in airtight rooms within a secondary BSL-4 containment do not increase biosafety, and are not necessary. Likewise, reduction of pressure zones from the outside into the secondary containment may also provide sufficient environmental protection. We encourage laboratory design professionals to consider technical simplification and policymakers to adapt corresponding legislation and regulations surrounding directional airflow and pressure differentials for technically airtight BSL-4 laboratories.

KEYWORDS

BSL-4 laboratory, differential pressure, directional airflow, biosafety, maximum containment laboratory, risk analysis (assessment)

Introduction

Handling and working with human pathogens, depending on their classification of risk groups 1–4, takes place in laboratories of respective biosafety levels 1–4 (BSL-1 to BSL-4). To reliably prevent cross-contamination of samples and exposure of employees and the environment, numerous safety measures are used in laboratories of the highest biosafety level 4 (BSL-4), also described as maximum containment laboratory (MCL), which have been developed and established over the past decades in step with the state-of-the-art in science and technology.

In general, two different types of BSL-4 laboratories have been developed: cabinet laboratories and protective-suit laboratories. In Germany, laboratories were built exclusively for use with protective suits. For this purpose, in the second half of the 20th century, the parallel technical development of positive-pressure suits to protect laboratory workers and biosafety cabinets (BSC) to prevent sample cross-contamination were used. To prevent the release of pathogens into the environment, both a directional airflow by constant (negative) pressure differentials was set up via room ventilation systems between the outside and inside areas of the laboratory (Figure 1A), as well as effective filtration of potentially contaminated exhaust air from the laboratory into the outside environment. The entirety of these measures has been implemented as a safety standard worldwide and included in the relevant recommendations and regulations (LBG, 1996; National Research Council Committee on Hazardous Biological Substances in the Laboratory, 1989; Biosafety in microbiological and biomedical laboratories, 1999). The aim of the directional airflow and the pressure differentials was to prevent the escape of potentially contaminated air from the laboratory through any structural leaks of the containment barrier between the laboratory and the outside (e.g., through doors, walls, floors, roofs, pipelines). In the course of the following decades, technical advancements allowing for tighter structures and thus also of MCLs have been employed, which enable the generation and monitoring of controlled and constant air flow and differential pressure gradients between adjacent rooms. Of note, these advancements also resulted in an increased technical complexity, and dramatically increased construction, operation and maintenance expenses.

Contemporarily, the planning, construction and operation of BSL-4 laboratories are subject to very rigorous quality and technical requirements. To prevent the release of human pathogens to the external environment, high-efficiency particulate air (HEPA) filters are used to filter exhaust air from BSL-3 and BSL-4 laboratories, and the laboratories are operated at a constant negative pressure. The requirements for air tightness of the laboratories also increase with increasing biosafety levels. In addition to all other technical requirements and safety measures, a defined, technically airtight and appropriately monitored containment is an absolute necessity for BSL-4 laboratories consistently worldwide (Figure 1B) (Canadian Biosafety Standard, 2015; Biosafety in microbiological and biomedical laboratories, 2020). The requirements of the air tightness criteria (e.g., the generally accepted "Canadian Biosafety Standards and Guidelines") are extremely high and therefore the air leakage volume is correspondingly small, while the air exchange rates within the rooms are maintained as high as possible. The advantage of an airtight and appropriately tested containment is, among other things, the increased protection of the environment, also in the event of a failure of the ventilation system or the occurrence of possible positive-pressure situations within the containment caused by technical faults. In Germany, this development led to the present legislation and regulations (BioStoffV 2013; Technische Regeln für Biologische Arbeitsstoffe, 2013; GenTSV, 2019), stating that access to the BSL-4 laboratory must traverse four airlocks (outer change room, personal hygienic shower, suit room, decontamination shower) with a differential pressure gradient. Furthermore, the established principle of directional airflow and pressure differentials is also requested within the laboratory depending on the contamination risk and was established from areas with potentially lower contamination risk to areas with highest contamination, e.g., from the main laboratory to animal rooms to necropsy rooms (Figure 1B). Similar requirements have been established worldwide (for text excerpts see Supplementary Table S1). However, an experimentally or computationally determined basis for evaluating the risk of potential exposure to biomaterials under normal operation or accident situations in a BSL-4 laboratory, animal holding or necropsy rooms are not considered in any of the regulations cited. Such a risk analysis of the alleged increased safety by directional airflow and the pressure differentials has not yet been published since the beginning of the operation of BSL-4 laboratories.

The technical implementation of differential pressure gradients between technically airtight rooms for BSL-4 laboratories, required by current regulations, is achieved by a specifically adjusted and controlled ratio of supply and exhaust air for each individual room. The air exchange rate per room of 12–15 times per hour ensures the dilution and removal of air contaminated by infectious microorganisms via the exhaust air through downstream HEPA filtration. For additional protection of staff within the laboratory, it is required that infectious material be processed only under a BSC (or comparable equipment), regarded as primary containment, while staff wear a ventilated positive-pressure protective suit. Following the successful technical implementation of defined, airtight containments for BSL-4 laboratories, the benefit or necessity of directional airflow and pressure differentials has not been evaluated to date. Due to the lack of experimental data, international and national microbiological guidelines do not suggest levels of negative pressures and the levels of pressure

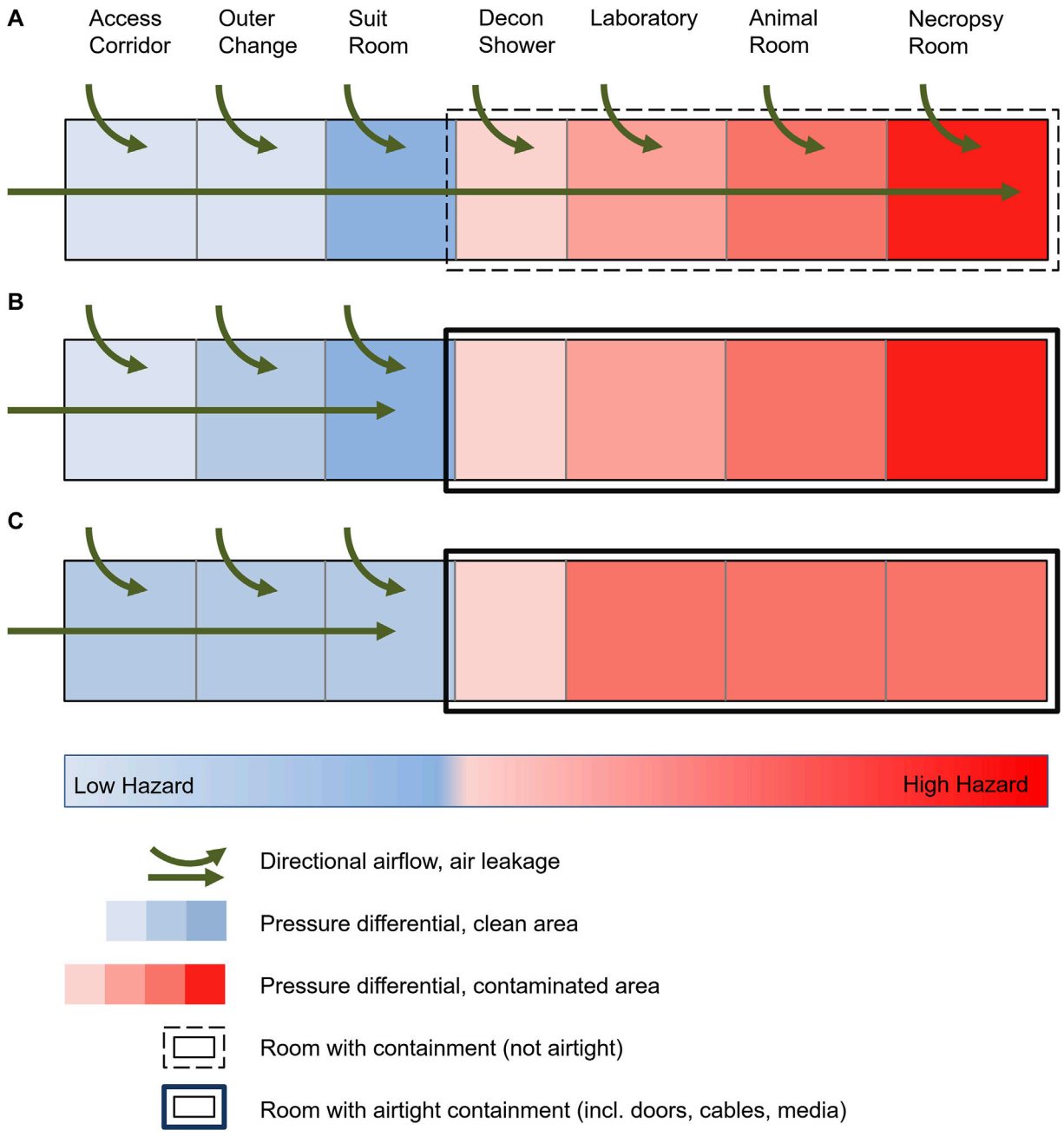

FIGURE 1
Schematic of a high-security laboratory with targeted air flow and pressure differentials. **(A)** Historical laboratories with common leakage due to standard construction practices, targeted air flow and pressure levels up to the area of greatest contamination. **(B)** Modern laboratories with technically airtight containment and maintenance of pressure levels up to the area of greatest contamination. **(C)** Risk assessment-based reduction in the number of pressure levels in a BSL-4 laboratory with airtight containment despite unchanged protection against cross-contamination and protection of the environment.

differentials required to effectively prevent cross-contamination with risk group 4 pathogens. Also, there are no data for the potential safety impact of changing the level of pressure differentials on potential cross-contamination. Only a 2005 study by (Bennett et al.,2005) addresses the relationship between negative pressure and protection from cross-contamination in BSL-3 laboratories in an evidence-based manner and concludes that pressure differentials has no effect on protection from cross-contamination. Only directional airflow into a laboratory (inflow velocity) had a positive effect

and is still used today to protect against cross-contamination in non-technically airtight rooms (e.g., BSL-3 laboratories).

The currently established and practiced differential pressure gradients are operated within the technical limits of the available measurement, control and regulation technology and have no empirical or biological basis. Worldwide, pressure differences of 30–60 Pa between adjacent rooms are used in BSL-4 laboratories, depending on technical possibilities. Both the actuating forces of doors in existing negative pressure cascades must be controllable and the air pressure controls for adjacent rooms (Δp) must have a sufficient limit distance from each other to avoid pressure disturbances. If the aforementioned "Canadian Biosafety Standards and Guidelines" for the tightness of the containment are complied with, the remaining air leakage is no longer relevant in this respect and is to be disregarded. Consequently, the question arises as to whether a reduction in the target directional airflow and the pressure differentials would result in an increased risk of contamination, which in turn raises the question of the extent to which a target directional airflow and the differential pressure gradients between technically airtight rooms fundamentally contributes to a reduction in the risk of contamination.

Considering the technical development of BSL-4 laboratories, we discuss in this article whether a directional airflow and/or differential pressure gradients are still necessary to minimize a contamination risk. To do this, we calculate the likelihood of contamination within a room and its spread to adjacent rooms, considering leakage volumes in airtight rooms, pressure equalization when a sealed door is opened, and the "displacement" of air when a person passes through a door.

Basis and calculations

The BSL-4 laboratory at the Robert Koch Institute, Berlin, Germany, was used as the basis for the following calculations. The laboratory was built according to the air tightness criteria of the Canadian Guideline (Canadian Biosafety Standard, 2015) and has been in regular operation since 2018 after construction completion in 2015.

Access to the laboratory rooms is via four airlocks with differential pressure gradients (−20 Pa, −40 Pa, −80 Pa [suit room], −120 Pa [decontamination shower]). In this process, the negative pressures in the respective airlocks increase towards the laboratory rooms and are thus intended to protect the environment by targeted directional airflow from the outside to the inside. Within the laboratory, further differential pressure gradients are applied to the areas with the highest probable risk of contamination (−160 Pa [cell culture], −200 Pa [animal room], −240 Pa [necropsy room]). The determination of the differential pressure gradient values followed the national and international regulations for BSL-4 laboratories and were planned for in 2008 (laboratory planning) with no separate, specially prepared risk analysis. The pressure differentials were designed to allow the actuating forces of the doors to be manageable in existing differential pressure gradients and also to allow Δp controls for the rooms to have a sufficient limit distance from each other to avoid pressure disturbances.

To our knowledge, no data have been published about the quantity of generated infectious bioaerosols during normal BSL-4 laboratory operation or accident situations in a cell culture laboratory, animal room, or necropsy room. Furthermore, it is comprehensible that bioaerosol generation during animal husbandry depends on the animal model or infection model and the caging systems used. It is also comprehensible, that working with infectious viruses under a BSC, handling animals in individually ventilated cages (IVC) and changing stations, or performing a necropsy on a downdraft table, considering their protection factor, would generate less bioaerosols than an accidental release of virus in a room, e.g., while dropping and breakage of a sample flask or vial. Therefore, for the risk assessment presented herein, we evaluate a worst-case practical scenario of contamination in a BSL-4 laboratory, using experimental data with spores from (Bennett and Parks, 2006), as well as a constant hypothetical generation of bioaerosols during an animal experiment in conventional cages. Standard and accepted fluid mechanics and thermodynamics formulas were used for all calculations.

The study by (Bennett and Parks, 2006) describes a single release of biomaterials in a defined room during various laboratory accidents. The dropping of a sample vessel (50 ml) with a spore suspension of 2×10^9 spores/ml (total of 1×10^{11} spores) in an 18 m^3 room was investigated as the scenario of the highest potential for contamination, and an aerosol release of 1.03×10^3 spores/m^3 (in relation to the room dimension, a total of 1.9×10^4 spores) was measured. To simulate a comparable laboratory accident in the BSL-4 laboratory, the release of a maximum possible virus concentration in the laboratory was considered. The scenario assumed here is the dropping of a sample vessel (50 ml) with a virus concentration of 2×10^8 viruses/ml (total 1×10^{10} viruses) in the laboratory. This amount corresponds to the maximum of viruses per volume processed in the BSL-4 laboratory at the Robert Koch Institute. According to the ratio of the release measured by (Bennett et al.,2005) a total of approximately 2×10^3 viruses would be released as aerosols in a room. The remainder of the virus-containing suspension would remain surface bound and would be removed immediately after dropping by decontamination of the affected surfaces. All calculations made here are performed with the assumption of a maximum bioaerosol release of 2×10^3 viruses. Since a fully equipped laboratory filled with furniture will most likely not provide a situation for an optimal release and distribution of bioaerosol as performed by (Bennett and Parks, 2006), we believe the assumed maximum release of 2×10^3

viruses is rather exaggerated, but already considers an added margin of potential error.

For evaluation of a continuous contamination by infected animals, an extensive literature search was conducted. Despite robust evidence supporting the airborne transmission, and hence bioaerosol release, of many respiratory viruses, including measles virus, influenza virus, respiratory syncytial virus, human rhinovirus, adenovirus, enterovirus, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2, and Zaire Ebola virus (Weingartl et al., 2012; Wang et al., 2021), very limited data are published about the quantitative release of airborne pathogens. Of those, the majority of air samples are analyzed for the presence of viral genome copy numbers, which do not indicate the quantity of infectious virus. Some extrapolations from genome copy numbers to infectious particles have been presented in various aerosol study, ranging from 10:1 to as much as 10^5:1 (Hawks et al., 2021; Tellier, 2022), indicating the unreliability of such extrapolations. Direct infectious virus quantification from air samples was performed in a study of SARS-CoV-2 (concentration between 6 and 74 $TCID_{50}$ per liter air) in a hospital room with two COVID-19 patients (Lednicky et al., 2020), in an experimental infection study of Syrian hamsters with SARS-CoV-2 with an average emission rate per animal of 25 infectious virions/hour on days 1 and 2 post inoculation (Hawks et al., 2021), and experimental infection studies of ferrets with influenza virus H1N1 with average emission rates per animal of <4 and 11 PFU/min (Gustin et al., 2013) and 7 to 138 PFU/min (Singanayagam et al., 2020). For the risk assessment presented herein, the hypothetical virus-containing bioaerosol release is calculated for the maximum number of the largest animal used in commercially available conventional cages (without primary containment) with a polyester filter sheet (TECNIPLAST 2000P) at the BSL-4 laboratory at the Robert Koch Institute: 48 infectious adult guinea pigs (e.g., as a possible animal model for human disease) with an average emission rate of 100 viruses/minute. The calculations estimating possible aerosol and virus release are given in the text below.

Very low leakage volume in airtight rooms

To protect the environment, modern BSL-4 laboratories are built with airtight rooms (walls, doors and penetrations) that allow the lowest possible leakage. To calculate the leakage rate of a sample room with 60 m^3, the tightness requirement is based on the pressure drop method according to the recognized Canadian guideline (at 500 Pa negative pressure, this may drop max. to −250 Pa within 20 min).

Definitions:

LW Air exchange rate.

$\dot{V}_{Zu}$ Supply airflow.

$\dot{V}_{Ab}$ Exhaust airflow.

V_{Ri} Room volume.

P_A Low pressure at the start.

P_E Low pressure at the end.

V_A Initial volume at low pressure at the start.

V_E Final volume at low pressure

dV Leakage volume.

The sample room of V_{Ri} = 60 m^3 has an airflow of 900 m^3/h at LW = 15 1/h.

$$\dot{V}_{Zu} \Big/ \dot{V}_{Ab} = 900 \frac{m^3}{h}$$

According to Canadian guideline, at 500 Pa negative pressure and a maximum drop to −250 Pa within 20 min corresponds:

P_A = regular air pressure 100,000 −500 Pa = 99,500 Pa.

P_E = regular air pressure 100,000 −250 Pa = 99,750 Pa.

The allowable leakage rate at constant temperature and atmospheric pressure is given by the equations:

$$\frac{V_A}{V_E} = \frac{P_{AE}}{P_A}$$

As well as

$$V_E = V_A - dV \text{ (Formula by Boyle Mariotte)}$$

After conversion and merging, the following formula is obtained for the leakage volume:

$$V_E = \frac{P_A \cdot V_A}{P_E}$$

$$\frac{P_A \cdot V_A}{P_E} = V_A - dV$$

$$dV = V_A - \frac{P_A \cdot V_A}{P_E}$$

$$dV = -\frac{P_A \cdot V_A}{P_E} + V_A$$

$$dV = -60 \cdot \frac{99,500}{99,750} + 60 = 0.15\ m^3$$

This results in a leakage airflow/h with closed doors of:

$$\frac{60\ min/h}{20\ min} \cdot 0.15\ m^3 = 0.45\ m^3 \Big/ h$$

For a sample room of 60 m^3, the allowable leakage rate is 0.45 m^3/h (0.75%/h). Following the Canadian guideline, the sample room would have a very low leakage volume and a leakage rate under operation of less than 0.45 m^3/h. Therefore, within a BSL-4 laboratory with airtight doors, no directional airflow is applicable. The airflow controllers used for the individual rooms have a deviation of ± 5 % (45 m^3/h at an airflow of 900 m^3/h) and thus a deviation too large to accurately evaluate the room tightness. Therefore, a corresponding pressure test is carried out annually.

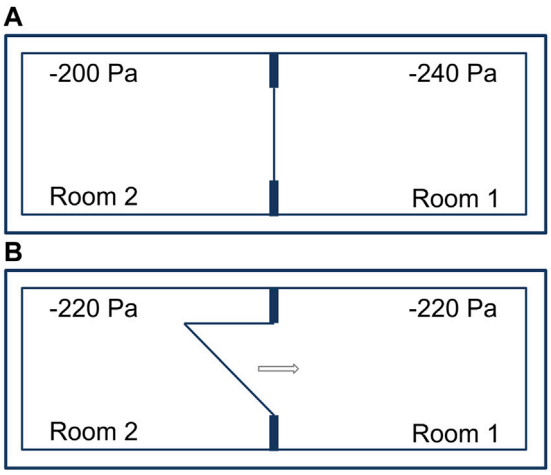

FIGURE 2
Adjacent laboratory rooms within a technically airtight BSL-4 laboratory. **(A)** Separated by an airtight door and operated with a pressure differential of 40 Pa. **(B)** Immediate pressure equalization when opening an airtight door.

Immediate pressure equalization when opening an airtight door

Adjacent laboratory rooms separated by an airtight door and operated with a pressure differential of 40 Pa (Figure 2A). Opening an airtight door inside a BSL-4 laboratory results in pressure control circuits being activated. The control circuits of the two neighboring rooms with pressure differentials oscillate and lead to irrational, uncontrollable pressure fluctuations. To avoid this, when a door is opened, both control loops of the rooms are "frozen" for the time the door is open, i.e., the controllers remain in the control position that existed before the door was opened and do not resume operation until the door is sealed. The amount of supply and exhaust air thus remains the same in both rooms during door actuation. After opening a door and interrupting the control function, there is inevitably a rapid pressure equalization between the two rooms, occurring in less than a second (Figure 2B). This involves extremely low air volumes of 0.3% or 0.4 %, depending on the rooms, relative to the total volume of the two rooms. Therefore, during the time of door opening, no directional airflow is applicable. For a pressure difference of 40 Pa between two rooms (−200 Pa and −240 Pa), the volume for pressure equalization is calculated as follows:

P_A = regular air pressure 100,000 −240 Pa = 99,760 Pa.

P_E = regular air pressure 100,000 −200 Pa = 99,800 Pa

$$\mathbf{dV} = \mathbf{60} - \frac{\mathbf{99,760}}{\mathbf{99,800}} \cdot \mathbf{60} = \mathbf{0.024}\ \boldsymbol{m^3}$$

When a door from a 60 m³ room is opened, 0.024 m³ of air flows from laboratory room 2 (−200 Pa) to laboratory room 1 (−240 Pa). Pressure equalization takes place immediately and even before the door is open wide enough for a person to pass through.

Person "dragging" air when passing through a door

When passing a doorway from laboratory room 1 to laboratory room 2, a person is dragging approximately 0.76 m³ of air (Figures 3A–C). The air volume was calculated by a computational fluid dynamics (CFD) simulation (Figure 3B). For this purpose, a model was chosen in which a person with a height of 1.80 m is moved through a door (door size 2.1 m × 1.1 m) at a speed of 1 m/s (3.6 km/h). After 15 s the door is closed.

The dragged air volume caused by the person is 32 times greater than the air movement caused by pressure equalization (calculated above) and can also occur in a direction opposite to the airflow caused by the pressure equalization in response to a door opening. Likewise, the movement of the person through the door will cause a small increase in pressure in laboratory room 2, resulting in the backflow of air into laboratory room 1 until the pressure is equalized again.

Virus distribution after contamination within a room

Two different scenarios were considered for the risk assessment in the event of the release of a maximum possible virus concentration in the BSL-4 laboratory. The virus distribution is calculated in scenario A for the case of a laboratory accident and in scenario B for the case of animal husbandry in conventional cages without primary containment. The influence of homogeneous distribution in the room as well as air exchange is considered.

In scenario A, the release of bioaerosols containing a total of 2×10^3 viruses in a room of 60 m³ is assumed after the breakage of a sample vial on the floor (50 ml virus suspension). If a sample of 50 ml breaks, only a portion of the virus is resultantly aerosolized. The largest portion wets the floor or other surfaces. Droplets sink back to the ground after breakage, a portion sticks to surfaces, another portion floats in the air (actual aerosols). After 10 min, an approximately homogeneous distribution of the suspended aerosols in the room can be assumed. The air exchange rate is 15 times/h. To dispose of the broken sample vessel and decontaminate the site following standard operating BSL-4 procedures, the person remains in the room for at least 15 min without opening any door. Within 15 min, 225 m³ of air is exchanged. To calculate the remaining number of virus particles in bioaerosols in the room, the formula for recovery time equation (Raatz and Luftwechsel, 2006) was used:

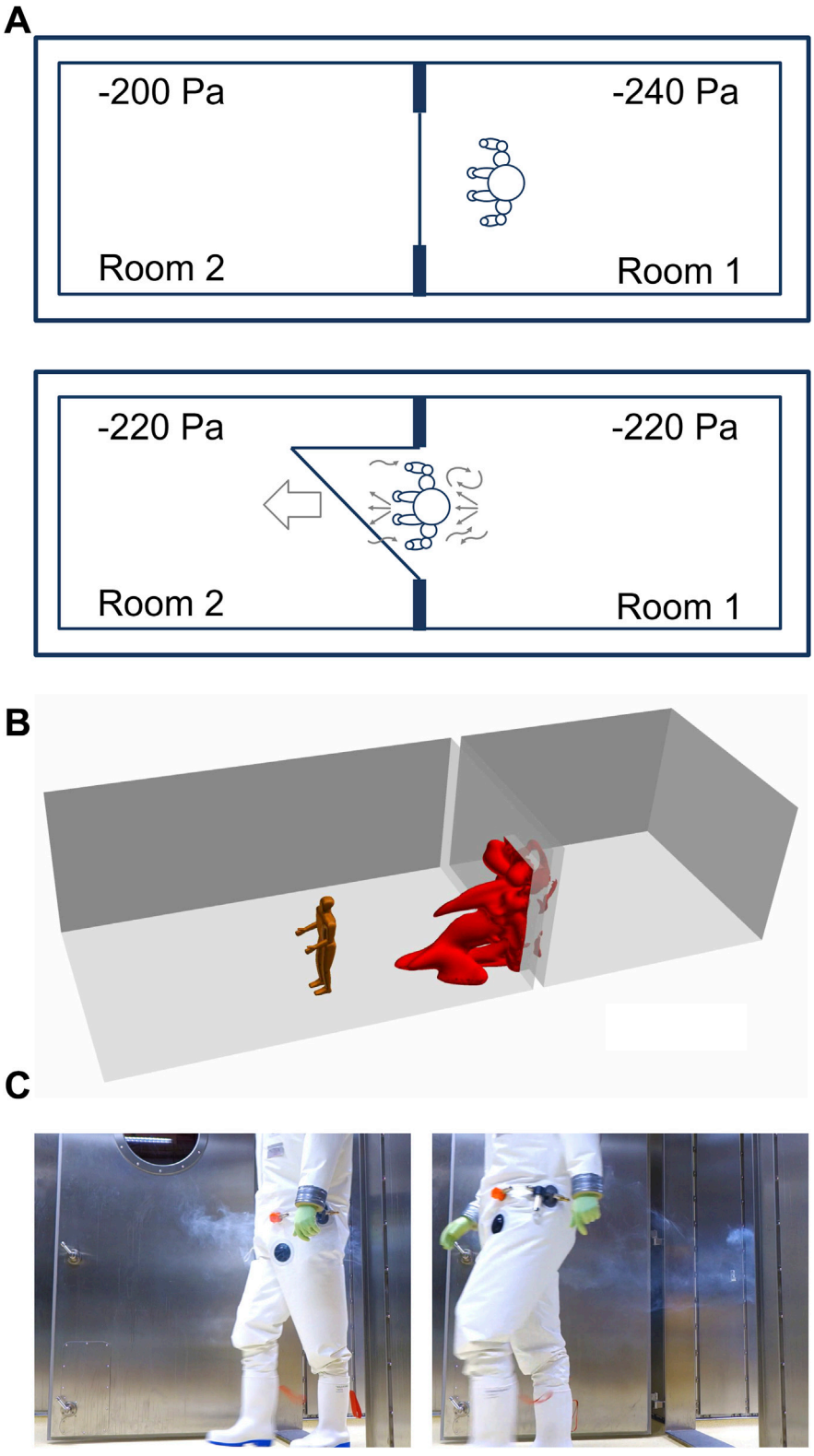

FIGURE 3
A Person is walking between adjacent laboratory rooms within a technically airtight BSL-4 laboratory. **(A)** Person "dragging" air when passing through a doorway. **(B)** Simulation of air dragged into a room 15 s after a person is passing through a doorway. **(C)** Illustration of dragged air by a person walking from room 1 into room 2 distributing smoke before the person began to walk.

$$C_{NT} = C_{N\infty} + (C_{NO} - C_{N\infty})e^{-\beta \cdot \varepsilon \cdot t}$$

β = 0.25 1/min (Air exchange rate).
$\mathfrak{E}$ = 0.8 (Ventilation efficiency).
t = 15 min.
C_{NT} = Current particle concentration.
C_{NO} = 34 viruses/m³ (2×10^3 viruses released in 60 m³).
$C_{N\infty}$ = 0 (Estimated final concentration)

$$\mathbf{C_{NT} = 0 + (34 - 0)e^{-0.25 \cdot 0.8 \cdot 15}}$$

$$= \mathbf{34 \cdot 0.0498}.$$

$$= \mathbf{2\ viruses/m^3}$$

After breaking a sample vial on the floor and waiting for 15 min, there are approximately 2 virus particle/m³ as bioaerosols in the room.

In scenario B, the hypothetical virus distribution is calculated for the case of a large animal husbandry situation of 48 adult guinea pigs (occupancy with 12 cages of four animals each as a possible animal model for human diseases) in conventional cages with polyester filter sheet cover. For lack of data on aerosol excretion of risk group-4 pathogens in experimental animals, a value of 100 viruses/minute/animal, comparable to SARS-CoV-2 in hamsters or influenza virus in ferrets (see details above), is hypothesized for the following calculations. In this hypothetical respiratory infection, 48 adult guinea pigs would exhale 2.9×10^5 viruses as bioaerosol within 1 h. The aerosol reduction by the polyester filter sheet of approximately 92% (TECNIPLAST Conventional Cages), 2.3×10^4 viruses are released into the room per hour. The dilution in the room of 60 m³ results in a release per hour of 384 viruses/m³.

To calculate the virus concentration after homogeneous distribution at an assumed maximum released quantity of 384 viruses/h/m³ in the laboratory with 60 m³ and an air exchange rate of 900 m³/h follows:

$$C_{NT} = C_{N\infty} + (C_{NO} - C_{N\infty})e^{-\beta \cdot \varepsilon \cdot t}$$

C_{NT} = Concentration after homogeneous distribution, current particle concentration.
C_{NO} = Input, original particle concentration
β = 0.25 1/min (Air exchange rate).
$\mathfrak{E}$ = 0.8 (Ventilation efficiency).
t = 20 min (Time span for safe homogeneous distribution)

$$\mathbf{C_{NT} = 0 + (384 - 0)e^{-0.25 \cdot 0.8 \cdot 20}}\ \text{(Value for permanent input)}$$

$$= \mathbf{384 \cdot 0.0183}.$$

$$= \mathbf{7.0\ viruses/m^3}$$

Considering the 15 air exchanges per hour, it can be assumed that a virus load in bioaerosols in the room caused by conventional animal caging will remain comparable to the accidental release of bioaerosols from scenario A. In the case of open animal housing and fleece paper, the concentration is reduced to below 7 viruses/m³ for the duration of maximum bioaerosol excretion.

Spread of viruses to adjacent rooms

First, the influence of the opening time of an airtight door connecting 2 neighboring laboratory rooms is discussed. It is to be noted that the door opening time has practically no influence on the entrainment of air (and aerosols), since the entrainment is decisively influenced exclusively by the movement of a person or objects through the doorway. The minimal air exchange (see calculations under 2.2), which occurs in case of existing pressure differentials between rooms, has no significant entrainment effect and is physically absent in case of connecting rooms with equal pressure. Also, the room pressure condition is not affected by room temperature if the negative pressure control per room is well adjusted, even for small and common room temperature differences. As already stated above, no directional airflow is applicable between individual airtight rooms.

First, we consider the influence of waiting time (5, 10, and 20 min) on virus concentration after maximum room contamination (scenario A) before a door to an adjacent room is opened (Figure 2A). When a person (0.76 m³) passes through a doorway from room 1 to room 2 (Figure 3A), 0.76 m³ of air in rooms without a pressure differential, or 0.736 m³ (0.76 m³—0.024 m³) of air in rooms with pressure differential is carried over.

a) $C_{NT} = C_{N\infty} + (C_{NO} - C_{N\infty})e^{-0.25 \cdot 0.7 \cdot 5}$

$= 0 + (34—0)\ e^{-0.25 \cdot 0.7 \cdot 5}$

= 14.3 viruses/m³ (after a 5 min waiting time).

= 0.4 viruses displaced by pressure equalization only (with pressure differential).

= 10.5 viruses displaced by a person (with pressure differential).

= 10.9 viruses displaced by a person (without pressure differential).

Of note: with 5 min of waiting time, an uneven distribution in the room is assumed (0.7 instead of 0.8)

b) $C_{NT} = C_{N\infty} + (C_{NO} - C_{N\infty})e^{-0.25 \cdot 0.8 \cdot 10}$

= 4.6 viruses/m³ (after a 10 min waiting time)

c) $C_{NT} = C_{N\infty} + (C_{NO} - C_{N\infty})e^{-0.25 \cdot 0.8 \cdot 20}$

= 0.6 viruses/m³ (after a 20 min waiting time).

A waiting time after a virus contamination leads to a reduction of the virus load due to the air exchange, whereby the absolute virus load of 14 viruses/m³ after 5 min is negligibly low despite a maximum release within the containment. After a maximum release of virus and opening of an airtight door, no virus (0.4) would move into the other room despite a pressure differential of 40 Pa. The amount of air dragged by a person, and therefore potential virus cross-contamination (11 viruses) is also negligible and does not differ in the presence or absence of pressure differentials between adjacent rooms. With a maximum

virus load of 7 viruses/m³ during animal experiments, the same insignificant risk for cross-contamination into the adjacent room can therefore be expected.

Discussion

When the first BSL-4 laboratories were built in the 1960-80s, necessary structural safety barriers were established to prevent pathogens from escaping the laboratories. These included an individual supply and exhaust air system that could create pressure differentials and directional airflow to prevent contamination from areas within the laboratory with the highest potential risk toward areas outside the laboratory. Accordingly, the directional airflow gradient was established from the area of lowest exposure risk to the area of highest exposure risk to biosubstances (outside area → decontamination shower → laboratory → animal holding; Figure 1A). This was assumed necessary to avoid contamination of the environment or cross-contamination to adjacent laboratory rooms due to the technological air leakage of the laboratories. With the development of airtight doors and sealed pipelines, laboratories with increasing airtightness could be built starting in the 1980s. Today, this is state-of-the-art, with the degree of airtightness being high and evaluated annually. However, the necessity and usefulness of the originally required pressure differentials and a directional airflow gradient has not been questioned or re-evaluated. The relevant requirements remain unchanged in national and international guidelines in this respect (Technische Regeln für Biologische Arbeitsstoffe, 2013; BioStoffV 2013; Canadian Biosafety Standard, 2015; GenTSV 2019; Biosafety in microbiological and biomedical laboratories, 2020). Only the most recent version of the WHO Laboratory Biosafety Manual (Laboratory design and maintenance, 2020) re-evaluated the strict determination of risk groups and biosafety levels, instead encouraging the evidence-based and transparent assessment of the risks to allow safety measures to be balanced with the actual risk. It is stated, that controlled pressure differentials should be designed for a MCL from the least to the most contaminated area when necessary, indicating possible unspecified scenarios when pressure differentials might not be necessary or even useful.

Due to the construction of airtight rooms, which results in a very low leakage volume of no more than 0.75% of the room volume per hour with the doors sealed, the benefit of a directional airflow is insignificant to the maintained air exchange rate. When a door is opened, only a very brief, negligible directional airflow occurs into an adjacent room with a pressure gradient (1/30th of what is caused by a person traversing a doorway). Thus, directional airflow loses its intended benefit of preventing cross-contamination into adjacent laboratory spaces. This means that pressure differentials between airtight rooms within containment does not reduce the risk of aerosol carryover. Therefore, passive air exchange with open doors or air displaced by people and moving objects are the sole factors to consider for possible cross-contamination into adjacent laboratory rooms.

During normal operation of a BSL-4 laboratory, the use of primary containment (safety cabinets, downdraft tables with filtered air exhausts, IVC cages for animal containment, or animal changing stations) reliably prevent significant contamination within a room. The use of positive-pressure suits provides further protection for laboratory personnel. The additional high air exchange rates ensure a contamination-free laboratory area. Cross-contamination is only conceivable in special situations (e.g., release of virus-containing samples outside the safety cabinet, animal husbandry without primary containment, failure of the ventilation system). To our knowledge, there are no data on cross-contamination in BSL-4 laboratories, although our theoretical calculations suggest that such contamination would not be measurable. In general, the amounts of viral material processed in a BSL-4 laboratory are very low. Hence, the maximum amount of bioaerosols released during an accident (2×10^3 viruses from a of total 1×10^{10} viruses in a vessel) implies a small biosafety risk, compared to situations in clinical settings. It is therefore not surprising, that even after release of the largest possible amount of virus by breaking a sample vial and a waiting time of 20 min, our mathematical model shows no bioaerosol presence (arithmetically 0.6 viruses/m³ in a 60 m³ room) due to the high air exchange rate. Even in the most unfavorable case of a maximum release without a waiting time, the number of aerosol-contained viruses (arithmetically 34 viruses/m³ in a 60 m³ room) is too low for a possible contamination of adjacent laboratory rooms. The theoretical calculations in this study clearly shows that there is no difference of the contamination risk into adjacent laboratory rooms with open doors with or without pressure differentials, even after the maximum release of viruses and only 5 min of waiting time (arithmetically 0 versus 0.4 virus from a 60 m³ room) or by air displacement by a person (arithmetically 10.5 versus 10.9 viruses/m³ after 5 min of waiting time in a 60 m³ room). A change in the hazard potential could arise, for example, when processing large quantities of virus or using large animals with a correspondingly high aerosol release. A detailed risk assessment for any individual BSL-4 laboratory should be carried out to evaluate the level of protection of laboratory personnel and the environment before requiring directional airflow and pressure differentials.

As a result of the above investigation and calculations, pressure differentials outside of the secondary containment areas remain necessary. A pressure differential in the decontamination shower as the outer secondary containment boundary and transition to the inner containment spaces are justified and reasonable in the event of a door leakage. In contrast, differential pressure gradients in entrance airlocks do not represent an additional increase in safety. As a logical consequence, the authors consider a total of three pressure levels to be sufficient if it can be excluded that the suit room could be potentially contaminated (e.g., by overriding the door in an emergency). This could be guaranteed if the door from the decontamination shower to the suit room can only be opened after complete decontamination (with a shortened

decontamination cycle, implying no emergency egress). This would have to be substantiated by a risk assessment of the respective user. If this question cannot be answered unambiguously and clearly, a further, fourth pressure level is required. This results in a minimal 3-zone differential pressure gradient (possibly four zones), which represents a significant reduction of current practice (Figure 1C): access corridor/outer change/suit room → decontamination shower → laboratory (possibly corridor/changing room → suit room). In principle, in the authors' opinion, a fixed number of pressure levels in legal regulations or ordinances is not practical and does not add to safety. The necessity and usefulness of the number of pressure levels depending on laboratory operation should always be assessed, proven and confirmed on the basis of risk assessments.

Considering the calculations presented above and the risk assessments carried out, the following conclusions can be drawn for the operation of the BSL-4 laboratory at the Robert Koch Institute, and likely apply to other BSL-4 laboratories throughout the world:

1) Due to the airtight construction with airtight doors and sealed pipelines, there is no actual directional airflow within the containment facility; not even when a door is opened.
2) An accidental release of a virus-containing sample outside a biosafety cabinet (e.g., dropping/brakeage of a cell culture vessel) represents the situation for the highest room contamination.
3) Regardless of the animal model and virus, an animal holding with primary containment (IVC) has no increased room contamination potential compared to normal laboratory operation.
4) Depending on the animal model and virus, animal husbandry in conventional cages without primary containment most likely results in lower or similar room contamination than point 2.
5) By processing animals individually in the necropsy room using a downdraft table with filtered exhaust air, room contamination is comparable to normal laboratory operation and lower than point 2.
6) Due to the low residual bioaerosol contamination of a maximum of 14 viruses/m^3 after the highest possible room contamination and a waiting time of 5 min or during an animal experiment using conventional cages, the air displacement of a person (including a maximum of 10 viruses), the risk of cross-contamination to an adjacent laboratory room is negligible.
7) The risk of bioaerosol movement from an area with potentially higher contamination to areas with lower contamination is insignificant due to the low virus concentrations and limited air displacement.
8) An increase of biosafety risk by potential contamination due to the elimination of pressure differentials within a secondary containment with airtight doors is excluded.
9) A uniform pressure level within the secondary containment including laboratory, animal room and necropsy room does not increase the safety risk.
10) Since contamination in the suit room is excluded, three pressure levels (suit room, decontamination shower, laboratory) provide a sufficient environmental protection.

Conclusion

An attempted directional airflow between technically airtight spaces does not contribute to reducing the risk of cross-contamination due to the very low leakage volume. Thus, directional airflow or a differential pressure gradient in airtight rooms within a secondary containment area do not increase biosafety and are no longer necessary. The only decisive biosafety factor is sufficient tightness of the secondary containment and the unconditional maintenance of the prescribed air exchange.

This simplifies the necessary operation and monitoring technology and workflows when the pressure differentials within the secondary containment are eliminated. At the same time, the simplified use of the laboratory increases occupational safety for the personnel working in the containment. Also, the control and regulation processes for controlling the pressure conditions of the secondary containment are simplified, and complex, highly sophisticated technical solutions for error-free door opening and closing are no longer required. This significantly reduces the probability of failure and significantly increases the availability and passive safety of these laboratories. Following the same rationale, a reduction of pressure levels from the outside into the secondary containment may also provide a sufficient environmental protection.

Adaptation of the legislation and regulations should occur for directional airflow and pressure differentials for technically airtight BSL-4 laboratories.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

DR, UW, and AK performed study and analyzed data, AK prepared figures, AK wrote the paper, UW and DR edited the paper.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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New genomic techniques and their European Union reform. Potential policy changes and their implications

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The article discusses amendment options (no significant change, lowering of administrative burdens or exemption of certain products from the legislation) for the European Union (EU) authorization procedures of New Genomic Techniques' (NGT) products and their consequences for the sector and research institutions, particularly in the context of internal functioning, placing products on the market and international trade. A reform of the EU regulatory system requires a change in the procedures for the authorization of NGT products, otherwise EU researchers and investors may still be at a competitive disadvantage (as compared to Argentina, Brazil, Canada, United States or the United Kingdom) due to the inefficiency of the current system and the committee procedure for authorization. New legislation, currently being adopted in the United Kingdom is also presented for comparison.

KEYWORDS

GMO, new genomic techniques, biosafety, authorization, committee procedure, bioeconomy, precautionary principle

1 Introduction

A process of revising the GMO legislation is currently ongoing in the European Union (EU). After preparing a study and two rounds of consultation, the European Commission (EC) plans to have a project ready in the second quarter of 2023. In the study (European Commission, 2021), the Commission mentioned that the current legislation may not be adequate to regulate research and marketing involving some products of "New Genomic Techniques" (NGTs) and indicated a need to alter it. In the new legislation the restrictions on research and use of regulated products are supposed be proportional to the risks connected with their use. The amendments also should contribute to the achievement of the goals of EU Green Deal and Farm to Fork strategies, which would require a more widespread use of such products, a higher throughput in authorization, and a higher level of legal certainty as to the outcomes of an authorization process. The term NGTs, is "an umbrella term to describe a variety of techniques that can alter the genetic material of an organism and that have emerged or have been developed since 2001, when the existing GMO legislation was adopted" (European Commission, 2021, 62). The glossary explains

that it means at least: gene editing techniques either through the application of oligonucleotide mediated mutagenesis (ODM), site directed nucleases (SDN) (Zimny and Sowa, 2021) and RNA-directed DNA methylation (European Commission, 2021, 62–63), although this classification of methods and their products is not uncontroversial (Vives-Vallés and Collonnier, 2020; Van Der Meer et al., 2021).

According to the current EU GMO legislation any GM product requires authorization as food or feed (Regulation 1829/2003/EC, 2003) or another type of product [e.g., for cultivation (Directive 2001/18/EC, 2001)]. Such products need to undergo rigorous risk assessment (see e.g. Regulation 503/2013, 2013), and need to meet traceability and labelling criteria afterwards. Member states have significant flexibility in opting out from authorization of products meant for cultivation in the EU (Directive 2015/412/EU, 2015). Both the study and the amendment initiative are a result of a judgement of the Court of Justice of the EU, according to which only products of methods of mutagenesis routinely used until 2001 are exempted from the EU GMO legislation (CJEU C-528/16, 2018). Since the passing of this judgment multiple stakeholders proposed changes to the EU legislation, usually through exclusions or exemptions of certain classes of organisms, (e.g., featuring single nucleotide variants products of SDN 1 or 2 techniques or cisgenesis), (Zimny and Eriksson, 2020).

A thorough critique of the current EU regulatory system was performed by Eriksson and others in 2020 (Eriksson et al., 2020c; 2020b; 2020a). The authors indicated problems ranging from the current legislation's unclear scope and conditions for authorization of products, through risk assessment procedures and their fitness for the purpose of performing proper risk management with regard to regulated products, and also problems with the post-authorization functioning of the products on the market. Proposed solutions to the problems involved: reconsideration of the current labelling requirements for authorized products, amendment of rules for the certification of organic products (Eriksson et al., 2020a), adding flexibility to the risk assessment procedures (to make the required steps dependable on the features of the examined product), switching from maximum to minimum harmonization in risk management (Eriksson et al., 2020b) and changing the approach to the regulation of organisms to a more product-oriented one, coupled with institutional and legal changes aimed at an increase of the certainty of law with regard to the development and marketing of regulated products (a pre-approval system) (Eriksson et al., 2020c). Issues, connected with the feasibility of the current legislation for the regulation of certain NGT products (in particular connected with detection and traceability), were risen by other authors (Emons et al., 2018; Broll et al., 2019; Sowa et al., 2021). Others postulate that risk assessment requirements should be altered with respect to products of targeted mutagenesis featuring small changes in the genome

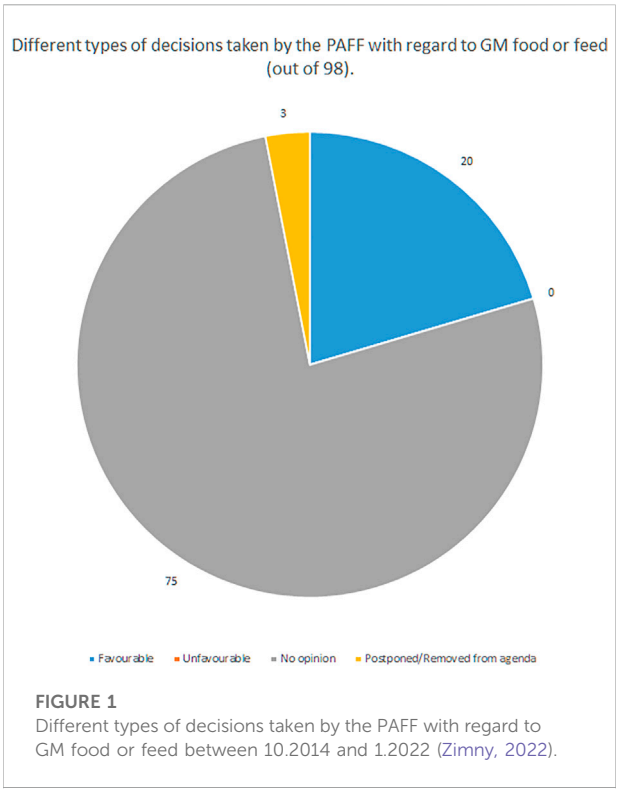

FIGURE 1
Different types of decisions taken by the PAFF with regard to GM food or feed between 10.2014 and 1.2022 (Zimny, 2022).

(Naegeli et al., 2020; Garcia-Alonso et al., 2022). The current authorization procedures also take ca. 5–6 years to complete (Smyth et al., 2014; Garcia-Alonso et al., 2022) and are rather costly [over 11 million € (Garcia-Alonso et al., 2022)], creating a high entry threshold for potential developers.

Criticisms of the current authorization system of GMOs also include the fact that the draft decisions by the EC may be accepted or rejected by a political body – a committee. The decisions regarding authorization of GM products in the EU are taken in "the committee procedure", where a draft decision of the EC is submitted for deliberation to a committee comprising representatives of the member states of the EU. In case of GMOs it is the Genetically Modified Food and Feed and Environmental Risk section of the Standing Committee on Plants, Animals, Food and Feed (PAFF). The committee can either accept or reject the Commission's decision or adopt no opinion. Acceptance and rejection require a qualified majority, hence if it is not reached, the Committee does not pass an opinion. In such a case or in the case of rejection, the draft is submitted to the Appeal Committee, operating on the same principles. If at this stage the opinion is favourable or no opinion is passed, the Commission can adopt the draft decision (see further Zimny et al., 2019). The role of the committee procedure is to involve member states in the decision-making process, when the EC issues a delegated or implementing act (e.g., a decision). The procedure is regulated by articles 290 and 291 of the Treaty on the Functioning of the

European Union and the Regulation 182/2011 of the European Parliament and the Council (European Parliament and the Council, 2011). Currently there are multiple committees operating under the auspices of different directorates of the EC, and these committees are divided in to thematic subsections. This structure would suggest that the opinions of a committee have a form of a quasi-expert opinion, since particular subsections make decisions in a particular area of regulation. This does not seem to be the case, however, in the area of authorisation of genetically modified organisms.

Committee members rather act upon the directives from their respective governments than basing on the scientific data. The committee can adopt or reject a decision with a qualified majority (55% of member states, no less than 15, comprising min. 65% of population). Notably this same majority is required for the adoption of changes to the GMO release directive, and some scholars indicate that reaching it after Brexit may be difficult due to the fact that the United Kingdom usually was generally in favour of transgenic crops (Purnhagen and Wesseler, 2021, 1631). An analysis of decisions taken by the PAFF between October 2014 and January 2022 shows that out of 98 decisions taken, 75 failed to reach the qualified majority, hence resulted in no opinion. Out of the remaining 23, 20 contained a favourable opinion, there were no unfavourable opinions (see Figure 1). All the favourable opinions were passed on purely formal issues, like the changing of the data of the applicant's representative (Zimny, 2022).

The EU legislation on GMOs is based on the precautionary principle (PP), which obliges decision makers to undertake preventive measures in situations, where the knowledge about the undesired outcomes of a planned action (e.g., introduction of a new product to the market) is insufficient. The principle defines the EU's approach to the protection of human health and the environment and is mentioned in art. 191.2 of the Treaty on the Functioning of the European Union. The CJEU mentioned PP as one of the reasons for its decision in the C-528/16 case (par. 50, 52, 53, 83), yet without going into a detailed analysis of its applicability to particular methods of gene editing, rather deciding about the products of such methods *en masse*. It should be noted that not every situation of uncertainty justifies the application of the PP. Its application should be necessary in the context of the lack of knowledge about the consequences of a given action (Zetterberg and Edvardsson Björnberg, 2017, 36). Where risks are known, preventive rather than precautionary measures, tailored to those risks, should be applied (Bergkamp and Hanekamp, 2018, 219). Excessive regulation (unjustified in view of possessed scientific knowledge) of an area of human activity may be viewed as a violation of the principle of proportionality (mandating that restrictions of basic freedoms should be genuinely necessary and justified), mentioned in art. 5.1 of the Treaty on European Union and art. 52.1 of the EU Charter of Fundamental Rights. For instance, if two groups of products are comparable in terms of risks connected with their use, and one group of such products is lightly regulated (e.g., products of conventional breeding or random mutagenesis), while another group is heavily regulated, then the regulation of the latter group may be seen as a violation of some basic freedoms (e.g., to conduct a business or freedom of arts and sciences). When drafting the new provisions on the authorisation of various NGT products, the EC needs to consider the PP on the one hand and the principle of proportionality on the other. The application of PP to certain products may no longer be justified, or certain regulations supposedly based on it, may no longer be necessary. This is a position taken *inter alia by* some of the EU's major trade partners genetically modified goods, who have decided to lessen the regulatory burdens placed on plants not featuring stable insertions of foreign DNA fragments (Dederer and Hamburger, 2019).

Recently, the United Kingdom seems to have reacted to the criticisms of the current GMO legislation, by changing its laws with regard to certain NGT products. The amendment to the regulation on the deliberate release of genetically modified organisms (UK Parliament, 2022a) allows for an exemption from the risk assessment before experimental release of a "qualifying higher plant" (*inter alia* SDN 1 or 2 products, plants with epigenetic changes or certain cisgenesis products (ACRE, 2022). The second stage of the reform planned in the United Kingdom encompasses changes regarding obtaining, importing and marketing products of "precision breeding". A bill proposing changes to the existing legislation was read in the House of Commons on the 25th of May 2022 (UK Parliament, 2022b). The act (which shall apply to plants and animals–subject to welfare assessment) introduces a concept of a "precision bred organism". Marketing of such organisms, will only be allowed, when such an organism would be a "marketable precision bred organism" (Art. 5 (1a)) or its "qualifying progeny" (Art. 5 (1b)) and 24), subject to a confirmation issued by the Secretary of State upon receipt of a report of an advisory committee (issued within 90 days). Marketing of food and feed products shall to a large extent be subject to regulations, which may impose obligations regarding obtaining a marketing authorisation and impose traceability requirements (Part 3 of the bill). It is yet too early to predict if the bill will be passed in the form it was submitted to the House of Commons, but its tenor indicates that the United Kingdom wishes to follow in the footsteps of other EU's important trading partners, who severely lessened the regulatory burden placed on NGT products, which would otherwise be obtainable through conventional breeding or random mutagenesis, or do not feature stable inserts of foreign DNA fragments—e.g., Argentina, Brazil, Canada, the United States (Dederer and Hamburger, 2019; USDA, 2020; Zimny and Sowa, 2021). Lack of harmonization of regulations

with such countries might result in serious cost increase and regulatory burdens placed both on the EU authorities and entrepreneurs (Ryan and Smyth, 2012).

2 Policy options and implications

The outcome of the Commission's initiative to amend the legislation is currently uncertain. The questionnaire for the recently concluded poll contained a whole spectrum of options, from a lack of changes, to changes envisioning a departure from risk assessment requirements for certain products. The project may not be adopted by the EU before the Commission's term of office runs out in October 2024. Given that the Commission has declared a need for a legislation, in which the regulatory burdens would be proportional to the risks connected with the use of the product in question, it needs to prepare a project that would comply with both the PP and the principle of proportionality. Taking this into consideration one can distinguish three policy options: 1—no change or negligible changes to the legislation, 2—limited changes, in particular through restrictions in the risk assessment requirements, 3—exemption of certain products from the legislation, in particular products featuring changes that would also be achievable through conventional breeding or random mutagenesis.

2.1 No changes or negligible changes

This option essentially means the maintenance of the *status quo*, which is not a scenario desired by the EC or the stakeholders advocating a reform of the legislation. According to the current interpretation of the definition of the GMO, products of modern methods of gene editing will fall under the current GMO legislation with all its drawbacks (see above). This scenario would be marked with a low throughput of the authorization procedures (Smyth et al., 2014; Garcia-Alonso et al., 2022), uncertainty of their outcomes strengthened by the politicization of the decision-making process (Purnhagen, 2019), problems with international trade of such goods (Purnhagen and Wesseler, 2021; Zimny and Sowa, 2021) as well as increased costs of authorization, but also potential occurrence of unauthorized products imported from third countries (Ryan and Smyth, 2012; Purnhagen and Wesseler, 2021). These consequences would significantly limit the economic justifiability of choosing an NGT for the development of products for the EU agricultural market. The application of the current GMO regulatory framework to some of the NGT products (an inevitable consequence of a lack of changes in the regulatory approach) can be seen as overregulation, not justified by the PP nor the proportionality principle (see below). The mere fact that a certain requirement can technically be introduced, does not make such a requirement scientifically or legally justified.

2.2 Limited changes

The actual contents of the EC project are not known yet, however the questionnaire for the survey, which ended in July 2022 contained a wide variety of options, including, *inter alia*:

- adapting risk assessment requirements for plants produced through targeted mutagenesis or cisgenesis (Question 3);
- introduction of a fast track authorization system or fee reductions for plants with traits contributing to sustainability (Question 7);
- waiving or limiting the duty to develop a method for detection and differentiation of plants produced by cisgenesis or targeted mutagenesis, where such a method cannot be provided (Question 11);

and others (European Commission, 2022). The practicality of such solutions and their actual content is still subject to speculation, (e.g., the meaning of "traits contributing to sustainability"). In view of the European Food Safety Authority's (EFSA) opinions regarding the applicability of the current GMO risk assessment requirements to products developed through SDN 1-3, ODM or cisgenesis, even if they are sufficient for the assessment, parts of those requirements may not be applicable or necessary for the determination of the safety of such products. Particularly, the assessment of SDN 1-2 and ODM products from the point of view of the safety of gene products could depend on the allele that was edited. Should the allele and the trait associated with it be already present in a cultivated variety, the risk assessment could be focussed on the history of safe use of said variety rather than on the specific data on the edited gene. This would not be the case for a completely new allele and trait (Naegeli et al., 2020, 8). Similarly, it is expected that the number of off target mutations for such products may be comparable with those of conventional breeding methods, and the existing environmental risk assessment requirements, while sufficient for the evaluation of SDN 1-2 and ODM products, would only partially be applicable to them, due to them featuring a modification of an endogenous sequence rather than an insertion of a transgene (Naegeli et al., 2020, 10).

While resignation from some risk assessment elements, justified by the lack of a stably present insert would not be appropriate for cisgenic products, there is still a leeway when it comes to such products, on a case by case basis, particularly if the familiarity of the plant and introduced gene were to be taken into consideration. Requirements justified by risks connected with the introduction of a foreign gene could be to an extent limited for such products as well. EFSA deemed parts of the abovementioned

requirements currently applied for "classic" GMOs not applicable to cisgenic products (EFSA Panel on GMO, 2012, 18–19).

Changes in the authorisation procedures in this scenario could then encompass at least (EFSA Panel on GMO, 2012, 19; Naegeli et al., 2020, 8, 11):

- lower requirements for experimental data for SDN 1-2 and ODM products (lack of transgene or cisgene);
- lower data requirements on the safety of gene products SDN 1-2 and ODM products basing on the familiarity of the altered alleles and traits;
- no risk assessment of the transgene itself (due to the lack of it);
- on a case by case basis: lower data requirements for cisgenic products, basing on their familiarity;
- and additionally a system that would facilitate the authorisation of the abovementioned products, at least through an *ex ante* status confirmation.

The adoption of such solutions (reduction of risk assessment requirements, a "fast track" for certain known products) would definitely lessen the administrative burdens placed upon researchers and developers of such products. Among the benefits, from their point of view, one can mention an increased throughput of the authorization process, lower uncertainty of as to the outcomes of that process, lowering of the costs of performing the risk assessment and obtaining the authorization, particularly if a pre-approval system for some products would be introduced. However, the ultimate decision would still depend on the political vote within a committee. If labelling requirements for GMOs would be maintained also for products of targeted mutagenesis or cisgenesis, this would still hamper the international trade with countries not having such requirements (see above) and result in potential stigmatization of labelled products, their removal from production chains, hence cause a lowered demand for such products.

2.3 Exemption of certain products from the legislation

Exemption (the way products of random mutagenesis are currently exempted) of certain products from legislation (e.g., SDN-1 and 2 - Vives-Vallés and Collonnier, 2020; see also Zimny and Eriksson, 2020) or even an interpretation of the GMO definition in such a way that it would not cover such products (Van Der Meer et al., 2021) has been postulated not only by researchers, but also stakeholders and some organizations. The adoption of such a policy would definitely have the most benefits from the point of view of researchers and developers of products covered by the exemption. An exempted product does not need to undergo any authorization procedures, which are also not required for non-regulated products [e.g., variety evaluation for the purposes of its placing in the Common Catalogue–an EU database of registered plant varieties, which are no longer subject to marketing restrictions (European Parliament and the Council, 2002)]. Access to the market of such products and the costs of their marketing would be greatly improved. Also the legal certainty of investors would be significantly enhanced, since the access to the market would no longer depend on a decision of a political body. Such a solution would also be harmonized with the systems adopted by the aforementioned trade partners, including the new legislation currently discussed in the United Kingdom. Introduction of a pre-approval system that would determine the legal status of a product before its development, as has been postulated in the literature, (Eriksson et al., 2020c), would further facilitate the decision making process on the side of the researchers and investors.

It needs to be stressed that with sufficient information available, an exemption of some products from the regulation does not need to result in a violation of the PP. If the risks connected with the use of a certain plants for their intended purpose were to be sufficiently known, and if they were deemed to be comparable with those associated with the use of already exempted plants, then preventive measures, such as a status confirmation system or supervision at the development level, could be sufficient to satisfy the safety requirements. Particularly if this solution were to be applied to products of SDN 1-2, ODM edition of known alleles with a history of safe use. The prerequisites for such an exemption should be cautiously determined by an expert body (e.g., EFSA GMO Panel), taking multiple factors into consideration, and be subject to periodical review.

The adoption of this policy option, even for a limited group of plants, would however have some significant drawbacks. Firstly the official control over such products would be much lower than in the remaining scenarios discussed here. Transparency, particularly perceived by the general population would also suffer, with lack of official oversight and reporting or labelling duties. This might lower the trust in the biosafety system as such. These features may render this policy option the least likely to be adopted, since it may be difficult to find political support for it (Purnhagen and Wesseler, 2021, 1631–1633). Another potential drawback may be the fact that as per the CJEU judgment, the member states of the EU are able to introduce national restrictions on exempted products. This policy option would be the easiest to implement, due to the lack of administrative burdens and special regulatory provisions connected with them.

3 Actionable recommendations

There are actually two stages of actions to be taken, depending on the state of adoption of the prospective

amendment of the EU legislation regarding NGT products. The first stage –before the adoption of a project and with the preliminary consultations already closed, would involve participation in public activities for the stakeholders, aimed at the preparation of a project that would ease the administrative burdens, as well as harmonize the legislation with that of EU's closest trade partners and neighbours.

Should the EU succeed in adopting a new legislation that will comprise at least the solutions presented in options 2 or 3, the formal situation of researchers will become more complicated than currently. Instead of having to consider three categories of organisms, as is currently the case [non-GMOs, regulated GMOs and GMOs exempted from legislation (Custers, 2017)] they may need to consider several additional categories–various NGT products that will legally be GMOs with an altered level of regulation. The legal status of a given organism will heavily influence its future viability as a product, depending on the requirements for research and marketing placed on it. Given that many R&D units will still employ a variety of methods in their activities, two types of solutions may help with the inter-institutional decision making process, as regards the choice of breeding methods and compliance. Firstly the development of an internal policy document, or even an algorithm that would help researchers with determining the legal status of their products depending on the methods and nature of intervention into the plants' genome. Secondly, the decision making process may be supported by establishment of an advisory body comprising compliance officers or persons otherwise competent in the assessment of the regulatory status of certain products, whose opinion would facilitate the decision-making process within the institution.

4 Conclusion

Despite the declarations of the EC regarding the amendment of the legislation, the future of NGT products in the EU still remains uncertain. Even if changes lessening the regulatory burdens placed on plants resulting from the use of NGTs are going to come into force, it is not clear that they will satisfy the needs of the R&D sector. The United Kingdom seems to follow into the footsteps of other EU's trade partners in agricultural goods, through a significant lessening of regulation of products, which could otherwise be obtained through methods of conventional breeding or random mutagenesis. Adoption of any amendments in the EU will require a proper response and policy adjustment on the part of the research institutions as well.

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Supplementary material

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Biofoundries and citizen science can accelerate disease surveillance and environmental monitoring

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A biofoundry is a highly automated facility for processing of biological samples. In that capacity it has a major role in accelerating innovation and product development in engineering biology by implementing design, build, test and learn (DBTL) cycles. Biofoundries bring public and private stakeholders together to share resources, develop standards and forge collaborations on national and international levels. In this paper we argue for expanding the scope of applications for biofoundries towards roles in biosurveillance and biosecurity. Reviewing literature on these topics, we conclude that this could be achieved in multiple ways including developing measurement standards and protocols, engaging citizens in data collection, closer collaborations with biorefineries, and processing of samples. Here we provide an overview of these roles that despite their potential utility have not yet been commonly considered by policymakers and funding agencies and identify roadblocks to their realization. This document should prove useful to policymakers and other stakeholders who wish to strengthen biosecurity programs in ways that synergize with bioeconomy.

KEYWORDS

biofoundry, biosecurity, biosurveillance, citizen science, policy

Introduction

Humans have always been at prey to natural pathogens. There have been at least fifteen epidemics with a death toll over 1 million in the last 500 years (one every 33 years on average). Two occurrences of bubonic plague, a bacterial respiratory infection, in the 6th and 14th century wiped out an estimated half of the worldwide population. Spanish flu, a viral respiratory infection, caused tens of millions of deaths in the early 20th century. More recently, the coronavirus pandemic caused millions of deaths worldwide. While the most shocking due to their rapid development, pandemics are only one of major global health risks. Another global health risk is due to antibiotic resistance. Increasingly prevalent among pathogens, it is causing an increase in the number of deaths due to bacterial infections globally (Zhang et al., 2022). Furthermore, as we become increasingly able to edit and engineer living organisms, man-made pathogens could be at the source of future health threats as well. Driven to protect ourselves from the often-lethal forces of nature, we as humans have learnt to shape our environments in many ways early on. From building shelters to growing crops, these efforts have paid out wildly, testified by how well we have done as a species. It has been only very recently, however, that we are developing more appreciation for how we have influenced and continue to influence the natural environment around us in this process. Environmental pollution, climate change and biodiversity loss are just some examples. One of the less known consequences is an emergence of novel urban ecosystems that give rise to novel species (Danko et al., 2021). Risks to the health of humans and our environment must be monitored, as any attempts to manage and contain them in the future will have to rely on data to be effective. Biosurveillance (detection of biological

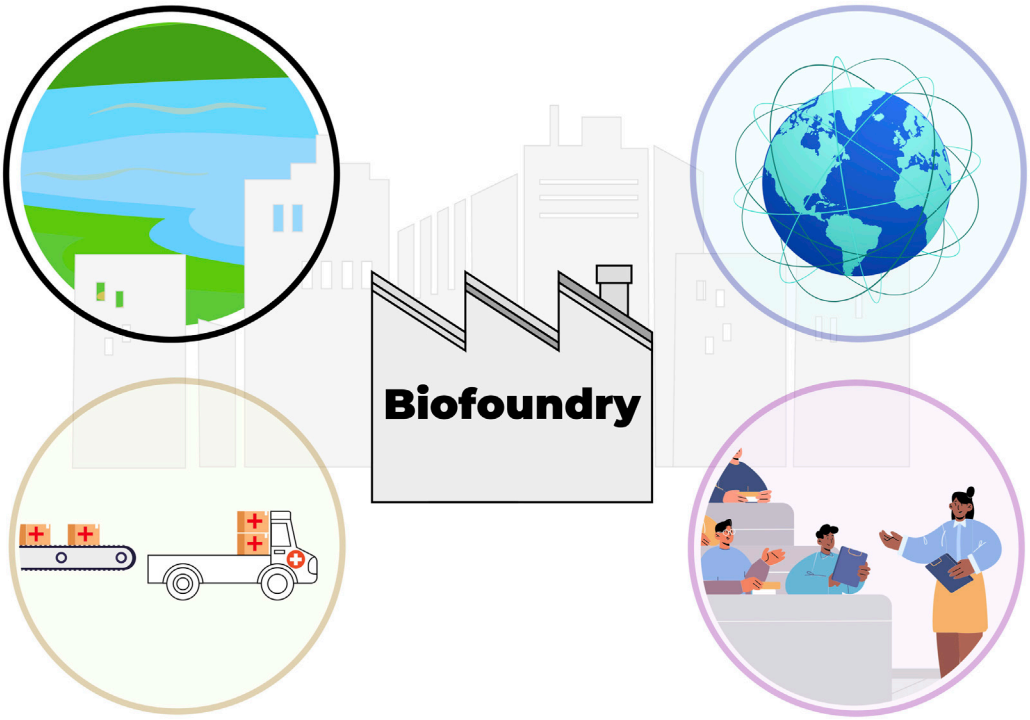

FIGURE 1
A vision for the future role of biofoundries and citizen science. Biofoundries are local hubs that are close to urban areas, and foster citizen engagement through citizen science (top left) and education (bottom right). Global network of biofoundries cooperates to share protocols and data (top right), which further strengthens the capacity of individual biofoundries to safeguard biosecurity and implement interventions.

threats to human health) and environmental monitoring (observation and characterization of the natural environment) are both processes of provisioning this data. In the recent case of coronavirus pandemic, biosurveillance through routine testing and contact tracing on the level of individuals has proved to be crucial to the coronavirus pandemic response worldwide. Additionally, aggregate monitoring of coronavirus through wastewater sampling has proved to be a predictive signal to case counts and hospital load independent of direct diagnostic data (Venugopal et al., 2020; Zhu et al., 2021; Calderón-Franco et al., 2022; Calderón-Franco et al., 2021). In a similar fashion, the benefits of biological monitoring have been seen for targets other than infectious disease such as tracking of bacterial antibiotic resistance in the environment (Huijbers et al., 2019), and even conservation efforts through the analysis of environmental DNA (Francis Thomsen and Willerslev, 2015).

Current bottlenecks in biological monitoring

Despite some successes, biological monitoring programs generally fall short on a multitude of levels when it comes to preparedness for detection and prevention of future biological risks. While, to our knowledge, there is no resource comprehensively reviewing and comparing biosecurity programs across the world, Nuclear Threat Initiative (NTI) has compared 195 countries in terms of preparedness for pandemics and epidemics in Global Health Security Index (www.ghsindex.org). The United States ranked number one in 2021 and this, together with its being relatively well researched in academic literature, is one of the main reasons why we use it as an illustrative example. It is likely that the US system is average or above-average compared to biodefense systems across the world and that its shortcomings will reflect common shortcomings worldwide.

The main shortcomings of the US biodefense as reviewed by the Bipartisan Commission on Biodefense (Bipartisan Commission on Biodefense, 2022) are lack of quick response capability (Vickers and Freemont, 2022) and general lack of structured investment, lack of adequate data interoperability and data collection standards, and poorly developed regulatory structure. Several biosurveillance bottlenecks, such as insufficient testing and processing capacity, where at one point a single facility was responsible for handling samples nation-wide, became manifest during the COVID-19 pandemic and limited the speed of delivering public health interventions (Boeckh et al., 2022). This ultimately encouraged establishment of more distributed testing sites and accessing unconventional sequencing facilities for diagnostic work, such as academic laboratories (Kim et al., 2020). Coupled with the increased public awareness of biosecurity as result of the pandemic, along with the identification of bottlenecks in current biosurveillance programs, the question arises: Is there a different way to structure biosurveillance programs that could improve outcomes? In this paper we argue for options to do so by considering the newly emerging infrastructure of highly automated facilities for processing of biological samples, biofoundries (Box 1; Figure 1). In the following sections we discuss how this infrastructure can be exploited to benefit not only response to disease outbreaks, but also the response to more subtle targets in health, ecology, and biosecurity. We identify several opportunities at this interface, most of which have not been commonly considered by policymakers and funding agencies. These include developing measurement standards and protocols, engaging

citizens in data collection, decentralized manufacturing (Box 1), and processing of samples. We then finish by highlighting roadblocks to their realization. In this vision we focus on biofoundries that are run and funded by the public sector. While industry-owned biofoundries exist and undoubtedly deliver value, they may be subject to unique agendas of their owners and we do not see them as a suitable foundation of national biosecurity. In contrast, we believe that less-formal infrastructure for biological experimentation, such as bio-hack spaces and bio-DIY labs, can contribute to these ends in various ways, including increasing the impact of citizen scientists, as well as encouraging safe practices, through collaboration with biofoundries and community engagement. However, due to specific challenges these spaces currently face, including lack of appropriate regulatory schemes, issues with securing suitable lab space and equipment, as well as negative sentiment among broad public, we anticipate that their contribution will develop only as they mature on medium and long term. We therefore leave them out of scope of the present discussion and refer interested reader to recent reviews on the topic (Seyfried et al., 2014; Meyer., 2013; Landrain et al., 2013; Keulartz and van den Belt, 2016).

Standardized and automated measurement workflows facilitate biosurveillance

The cornerstone of biofoundry operations is the melding of automation of standardized bioengineering workflows and the design, build, test and learn cycles. Without these principles implemented, the difference in throughput achieved by biofoundries as compared to typical laboratories would not be possible (see Box 1 for a general introduction to biofoundries). The outcomes of engineering biology can be variable due to the complexity of biological systems and the magnitude of unknowns and confounding factors. Thus, by leveraging automation technologies throughout sampling, processing, and analysis, as much human variability is removed from the process which allows for gains in consistency of results while shortening the timescale of workflows. This approach is also suitable for processing many samples at the same time, allowing to explore unprecedented breadth of genetic variability. While mainly employed for sample processing, experimentation, and analysis by academics and researchers, biofoundries are also well suited to boost our ability to rapidly collect and analyze samples originating from patients, or the environment. In a recent example, Ginkgo Bioworks has used its high-throughput sequencing capabilities to support nation wide efforts in COVID-19 testing, as well as supported vaccine manufacturers in optimizing their products (Cho, 2020). On a similar note, automatized routines adopted at biofoundries, as well as their equipment, make them good candidates for handling samples with pathogenic potential. Aside from automated processing of high numbers of samples, biofoundries are particularly suited for development of measurement standards and standardized calibration samples. Their nature as a collaborative platform, that can interface with governmental entities, further facilitates encouragement and adoption of so developed standards (Mao et al., 2021). In the context of biosurveillance, adoption of these standards enables comparison of results across time and geographical regions and enables their users to harmonize interventions. An example is provided by London Biofoundry, which developed a rapid automated SARS-CoV-2 testing platform that was deployed and scaled in national diagnostic labs and could be also adopted by other biofoundries (Crone et al., 2020).

BOX 1 | Tools for Rapid and Robust Biological Surveillance

Biofoundries

A biofoundry is a highly automated facility for processing of biological samples. In that capacity it has a major role in accelerating innovation and product development in engineering biology by implementing design, build, test and learn (DBTL) cycles (Hillson et al., 2019) (Figure 2). The equipment in biofoundries typically include automated liquid handling systems, high-throughput sequencing and chemical analysis equipment, and a software ecosystem for data and personnel management (Hillson et al., 2019). For example, one of the largest biofoundries and synthetic biology companies in operation today, Ginkgo Bioworks, has leveraged their integrated system of automated bioengineering to evaluate on the order of tens of thousands of engineered strains (Ginkgobioworks, 2022) — a quantity that can not be achieved with bench-scale workflows alone. Dropping costs of DNA synthesis and sequencing, development of facile technologies for genome editing, lab-on-chip microfluidics, and expanding ecosystem of hardware and software automation tools are some of the main factors that contribute to synthetic biology as an engineering discipline. The growth of bioeconomy enabled by these technological advances goes hand in hand with the increasing popularity of biofoundries. The establishment of the Global Biofoundry Alliance (GBA), which has grown to over 30 members since 2019 (Hillson et al., 2019), including 14 biofoundries in Australia and Asia, nine in North America and 10 in Europe, is a sign of the continued growth of this sector. Importantly, first steps towards establishment of biofoundries in Latin America (The Bridge Biofoundary, 2022) and Africa (Thimiri Govindaraj, 2022) are already underway. Aside from their direct role in biological experimentation, biofoundries serve as platforms that bring public and private stakeholders together to share resources, develop standards and forge collaborations on national and international level (Vickers and Freemont, 2022). In that capacity they can gather sufficient momentum to realize collaborative projects that may need top-down incentive or broader consensus for economical viability (e.g., projects contributing to environmental sustainability), contribute to development of legal and ethical frameworks by shaping governance of emerging fields (Mao et al., 2021) and manage the relationship with the public. Despite their obvious utility, the high establishment, personnel and overall running costs make the business case for biofoundries difficult. While there is early evidence that biofoundries deliver high added value through innovation and knowledge creation (Winickoff et al., 2021), it is useful to consider additional roles for biofoundries that could strengthen their business case, which could further rationalize their establishment in countries with lower research budgets.

Citizen science

Citizen science, which is the involvement of the public in scientific research, can range from collecting and analyzing data to prototyping low-cost sensing devices. Digitalization of our society and adoption of open-data and open-innovation paradigms are the main contributors to its rise in recent two decades (Maccani et al., 2020). The main benefits of citizen science are two-fold: 1) citizen science contributes to and expands research, and 2) it shapes the relationship between scientists and the public in an engaging, two-way interaction (Hecker et al., 2018; Den Broeder et al., 2016). The first benefit enables a larger breadth of research than what is achievable by an academic laboratory alone, e.g. collection of data at higher spatial resolution, or making measurements of completely new parameters. The latter allows citizens to familiarize themselves with the scientific method and gain insight on interpretability and accuracy of collected data, as well as reciprocally provide feedback on collected data and the process of its acquisition. Recent incorporation of citizen science concepts into university (MOOC, 2022; UZH, 2022) and high-school (Developer Community, 2018) curricula suggest that its impact will continue to rise.

Cell-free synthetic biology

Standardization could be facilitated by adoption of cell-free systems (CFS). CFS could also contribute to a shift towards decentralization of manufacturing. Cell-free gene expression is gaining popularity in synthetic biology and bioengineering (Garenne et al., 2021). Diverse applications including protein production, therapeutics manufacturing and biosensing all can benefit from by-passing living cells. Benefits include facilitated rapid prototyping and condition screening,

(Continued on following page)

BOX 1 (*Continued*) |
Tools for Rapid and Robust Biological Surveillance
reduction of reaction volumes, higher predictability and amenability to mathematical modeling. Consequently, cell-free biomanufacturing is of imminent interest also beyond academia. Furthermore, engineered cell-free systems are not classified as genetically engineered organisms (Khambhati et al., 2019; Taylor and Wieden, 2021), which simplifies biosafety and biosecurity of their application. Adoption of cell-free systems further decreases batch-to-batch variability (Kumar Dondapati et al., 2020), reduces sample volumes and lowers regulatory barriers. Biofoundries are particularly suited to drive the transition to decentralized biomanufacturing through adoption of cell-free systems. The integrated design, build, test and learn cycle, the automation facilities for liquid handling, and the standardization in biofoundries are all vital to rapid, scalable and reproducible processes. Geographical distribution of biofoundries allows them to serve as local hubs (Hillson et al., 2019), out of which products based on CFSs can be rapidly deployed, for instance in the case of response to health and environmental crises.

Biosurveillance enabled by biofoundries and citizen scientists

Areas that can benefit from citizen science (Box 1) are diverse. With an aging population and increasing obesity rates on one hand, and ongoing prevalence of malnutrition, in both developed and developing countries, on the other (Jain et al., 2021), public health monitoring emerged as an important area for application of citizen science. In The American Gut project (The Microsetta Initiative, 2022) scientists receive stool samples from the public with the aim of identifying the relationships between health and lifestyle and the microbiome. The 100 For Parkinson's project (The Parkinson's Blog, 2022) invited people across the United Kingdom and United States to track their health for 100 days with a mobile app, with the aim of understanding how technology can support Parkinson patients. The Seattle Flu Study (Seattle Flu Alliance, 2021) focuses on studying seasonal influenza, aiming to understand how it develops and spreads in the Seattle area. Participants are typically asked to regularly answer simple survey questions and if they are identified as high-risk, they are sent a testing kit and asked to submit the swab back by post or to report the result of a self-test. Thanks to the high number and broad distribution of samples, The Seattle Flu Study was among the first to discover and identify COVID-19 in the Seattle area (Chu et al., 2020), clearly highlighting the utility of citizen science in public health monitoring and protection. Overall, these examples demonstrate the utility of citizen science programs outside of conventional academic and medical studies on assessing healthcare outcomes and impacts.

Synthesizing the capabilities of biofoundry facilities with the breadth of sampling possible with citizen-based science programs described above brings a new conception for biological monitoring and surveillance to light. When considering the limitations of citizen science programs, in terms of the input variability and the magnitude of samples collected, leveraging the processing pipeline of a biofoundry may allow more consistent results to be obtained. Furthermore, biofoundries could act as formal knowledge hubs which if engaged appropriately with the local community could facilitate the quality of input from citizen scientists. Both these aspects could encourage the establishment of more citizen science programs as biofoundries can effectively reduce some of the technical hurdles associated with citizen science. As another consideration, the automation technologies leveraged in biofoundries also enable the incorporation of additional engineering controls in the handling of hazardous samples that could de-risk many hazardous biosurveillance targets. Overall, the synergies between biofoundry automation and standardization, and the collaborative nature of biofoundries as interface between public and private sectors are all factors that point to utility and feasibility of expanding the applications of

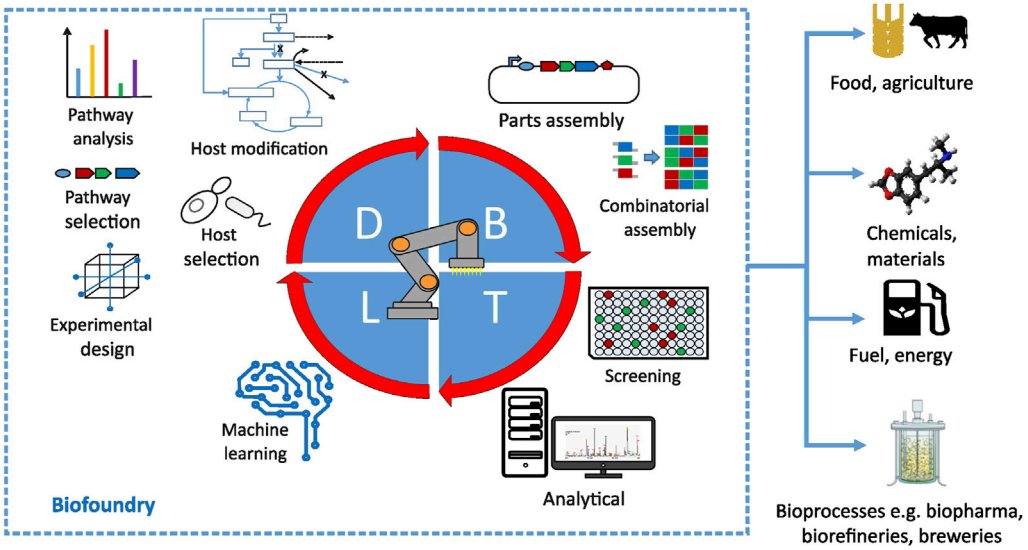

FIGURE 2
Overview of major processes in a biofoundry happening at design (D), build (B), test (T) and learn (L) stages of the development cycle. Reprinted with permission from ref. (Philp, 2021).

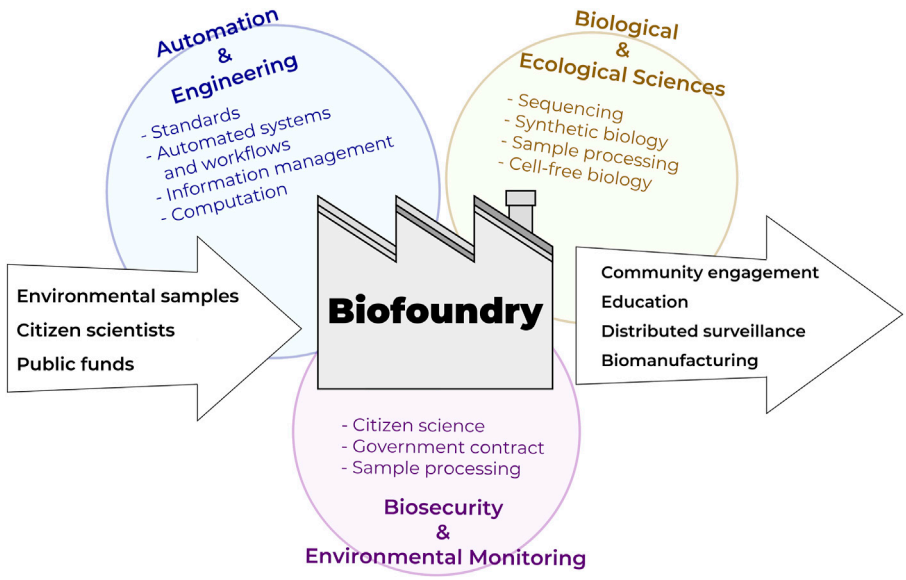

FIGURE 3
Biofoundries at the nexus of automation technologies, bioengineering, and biological/ecological monitoring interfacing with citizen science programs.

citizen science to more elusive biosurveillance targets that could strengthen existing biodefense programs and could have positive impacts on our ability to monitor the environment and public health.

Biosecurity-related activities are source of funding and direction of development for biofoundries

Biofoundries are useful to the communities of their users as hubs with dedicated instrumentation and support of skilled staff. Sample handling and processing can be automated and standardized, carried out at small and medium scale rapidly and reproducibly. Resulting data are appropriately stored and processed, often in cooperation with trained bioinformaticians. However, acquiring and maintaining dedicated equipment carries cost. Equally importantly, salaries of highly-skilled employees, together with costs for consumables for experiments, contribute to high running costs of a biofoundry (Holowko et al., 2021). Consequently, putting together a viable business model for biofoundry is challenging. Above we have outlined how biofoundries can foster and support biosecurity, bio- and environmental-surveillance efforts by various means including standardization of samples and protocols, engagement with citizen scientists, and interface with decentralized manufacturing facilities. We believe that these further strengthen rationale for structural public investment into biofoundries and that national security agencies, environmental protection agencies, and related institutions can reap substantial benefits from channeling some of their financial resources into biofoundry operations. Aside from enabling biosurveillance, such effort contributes to training of staff at the forefront of biological engineering and biorisk and environmental monitoring, which is a valuable asset for national economy and security both long and short term. Furthermore, such trained staff, at the disposition of biofoundry infrastructure, will be instrumental to establishment of biosecurity training programs for professionals across the fields of security, intelligence and law reinforcement. This was recently exemplified by hands-on introductory to synthetic biology developed in collaboration between the Federal Bureau of Investigation (FBI) and the Colorado State University (CSU) (Neil et al., 2019). Recent years have seen growing interest in and implementation of decentralized biomanufacturing facilities (also called biorefineries) (Kritharis et al., 2022). While decentralized manufacturing will likely develop infrastructure separate from biofoundries, there is potential for their synergy in bioeconomy as well as in health and biosecurity targets. Biofoundry-enabled surveillance would likely lead to shorter feedback cycles, earlier risk detection and ability to respond more locally to potential outbreaks. Such response could be further sped up by access to localized biomanufacturing facilities that would have the ability to develop therapeutic or other responses. Similarly as the ability to produce crops locally contributes to food supply chain security and sustainability, so will decentralized biomanufacturing contribute to local security and sustainability. The rise of the bioeconomy suggests that this contribution will play out on multitude of levels including therapeutics, materials, fuels and food.

Conclusion

Biological risks, including pandemics or rapid rise of antimicrobial resistance, are commonly regarded as potentially existential to humanity (FHI, 2022; CSER, 2021). Even if not fatal, biological catastrophes and engineered attacks have the potential to significantly impact lives of many, spreading rapidly to large geographical areas. Biosecurity therefore should be a critical priority for national security agencies (NSAs) worldwide. Similarly, climate change leads to gradual change of environmental conditions impacting ecosystems globally, also imposing existential threats to humanity. Accurate, wide-spread and time-resolved monitoring is crucial for effective interventions and policy making in these scenarios. Biosurveillance at the required level of spatial and temporal resolution remains challenging. Required number of samples and collection points is usually high. Moreover, samples may be perishable or pathogenic, complicating transport. In this paper, we have argued

that biofoundry facilities can support several ways to improve our ability to carry out biosurveillance. They can function as distributed hubs of data collection and analysis, empowering biosurveillance by reducing transport times. Their distributed nature further confers the system with robustness, e.g., in case of a targeted attack. They can play a key role in developing standard protocols and standardized samples and work with citizens to develop new sample collection schemes. Finally, they can collaborate with biorefineries for small scale rapid production of therapeutic compounds.

While there is potential for the vision presented in this paper (Figure 3), biofoundries worldwide are still in their early stages of development and such biosurveillance programs have challenges barring implementation. We have identified some key barriers, as well as some directions to address these below.

- **Develop biosecurity policy to leverage biofoundries.** Foremost, biofoundries may not be eligible for biosurveillance related operations and or funding as they may not qualify for the correct biosafety clearance in their jurisdiction. Regulatory frameworks and granting programs, which differ jurisdiction to jurisdiction, should be reviewed with biofoundries in mind so that appropriate amendments, that support the biosecurity capacity of biofoundries, can be identified. Additionally, with the continued creation of biofoundries worldwide, it is imperative that a unified development of standards be created and adopted such that the benefit of standardization can be preserved between nations.
- **Design biofoundries with sufficient biosafety level.** Bifoundries are currently mostly designed and classified at the biosafety level 1. In order to be able to use their capacities for broad-spectrum pathogen monitoring, they will have to classify for biosecurity level 2 clearance. There is a need for collaboration between biofoundries and biosafety regulators to apply and adapt the regulations to biofoundry use cases.
- **Expand use cases for biofoundries to include citizen science.** Citizen science programs may not be currently considered as a part of a biofoundry's use cases. Thus, a biofoundry's engagement with citizens and citizen science groups may not be adequate and could preclude their use by these groups. Therefore, it is recommended that established, and up and coming biofoundries, ensure that citizens and citizen science groups are included in the development of their facilities and invited to participate in biofoundry operations.
- **Create incentives to encourage biofoundry establishment.** As biofoundries are at the confluence of automation and biological technologies, they have the potential to closely cooperate with decentralized biomanufacturing facilities, and catalyze their further emergence. With the increasing growth in this sector, incentives for the establishment of biofoundries should be put forth as it could not only enable efforts in engineering biology, but could also help drive the transition to a circular bioeconomy.
- **Equip future biologists with quantitative and engineering skills.** While many universities have adapted their study programs and include increasing amounts of quantitative, programming and even hardware skills in their curricula, these efforts require broader adoption to build a future workforce that can effectively work at the nexus of technology and biology and continue to push it forward. As biofoundry operations and related facilities become more common, the need for such skills will continue to rise.

Biofoundries are growing in prevalence year over year, and this growth highlights the importance of assessing the role biofoundries can play in a nation's biosecurity program. Synergies with citizen science could potentially extend the breadth of biosurveillance to more subtle targets than before by leveraging biofoundry facilities. Should the concepts in this paper be implemented, it could have transformative impacts on the way we monitor health, ecology, and biosecurity, by distributing the load among a network of biofoundries.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MH conceptualized the research, carried out the interviews and wrote, reviewed and edited the manuscript. EA contributed to drafting the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Biosafety and biosecurity challenges during the COVID-19 pandemic and beyond

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As the world continues to battle the SARS-CoV-2 pandemic, it is a stark reminder of the devastation biological threats can cause. In an unprecedented way the global community saw a massive surge in the demand for diagnostic capacities, which had a substantial impact on biosafety and biosecurity. Laboratories had to cope with a surge in laboratory testing capacity, while resources and training possibilities were limited. In addition, the pandemic highlighted the impact biological threats can have, thereby giving rise to new dialogue about biosecurity and new biological threats. This paper aims to highlight some of the most pressing issues regarding biosafety and biosecurity observed during the COVID-19 pandemic with special focus on low and lower middle-income countries. The authors provide lessons learned, tools and recommendations to improve future biosafety and biosecurity and increase preparedness for the next global health crisis.

KEYWORDS

biosafety, biosecurity, online tools, emerging issues, low and lower middle-income countries

Introduction

The COVID-19 pandemic changed the world as we knew it. Not only our everyday life was profoundly shaken, also the way we perform and disseminate science faced massive overhauls. As demonstrated by the pandemic, it is essential that public health laboratories have the capacity to work safely and securely on emerging pathogens that can have high consequences. This is especially important for low and lower middle-income countries, classified by the World Bank as countries with a gross national income (GNI) *per capita* of $4,255 or less (World Bank). Due to the rapid spread of the disease around the globe and the excessive amount of potential infected patients, diagnostic laboratories faced a surge in specimen inflow. However, in the first months of the pandemic, certain characteristics of SARS-CoV-2 remained unknown and it lasted till May 2020 for the first laboratory biosafety guidance for SARS-CoV-2 to be published (WHO, 2019). New insights and developments during the pandemic led to changes in handling procedures (Kaufer et al., 2020; Naeem et al., 2022). This together with a massive growth in testing demand resulted in a series of biosafety and biosecurity issues.

Especially in the summer months of 2020 many laboratories and new established diagnostic facilities had to expand their capacities swiftly, often facing shortages in personal protective equipment and basic laboratory furniture.

Now in hindsight it is possible to identify three major topics, where biosafety and biosecurity policies may need to be adapted and improved to serve the laboratory manager and operative in a future pandemic. Those three topics are biosafety under resource limited conditions, training and communication of COVID-19 biosafety aspects, and biosecurity challenges under pandemic circumstances. The concept of biosafety is defined as the aggregate of measures, focusing on the prevention of an unintentional release of hazardous biological agents (World Health Organisation, 2020), and biosecurity as all measures focusing on the block of an intentional release of biological agents (National Research Council (US), 2009; Vennis et al., 2021; World Health Organization, 2006).

In this paper the authors describe their insights of issues and pitfalls in biosafety and biosecurity policies in practice observed in multiple countries and laboratories during the fight against the pandemic. It aims to foster a discussion on gaps and improvements in biosafety, biosecurity, and trainings by highlighting lessons learned and potential solutions.

Biosafety under resource limited conditions

The emergence of a new pathogen or a zoonotic microbe that mutated and changed its host range needs a new classification of its risk level by established experts. Such scientific studies are performed in high or maximum containment laboratories that are usually operated by governmental institutions. However, since the construction and maintenance of such laboratories is very expensive, many low and lower middle-income countries are dependent on the information provided by resource rich countries. As was observed during the COVID-19 pandemic, such information on safe handling of the virus came quickly from various laboratories. In the course of the pandemic, scientific institutions constantly gained new insights and shared them in the form of peer-reviewed publications, but also as preprints under review to timely enclose the information to the scientific community. Many publishers of scientific literature understood their role in educating people and made relevant publications about the virus free of access [Callaway, 2020; Wellcome]. Nevertheless, official global bodies such as the WHO took up to 6 months after the start of the pandemic to establish a universal list of recommendations and best practices for the safe handling of viral diagnostics (Timeline, 2019; Maxmen, 2021). Because of this delay in access to official recommendations, laboratories had to make their own biosafety protocols with limited scientific knowledge about the properties of the virus.

A safe handling of microorganisms in the laboratory is based on its risk categorisation and a risk assessment. Accordingly, operators of laboratories can select from listed techniques and SOPs suitable for them, appropriate selection of Personal Protective Equipment (PPE, primary barrier), and whether the use of a Biosafety Cabinet (secondary barrier) is advised. In resource-limited environments, not every laboratory is fully equipped with the appropriate equipment and it is a management decision for which activities to allocate the limited machine-pool. Several low and lower-middle income countries reported to be struggling with the right safety equipment such as sufficient appropriate biosafety cabinets (Faust et al., 2020). In addition to a lack in official technical information and limited biosafety resources, the halting supply of COVID-19 vaccinations to low and lower middle-income countries made it further impossible for many laboratory operators to protect their staff.

A potential solution to that issues represents the 'Sustainable Laboratories Initiative Prior Assessment Tool' an online tool supporting laboratory managers in allocating funding and laboratory equipment that is provided by the Chatham House think tank (chathamhouse, 2019). This tool is meant to help structure a conversation between funding partners and recipient countries on how to most effectively establish or repurpose laboratories in low-resource environments. The medium provides a structure for a conversation between the funding partner and recipient country early in the process. It is based on a local risk assessment, whereby laboratories are appropriately and optimally tailored to the local risks and to the resources available, both in the short and longer term, without compromising biosafety and biosecurity. It seeks to increase local ownership and help partners ensure they have given due attention to all the relevant aspects, including risks and benefits, that need to be considered at an early stage. It should provide clarity on what is needed and improve the sustainability of any laboratory project that might result from the discussions. The tool contains questions regarding national strategic engagement, general framing of the laboratory and four essential functional aspects that should be considered prior to embarking on establishing or repurposing a laboratory: finance, human resources, operations, and infrastructure and utilities (chathamhouse, 2019).

An alternative *ad hoc* solution for countries struggling with a massive outbreak of a disease include the deployment of a mobile laboratory operated by several countries or state unions like the EU, WHO and others (Wölfel et al., 2015; EU CBRN CoE). Such mobile laboratories are designed to operate in resource-limited areas and are rapidly deployable. They contain equipment to perform basic diagnostic analysis on given pathogens and are intended to give a short time relief to governmental diagnostic laboratories until a stable operative infrastructure is built. However, mobile laboratories are very expensive to set up and are further dependent on highly qualified technical personnel. Policymakers from low and lower middle-income countries should know that many countries are operating such laboratories and are happy to support health systems in need. Nevertheless, the pandemic may serve as a wakeup call for many policymakers that the next global health crisis may be just around the corner and it needs funding and dedication from the governmental bodies to install the primary and secondary barriers to be physically prepared for the next outbreak.

Training and communication of COVID-19 biosafety aspects

Next to the physical preparation of a country to raise its resilience against the next pandemic it is paramount to invest into highly qualified and reliable staff operating in the laboratories and performing the diagnostic testing.

Staff working in a certified ISO 15189 or 17025 laboratory regularly needs to attend advanced training courses to keep their certification (OECD, 1998; Zimmermann et al., 2019). Copious

training courses are offered among others by several governmental institutions or non-profit organisations. However, the SARS-CoV-2 pandemic with its global traveling restrictions and the sudden need for more personnel brought such training to a standstill.

Over the timespan of a few months into the pandemic, many organisations started to offer online training courses. However, these solutions faced several challenges. Beside many technical hurdles that contained mostly limited access to computer hardware or insufficient internet connections, cultural challenges also had to be overcome. Offering such courses often faced difficulties to reach the correct audience. There is no point in teaching a laboratory manager the correct procedures in how to run a qPCR, when the technical staff never hears of this information. Hence, it was good to build on pre-existing networks and train the trainer initiatives to ensure the proper use of such online training courses. Several online initiatives by various national and international institutions were launched over the last 3 years. For instance, the German Biosecurity Programme funded by the German Foreign Office launched the "COVID-19 Digital Initiative". This consists of two main components 1) a COVID-19 Information Hub, and 2) a series of COVID-19 related digital self-study modules. While the first provided a demand-driven selection of scientific publications and regular newsletters informing about advances in fighting the pandemic, the latter focused on virtual and practical laboratory training. In total, seven modules available in three languages (English, French and Russian) taught the basics on how to safely handle swabs samples, isolate viral RNA, and conduct WHO approved PCR screening (Peintner, 2023). While this course was created as a self-study initiative, other initiatives hired a designated teacher that informed their participants in their native language about biosafety measures regarding the handling of SARS-CoV-2 in the laboratory (Zimmermann et al., 2019).

Another example is the "Biosafety/Biosecurity Hybrid Train the Trainers Program in Georgia" organized by the Netherlands National Institute for Public Health and the Environment (RIVM), co-funded by the Dutch Ministry of Foreign Affairs and CBRN Centres of Excellence Project 53. This program, available in English and Georgian, taught Basic Laboratory Biosafety, Biorisk Assessment, Dual-Use, and how to train new trainers in a hybrid manner, starting with interactive online sessions, followed by in person training once the travel restrictions were released.

A completely different approach in supporting policymakers and lab operators are online decision-making tools. For example, the Netherlands Biosecurity Office has developed a toolkit that can help to increase biosecurity awareness (bureaubiosecurity). Besides an informative film, and gadgets to raise biosecurity awareness (postcards and the 10 golden security rules), the biosecurity toolkit also includes the 'Biosecurity Self-scan Toolkit' and the "Vulnerability Scan". These are online tools to analyse biosecurity vulnerabilities in an organisation dealing with high consequence pathogens. Furthermore, as precise instructions for researchers on how to perform a dual-use risk assessment was largely lacking, the Biosecurity Office developed the "Dual-Use Quickscan". This tool aims to identify potential dual-use aspects in research and contributes to stimulating dual-use awareness. Increased international attention to examine pathogens with pandemic potential has been enhanced by the COVID-19 pandemic, hence monitoring of dual-use potential needs to be encouraged (Vennis et al., 2021).

Moreover, Biosecurity Central is a publicly available web-based library that helps users find relevant and reliable sources of information for key areas of biosecurity. The site aims to widely disseminate and share knowledge to help advance biosafety and biosecurity. The library is a searchable and filterable database designed to enable ready access to biosafety and biosecurity resources from around the globe, published by governmental, international, and non-governmental organisations (Biosecurity Central). Table 1 provides multiple examples of tools that support biosafety and biosecurity.

The COVID-19 pandemic has brought about preventive measures that have had a considerable impact on various dimensions of biosafety and biosecurity teaching and learning. While digital teaching and learning approaches cannot substitute in-person training, they have shown to be useful tools to complement other training formats, and can provide guidance during outbreak of newly emerging pathogens, such as SARS-CoV-2.

Biosecurity challenges under pandemic circumstances (in regard of physical and cybersecurity aspects)

The rise of the SARS-CoV-2 pandemic has sparked public interest in the biological sciences. In contrast to before the pandemic, non-professionals became familiar with concepts of incidence rates, incubation periods, herd immunity, vaccinations and PCR testing. In addition, the pandemic initiated new discussions about weaponization of biological entities and biosecurity gained new momentum (CTPN, 2021). Although the use of microorganisms and toxins as weapons is strictly prohibited by the Biological and Toxin Weapons Convention (BTWC) and United Nations Security Council Resolution 1540 (UNSCR1540), some think tanks see a potential rise in the interest of individual states of starting and pursuing new biological weapons initiatives. The Washington D.C. based council of strategic risks envisions three potential scenarios developing from the COVID-19 crisis (Bajema et al, 2022). In Scenario one they claim that the damage caused by COVID-19 leads to the rise of biological weapons as a significant component of deterrence for many nations, with these trends intersecting and feeding into greater security tensions. Scenario two envisions the exact opposite and predicts that fear of future biological threats bolsters international cooperation—states are driven to avoid another catastrophic biological event, working together to better utilise technologies and enhance diplomatic mechanisms. In Scenario 3 the think tank combines these two aforementioned scenarios and envisions a lack of momentum after the current pandemic translates into weak progress in strengthening healthcare systems, waning interest in developing global early warning systems, and a continued rise of biological threats. They claim that these scenarios may help policymakers by illustrating the plausible ways biological weapons could shape global affairs—and in turn, provide the foresight needed to make decisions and investments that avoid the worst of these realities.

Other institutions like the European Center of Disease Control (ECDC) sees the biggest danger in new forms of terrorism. Now the public is aware of the threats posed in a (zoonotic) outbreak (Episode

TABLE 1 Some examples of tools to support education and outreach on biosafety and biosecurity topics. The tools listed are created and maintained by either governmental or non-governmental organizations and have the common goal of assisting life science researchers and laboratory managers in creating a safe work environment.

Name of tool	Content	Access
Biosecurity Central	-Laboratory biosafety -Legal mechanisms and authorities -Risk assessment -Laboratory biosecurity -High-consequence pathogens -Laboratory research -Dual use -Animal health, Zoonotic diseases -Law enforcement -Medical diagnostics -Export, Sample transportation -Environmental safety	https://biosecuritycentral.org
Surge Capacity Assessment Tool	E-learning and assessment questionnaire and calculator	https://lms.sckcen.elonisas.dev/moodle/login
Dual-Use Quickscan	Freely available webtool to assess dual-use potential of life science research	https://dualusequickscan.com
German Online Platform for Biosecurity and Biosafety (GO4BSB): 'COVID-19 Digital Initiative'	-Newsletter -Wiki -Collection of publications -Self-study modules	www.go4bsb.de
Biosecurity pillars of good practice	Basics of working with high risk biological material	https://www.bureaubiosecurity.nl/en/node/531
Biosecurity Vulnerability Scan	Helps to detect weak spots in laboratory security. An extensive scan with questions, scenarios and best practices built around the eight pillars of biosecurity	https://www.biosecurityvulnerabilityscan.nl
Biosecurity Self-scan Toolkit	A relatively fast scan with a limited number of closed questions that can easily form an indication of strong and weak biosecurity aspects within your organisation	https://biosecurityselfscan.nl
Ten golden rules of biosecurity and biosafety	Explains the cornerstones of biosecurity	https://www.bureaubiosecurity.nl/en/information/10-golden-rules-of-security
Biosecurity Checklist	Laboratory Biosecurity Assessment and Monitoring Checklist for biosecurity monitoring and auditing of laboratories	https://www.liebertpub.com/doi/10.1177/1535676019838077 Brizee et al., 2019
International Gene Synthesis Consortium	A common protocol to screen DNA sequences	https://genesynthesisconsortium.org/
Basic cybersecurity measures	Several cases exploring potential cybersecurity issues	https://english.ncsc.nl/

23 - Paul Riley—Bioterrorism and biosecurity). Terrorists could instrumentalize these fears and cause mass panic among citizens. Even though terrorists are probably not able to successfully build and deploy biological warheads, the simple spraying of bacteria or viruses in a densely populated area or the poisoning of drinking water would be enough to terrify the public. There are also stories of panic caused by excessive faked coughing in a public gathering to disturb a political discussion (Arora et al., 2020). Bioterrorism should be seen as one of the new asymmetric challenges of the contemporary international security environment with the aim to impose concrete political, ideological and quasi-religious opinions mainly by non-state aggressive actors (Maisaia and Alika, 2020).

Although most terrorists are unlikely to be able to build a biological weapon, bioscientists do have the necessary skills. One of the greatest threats to the successful misuse of microorganisms is therefore rogue scientists, who pose a potential insider threat (Perkins and Fabregas, 1773). The fight against insider threat is largely based on personnel reliability. Insiders with fraudulent intent can look up information and have access to high consequence pathogens easily as they have been granted access to databases and pathogen inventories. Hence it is paramount to perform an in-depth security check of all existing and new employees in an institution that is handling sensitive information. One initiative to screen the activities of scientists rests in the surveillance on the orders of primers and gene sequences by the 'International Gene Synthesis Consortium (IGSC)' (IGSC, 2017). With regard to research with the virus and the production of (parts of) SARS-CoV-2, there are guidelines for ordering synthesised viral sequences (*e.g.*, primers for PCR). The IGSC is an industry-led group of gene synthesis companies and organisations and has established a "Harmonised Screening Protocol" to prevent abuse of synthetically produced sequences. It is their aim to protect the positive aspects of gene synthesis technology while minimising the risk of misuse.

Most life scientists probably do not have malicious intents, but it is important that they have sufficient awareness about biosafety and biosecurity to work safe and securely. For example, it is crucial for employees to be aware to never leave data unprotected and unattended. Still one of the most common ways to get behind the firewall of databases are phishing programmes on USB sticks or E-mail attachments. The best digital countermeasures can be easily bypassed by the thoughtlessness of the employees (Ferreira and Cruz-Correia, 2021; Mueller, 2021). As these examples demonstrate, security in the biological sciences is expanding to the cyberspace.

Hence, in the last decade the term cyberbiosecurity was termed (Adler et al., 2021). Richardson et al. describe cyberbiosecurity as *"addresses the potential for or actual malicious destruction, misuse, or exploitation of valuable information, processes, and material at the interface of the life sciences and digital worlds*" (Richardson et al., 2019). Key issues of concern include, among others, the privacy of patient data, the security of public health databases, the integrity of diagnostic test data, the integrity of public biological databases, the security implications of automated laboratory systems and the security of proprietary biological engineering advances.

But, as already briefly mentioned above, cyberbiosecurity does not only concern the public health sector but amongst others also the field of synthetic biology. Technologies in synthetic biology were rapidly advancing over the last decade and genetic sequences were openly published. With the new techniques and public genetic information whole stretches of sequences can be produced artificially. Even a bigger threat is the possibility of cyber-criminals remotely injecting malicious DNA sequences, resulting in life scientist unknowingly developing biological threats (Puzis et al., 2020). Another cyberbiosecurity example is the possibility to hack a negative pressure system with the aim to breach containment of dangerous pathogens. Researchers in the US sought to probe whether negative pressure systems could be hacked and succeeded (Poste and Gillum, 2023). This highlights the need for robust cybersecurity measures to protect vital healthcare infrastructure during a public health emergency.

During the COVID-19 pandemic, there were multiple reported cases of cyberattacks targeting healthcare organizations, including hospitals and research institutions. These attacks aimed to disrupt operations and steal sensitive information, such as patient data and research findings. Further, as a result of the pandemic, many organizations shifted to remote work, which increased the risk of cyberattacks such as phishing, malware and other forms of cybercrime. In 2020, several hospitals in India reported cyberattacks that disrupted their operations, including the theft of patient data (AFP, 2022; Wasserman and Wasserman, 2022) and also Brazil reported an increase in cybercrime, including phishing scams and ransomware attacks targeting individuals and organizations, including healthcare providers (Macedo and Singleton). In Africa there have been numerous reported cases of cybercrime targeting individuals and organizations in different African countries during the pandemic, including phishing scams, malware, and ransomware attacks (Chigada and Madzinga, 2021). These attacks took advantage of the increased reliance on digital systems during the pandemic, highlighting the need for improved cybersecurity measures, especially in healthcare organizations in low and lower-middle income countries.

The impact of the pandemic on biosecurity is discussed on many levels. WHO, for instance, aims to publish a new laboratory biosecurity guidance for biorisk management in the beginning of 2023 (Kojima, 2022), as the latest edition dates from 2006 (World Health Organization, 2006). The WHO saw that after the pandemic there is a need to develop a global minimum requirement for safeguarding global health security. WHO is calling for a consensus definition of global minimum requirements focused on biological risk management of laboratory activities. They call for consensus-based standards developed for global best practices, not to replace them. These claims follow three rationales: First, WHO identifies growing concerns for biosafety and biosecurity. WHO recommends their "WHO BioHub system biosafety and biosecurity: Criteria and operational modalities" (World Health Organization, 2022). Second, WHO calls for a review of existing legislation. They ask if the current national legislations are enough to prevent various scenarios? Finally, WHO wants to increase the focus on the Biological and Toxin Weapons Convention (BTWC). They call for a verification mechanism based on ISO35001 with a neutral third party assessment for safe and secure operations (ISO 35001:2019, 2019).

In addition, international initiatives such as the Global Health Security Agenda (GHSA), Global Biosecurity Dialog (GBD), and the Global Partnership Against the Spread of Weapons and Materials of Mass Destruction (GPWMD) play a major role in building biosecurity capacity and employing international legally binding biosecurity instruments (Vennis et al., 2022). Such legal instruments, as the BTWC (disarmament) and UNSCR1540 (UN Security Council, 1540) are international legally binding non-proliferation instruments to reduce dangers of deliberate disease outbreaks in humans, animals and plants. The BTWC also contributes to global disease surveillance as it requests international exchange of equipment, materials, and information to combat outbreaks of infectious diseases. UNSCR1540 emphasises safe and secure handling, use, transport, and storage of pathogenic material, thereby contributing to biosafety and biosecurity. Furthermore, the COVID-19 pandemic has increased attention toward the WHO's International Health Regulations 2005 (IHR) (World Health Organisation, 2005). IHR focusses on infectious disease outbreaks with a natural origin and covers some aspects of accidental and deliberate releases. However, independent of the origin of a disease outbreak, an effective public health response is necessary to control it.

(Vennis et al., 2022) identified overlapping and complementary issues in IHR, UNSCR1540 and BTWC with the aim to improve understanding of policymakers, civil servants, biosecurity experts, and practitioners regarding these instruments. This accommodates the enhancement of full employment of national resources to comply with international requirements, ultimately leading to an improved capacity to prevent, detect and respond to infectious disease outbreaks, independent of their origin.

Lessons learned and suggestions for improvements

As with the corona pandemic, previous outbreaks also highlighted weaknesses in laboratory preparedness. One of the examples of laboratory shortcomings during the SARS outbreak (2002–2004) are the reports on laboratory acquired infections in China and Singapore (Lim et al., 2004; WHO, 2004). The SARS outbreak demonstrated there are unforeseeable threats, whether natural emerging diseases or biosecurity threats. After SARS, the International Health Regulations (IHR) were revised with the aim to prevent and control public health threats while avoiding unnecessary interference with international travel and trade. The revised regulations included *"all events potentially constituting a public health emergency of international concern (PHEIC)"* (CDC, 2019). Monitoring and evaluation of IHR was mainly through the States' Self-Assessment Annual Report (SPAR). The Ebola outbreak (2014–2016) clearly demonstrated that this self-reporting mechanism did not provide an accurate representation of IHR implementation. The countries concerned with Ebola had

reported rather high levels of implementation, which appeared to be an overestimation once facing the outbreak. After the Ebola outbreak the JEEs (joint external evaluations) were established to move from exclusive self-evaluation to approaches that combine self-evaluation, peer review and voluntary external evaluations involving a combination of domestic and independent experts (WHO, 2005). In October 2019, the Global Health Security Index analysis found no country to be fully prepared for epidemics or pandemics (Vennis et al., 2022). The COVID-19 pandemic demonstrated that the world collectively indeed did not have sufficient capacity to prevent and control major infectious disease outbreaks, as also shown in the 2021 Global Health Security Index report. The report found "Although many countries were able to quickly develop capacities to address COVID-19, all countries remain dangerously unprepared for meeting future epidemic and pandemic threats." Towards the end of the pandemic statements were made that the IHR "are a conservative instrument that constrain rather than facilitate rapid action" (Sirleaf and Clark, 2021). WHO established a Review Committee on the Functioning of the International Health Regulations (2005) during the COVID-19 Response. The committee summarized that the IHR can certainly facilitate adequately, but many countries only applied the IHR in part and that WHO did not make fully use the established powers they have (WHO, 2021). The COVID-19 pandemic and previous outbreaks demonstrate that many international efforts were made to adhere to an international standard of preparedness. However, both the Ebola outbreak and COVID-19 pandemic clearly show that implementation of IHR in practise is still a weakness.

Furthermore, many countries, both low and lower middle-income countries and resource rich countries, faced difficulties to keep an overview on the maturing body of SARS CoV-2 knowledge, including biosafety and biosecurity measures. Still, it was apparent that many low and lower middle-income countries struggled to have equal access to diagnostic tools, safety equipment, training, and vaccine supply. Hence, it needs to be the focus of the global community to prepare for these issues in non-pandemic times. There is a need for a strategy on how to train more laboratory specialists, so that they are readily available in the next pandemic and to install a viable global stockpiling system of diagnostic materials and laboratory equipment to supply all countries equivalent.

So far, this paper elaborated on biosafety and biosecurity standards in public health, since that was the field that got challenged the most during the COVID-19 pandemic. However, SARS CoV-2 is a zoonotic disease and the hunt for the species that finally transduced the virus from animals to humans is still ongoing (Lytras et al., 2021). Hence, biosafety and biosecurity in the area of animal health play a critical role in preventing and controlling veterinary disease outbreaks that can pose significant risks to public health and the economy. Effective biosafety and biosecurity measures are crucial in preventing and controlling the spread of diseases in animals and reducing the risk of transmission to humans. The WHO propagates this in an one-health approach (WHO), however, in low and lower middle-income countries farmers and meat production companies often face the issue of a lack of resources such as funding, trained personnel, and infrastructures for animal health (Future of Animal Science Research, 2015). These existing infrastructures may not meet the necessary biosafety and biosecurity standards (Siengsanan-Lamont et al., 2019). This includes facilities for housing and caring for animals, as well as laboratories for disease diagnostics. These deficits in the hardware can be potentiated with a lack of awareness and education among relevant personnel and farmers about the importance of biosafety and biosecurity in animal health, and the measures that need to be taken to prevent and control disease outbreaks.

To address the above mentioned challenges, it is important to invest in building the necessary resources and infrastructure in a one-health setting, as well as in increasing awareness, education, and training about the importance of biosafety and biosecurity measures (Butucel et al., 2022). Additionally, international cooperation and collaboration are essential in sharing knowledge, best practices and resources to improve the implementation of these measures, particularly in low and lower middle-income countries. Furthermore, the authors argue that international regulations are important, but biorisk management could benefit from more emphasis on practical implementation of biosafety and biosecurity policies.

Conclusion

The ongoing COVID-19 pandemic highlights the need for laboratories that have the capacity to work safely and securely with emerging pathogens. International instruments from different disciplines address these health and security challenges, setting requirements for states to effectively prevent, detect, and respond to infectious disease outbreaks, either with deliberate or non-deliberate origin (Vennis et al., 2022).

In this policy and practice review the authors intended to highlight some of the initiatives that aim to tackle biosafety-, biosecurity- and training concerns provoked by the pandemic. However, the pandemic is only slowly coming to an end and it will take many more years to fully understand the impact of this event on how we will perform safe and secure science and diagnostics in the future.

Author contributions

IV, SR, and LP conceived the project. IV, SR, and LP wrote the manuscript. EW and VM contributed additional information. All authors read and supported the final version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Application of multi-criteria decision analysis techniques and decision support framework for informing select agent designation for agricultural animal pathogens

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The United States Department of Agriculture (USDA), Division of Agricultural Select Agents and Toxins (DASAT) established a list of biological agents and toxins (Select Agent List) that potentially threaten agricultural health and safety, the procedures governing the transfer of those agents, and training requirements for entities working with them. Every 2 years the USDA DASAT reviews the Select Agent List, using subject matter experts (SMEs) to perform an assessment and rank the agents. To assist the USDA DASAT biennial review process, we explored the applicability of multi-criteria decision analysis (MCDA) techniques and a Decision Support Framework (DSF) in a logic tree format to identify pathogens for consideration as select agents, applying the approach broadly to include non-select agents to evaluate its robustness and generality. We conducted a literature review of 41 pathogens against 21 criteria for assessing agricultural threat, economic impact, and bioterrorism risk and documented the findings to support this assessment. The most prominent data gaps were those for aerosol stability and animal infectious dose by inhalation and ingestion routes. Technical review of published data and associated scoring recommendations by pathogen-specific SMEs was found to be critical for accuracy, particularly for pathogens with very few known cases, or where proxy data (e.g., from animal models or similar organisms) were used to address data gaps. The MCDA analysis supported the intuitive sense that select agents should rank high on the relative risk scale when considering agricultural health consequences of a bioterrorism attack. However, comparing select agents with non-select agents indicated that there was not a clean break in scores to suggest thresholds for designating select agents, requiring subject matter expertise collectively to establish which analytical results were in good agreement to support the intended purpose in designating select agents. The DSF utilized a logic tree approach to identify pathogens that are of sufficiently low concern that they can be ruled out from consideration as a select agent. In contrast to the MCDA approach, the DSF rules out a pathogen if it fails to meet even one criteria threshold. Both the MCDA and DSF approaches arrived at similar

conclusions, suggesting the value of employing the two analytical approaches to add robustness for decision making.

KEYWORDS

multi-criteria decision analysis, decision support framework, select agent designation, agriculture animal pathogen, risk assessment tool

Introduction

Incidents of biological warfare have been historically well-documented (Geissler, van Courtland Moon, 1999; Carus, 2002). While most of these incidents have been directed against humans, biological agents have also been used by state programs against animals to promote sabotage and weaken the enemy. For example, during World War I (WWI), Germany covertly inoculated military horses and cattle, most extensively those belonging to neutral suppliers of the Allied Powers, with *Burkholderia mallei* (glanders) and *Bacillus anthracis* (anthrax) (Wheelis, 1999). After WWI, many countries [e.g., Canada, France, Germany, Hungary, Italy, Japan, Soviet Union, United Kingdom (U.K.), and the United States (U.S.)] started to develop biological weapons programs primarily as a deterrent or for retaliatory purposes (Wheelis et al., 2006). Beginning in 1940, the Germans took an active interest in countering a foot-and-mouth disease (FMD) threat to their own cattle while they explored the use of this virus as an offensive weapon. Defensive vaccine production began in 1940, and by 1943 they had experimented with ways to disseminate FMD virus using little bunches of grass or hay dropped from specific heights to create an inconspicuous dispersal (Geissler, 1999). Most belligerents entered World War II (WWII) with at least exploratory biological weapons programs against personnel and animals, and most increased their activities during the war (Wheelis et al., 2006). Apparently, only the U.K. mass-produced a usable biological weapon targeting animals, which consisted of 5 million cattle cakes comprised of linseed meal laced with spores of *B. anthracis*. It was expected the cattle cakes would be dropped from bombers onto German fields to cripple their domestic animal production in retaliation if the Germans used biological weapons against the allies (Wheelis, M. et al., 2006).

After WWII, the strategic use of biological weapons against animals by state programs was, for the most part, to reduce enemy food supplies or to cause economic damage (Millett, 2006). FMD virus was the subject of considerable research as a weapon by the U.S., U.K., Canada, and the Soviet Union among others but never used (Millett, 2006; Alibek and Handelman 1999). However, there were reports describing the use of zoonotic bacterial pathogens against animal targets. In 1978, Rhodesia with assistance from South Africa purportedly attacked cattle in the Rhodesian tribal trust lands with *B. anthracis*, which also resulted in numerous human infections caused by eating infected animals or encountering spores (Mangold and Goldberg, 1999; Martinez, 2003). By 1980, more than 10,000 Zimbabweans had reportedly developed anthrax and 182 had died (Martinez, 2003). In another incident, between 1982 and 84, the Soviet Union was alleged to have attacked the mujaheddin and their horses in Afghanistan with *B. mallei* on at least one occasion (Alibek and Handelman 1999).

Today, the deliberate misuse of biological agents by terrorists and criminals poses a threat not only to public health, but also to the agricultural sector and the food chain. The intentional use of biological agents to attack crops or animal agriculture has been termed agroterrorism (Ryan and Glarum, 2008). Agriculture and food systems are extensive, open, interconnected, diverse, complex structures providing terrorists and criminals targets for plant and animal diseases. Agroterrorism is viewed as a desirable option for terrorists and criminals for several reasons. First, pathogens exist in natural reservoirs and would be relatively easy to obtain. Second, security measures at facilities where livestock are raised, or fed (i.e., feed lots) are normally low. Simple methods may be used to introduce the pathogen and the high-density conditions under which livestock are raised today, together with their mobility, will enhance its spread. Third, the time between introduction of the pathogen and when disease is noticed would allow the perpetrator to get away from the scene of the crime. Fourth, most of the animal viruses (e.g., FMD virus, Rinderpest, African Swine Fever virus) of interest to terrorists are not infectious for humans, so terrorists would not have to worry about infecting themselves. Fifth, a terrorist attack on livestock could significantly damage the U. S. economy. FMD is the most economically devastating livestock disease in the world. It has been estimated that a single case of FMD in the U. S. would result in the loss of $12–20 billion due to restrictions on cattle exports from the U. S. that would be imposed, culling of animal populations exposed to the virus, decontamination and other expenses involved in regaining national FMD-free status (Schoenbaum and Disney, 2003). Outbreaks of animal diseases, regardless of origins, could undermine the capacity to export agricultural goods, thereby generating significant losses to the economy.

Many serious animal diseases that do not exist in the U. S. (i.e., foreign animal diseases) could be of interest to terrorists and are of great concern to U. S. animal health officials. The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture (2020) works with state animal health officials and veterinarians to identify, control, and eradicate these diseases. At the international level, the World Organization for Animal Health (WOAH, formerly the Office International des Epizooties/ Epizootics [OIE]), is responsible for tracking diseases throughout the world and provides rules for animal movement and disease control. The World Trade Organization recognizes WOAH as the international agency for setting animal health standards for conducting international trade. The WOAH maintains a list of diseases of concern; the current list combines the former Lists A and B (which were mentioned in Public Law 107-188, 2002) into one consolidated list that divides the diseases of concern by host (REPORT OF THE MEETING OF THE OIE WORKING GROUP ON WILDLIFE DISEASES Paris, 4-6, 2000). Inclusion criteria for the WOAH list include four considerations: potential for international spread; significant spread within naïve populations; zoonotic potential; and emerging diseases. The presence or absence

of confirmed WOAH reportable diseases in specific commercial livestock (i.e., cattle, sheep, goats, equine, swine), commercial poultry and aquaculture species are currently monitored in WOAH member states by domestic programs (e.g., National Animal Health Reporting System) (Ryan and Glarum, 2008).

So far, agroterrorism has not been a serious problem; however, the proliferation of terrorist groups with different agendas and the availability of biological agents in the environment heightens concerns (Keremidis et al., 2013). The complex global food trade and risks associated with livestock transport present vulnerabilities that may have undesirable economic animal and public (if zoonotic) health implications. Furthermore, an attack on animals is generally viewed as more restrained and less offensive than an attack against humans. Agricultural terrorism is not about killing animals; it is about crippling an economy. The outbreak of FMD in the UK in 2001 highlighted the enormous consequences, both economic and in animal health, that even a natural outbreak can have for a country (Gibbs, 2003).

These events and others have led to the promulgation of regulations to ensure the biosafety and biosecurity of animal pathogens. The effort began in 1996 when the U.S. Congress passed the Antiterrorism and Effective Death Penalty Act (Public Law 104-132, 1996) in recognition of the need for regulations to ensure the safe and secure transfer of hazardous biological agents and toxins when shipped between facilities. The legislation directed the Department of Health and Human Services (2020) to establish a list of biological agents and toxins (Select Agent Regulation. 42 C.F.R. Part 73, 2023), which included zoonotic pathogens, that could potentially threaten human health and safety. This list ultimately became part of the Select Agent Regulation, which was delegated by DHHS to be administered by the Centers for Disease Control and Prevention (CDC) (Morse, 2015). In the aftermath of the release of *B. anthracis* spores through the U.S. mail in the fall of 2001, Congress significantly strengthened and expanded oversight of Select Agents with the passage of the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (Public Law 107-188, 2002); among other things, this law expanded controls from shipment of hazardous biological toxins and agents to their possession and use. Subtitle B (Agricultural Bioterrorism Protection Act of 2002) of PL 107–188 directed the Secretary of the USDA to establish and maintain a list of biological agents and toxins that he/she determined have the potential to pose a severe threat to animal health or products. The criteria for inclusion on this list included: 1) availability and effectiveness of pharmacotherapy and prophylaxis to treat and prevent any illness; 2) economic impact; 3) inclusion on the then-OIE A and B lists (Ryan and Glarum, 2008); and 4) presence on the Australia Group List (Australia Group List, 2017). Non-biological criteria—economic consequences and effect on international trade agreements—were of paramount importance when considering agents for this list. Thus, these agents have been designated USDA Select Agents not because they necessarily pose a threat to animal health but because they pose a threat to national security (National Research Council, 2010). This contrasts with the DHHS list where the impact on public health and safety were primary factors for inclusion. Agents and toxins that appear on both the USDA and DHHS lists are referred to as Overlap Agents and are regulated by both agencies. The comparable USDA regulation 9 C.F.R. Part 121 governs select agents and toxins that have the potential to pose a severe threat to animal health or to animal products (Select Agent Regulation. 9 C.F.R. Part 121, 2023). Furthermore, Title 7 U.S. Code 8401 requires the Secretary of USDA to review and republish the list biennially, or more often as needed, and shall by regulation revise the list as necessary.

Recently, we explored the applicability of MCDA techniques and DSF logic tree analyses to assist the CDC Select Agent Program's biennial review of the Select Agent and Toxin List, applying the approach broadly to include non-select agents and toxins to evaluate its generality (Pillai et al., 2022; Pillai et al., 2022). A description of these methodologies, their advantages and disadvantages, and their prior use has been previously described (Pillai et al., 2022).

In this study we evaluated whether approaches used for HHS agents would be effective in assisting the USDA DASAT in their biennial review process. Two analytical approaches were developed and evaluated for classifying bacteria and viruses as USDA Select Agents: an MCDA framework and a DSF logic tree. Previous efforts by the USDA DASAT to review its Select Agent List relied solely on subject matter expert (SME) assessments to assess the agents and did not include non-select agent pathogens due to the additional burden placed on the SMEs. The analytical approaches we describe herein seek to provide a systematic approach and decision analysis techniques for assessing the impact on national security, and to reduce the burden on SMEs by documenting the supporting data from peer-reviewed literature in agent fact sheets to support the process.

Methods

Analytical framework

The starting point for the MCDA analysis was a set of 21 criteria (Table 1) that affect bioterrorism risk, including factors that would affect the public health impact of zoonoses. For convenience, these criteria were grouped into those that are relevant for agent production, agent exposure, exposure consequence, mitigation, or potential economic impact (Table 1). SMEs, or the analysis team, scored these 21 criteria on a scale of 0–10, based on the scoring definitions in Table 1, for each of the biological agents in Table 2. The scoring scale reflects relative concern as it pertains to the agent's designation as a select agent, with 0 corresponding to lowest concern and 10 corresponding to highest concern. For simplicity, a linear scale was chosen for this evaluation. Table 1 lists the scoring definitions for each of the criteria for even-numbered scoring options (0, 2, 4, 6, 8, and 10). In the event SMEs were not in agreement on an even-numbered score, which sometimes occurred for criteria with more qualitative data, we assigned odd-numbers as an intermediate score.

The scores for each agent were used to inform identification of pathogens for consideration as select agents as follows. Several of these scores had multiple components: first, scores for 1a, 1b, 1c, 1d and 1e (Table 1) were averaged to give a score for Ease of Production (Criterion 1); scores for 5a, 5b and 5c were averaged to give a score for Ease of Introduction (Criterion 5); scores for 12a and 12b were averaged to give a score for Farm Impact (Criterion

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TABLE 1 Criteria scoring definitions.

PRODUCTION	
Ease of Production (1)– The ease of producing agent in the laboratory as measured by the skill required, availability of growth media and equipment, time required, yield and storage stability.	
Production Skill Required (1a) – The level of training and agent-specific expertise needed to produce the agent and maintain pathogenicity:	
0	Difficult to produce
2	Expert-level training and agent specific experience
4	Expert-level training with similar organisms
6	Proficient in tissue culture and/or expert in aseptic technique
8	Basic microbiology training
10	Untrained
Growth Conditions (1b) – The availability of growth media, culture and/or equipment required to successfully grow the agent:	
0	No known cell lines available
2	Virus: Special post processing required
4	Virus: Special cell line required. Bacteria: Must be grown *in vivo* or *in vitro*
6	Virus: Common cell line required (e.g., Vero E6). Bacteria: Requires cell line or anaerobic conditions
8	Bacteria: Only grown in a single, complex broth or requires additional processing
10	Bacteria: Can be grown in common broths
Growth Time (1c) – The length of time to produce the agent based on growth characteristics of the agent:	
0	>1 month
2	14-28 days
4	10-13 days
6	7-9 days
8	3-6 days
10	2 days or less
Production Yield (1d) – Highest concentration (pfu or cfu/mL) achieved by experts using optimal production methods:	
0	$<10^2$ per mL
2	10^2-10^3 per mL
4	10^4-10^5 per mL
6	10^6-10^7 per mL
8	10^8-10^{10} per mL
10	$>10^{10}$ per mL
Storage Stability (1e) – The amount of agent lost during storage at 4°C:	
0	>1 log loss/day
2	1 log loss/day
4	1 log loss/week
6	1 log loss/month
8	1 log loss/year
10	<1 log loss/year
Ability to Genetically Manipulate or Alter (2) – The degree of difficulty of the techniques required to create a more virulent, transmissible, environmentally stable or countermeasure-resistant strain:	
0	No known method to genetically manipulate and maintain pathogenicity
2	Very difficult (e.g., negative strand RNA viruses)
4	Highly difficult (e.g., positive strand RNA viruses, gene reassortment or reverse genetics available)
6	Moderately difficult (e.g., DNA viruses and intracellular bacteria)
8	Low difficulty (e.g., plasmid insertion for bacteria)
10	No directed genetic manipulation required (e.g., can use selection for antibiotic resistance)
EXPOSURE	
Susceptible Hosts (3) – Number and type of livestock species that are susceptible to the disease	
0	None
2	Horses, goats, sheep or fish
4	Poultry
6	Cattle or pigs
8	Multiple agricultural animal hosts
10	Multiple agricultural animal hosts and/or zoonotic

(Continued on following page)

TABLE 1 (*Continued*) Criteria scoring definitions.

EXPOSURE	
Environmental Stability (4) – The extent to which the agent is stable in the environment (outside the host) in matrices such as soil and dried on surfaces	
0	Agent decays immediately upon dissemination
2	Agent persists in indoor environments for minutes to hours
4	Agent persists in indoor environments for days to weeks
6	Agent persists in indoor environments for months to years or outdoors for hours to days
8	Agent persists in outdoor environments for weeks to months
10	Agent persists in outdoor environments for > 1 year
Ease of Introduction (5) – The ease with which the agent can be introduced to the target host	
Route of Exposure (5a) – The routes in which the disease is infectious to livestock. Routes below limited to direct contact, cutaneous, vector, ingestion, inhalation. Vertical and trans-mammary transmission not included	
0	None
2	Direct contact, cutaneous and/or vector
4	Ingestion
6	Inhalation
8	2 different routes
10	3 different routes
Infectious dose (ID_{50}) (5b) – The dose or amount of agent (in cfu or pfu as appropriate) required to infect 50% of a healthy livestock population by inhalation or ingestion (score worst case):	
0	Not infectious by inhalation or ingestion
2	>10,000
4	1000-10,000
6	100-1000
8	10-100
10	1-10
Transmissibility animal to animal (5c) – The extent to which the disease can be transmitted from one animal to another within a farm	
0	Non-communicable and non-transmissible
2	Rare animal-to-animal transmission
4	Transmission via non-airborne vectors such as ticks or limited animal-to-animal transmission
6	Moderate animal-to-animal transmission
8	Relatively high transmission via airborne vectors such as mosquitoes and flies
10	Highly transmissible among one or more animal species
CONSEQUENCES	
Farm Production (6) – The impact on animal/farm production (meat, eggs, milk, hides, breeding) due to illness	
0	Little or no symptoms or impact
2	Minimal to no impact on production due to mild symptoms of short duration
4	Decreased production for up to 1 month
6	Decreased production among existing herd for months to a year due to ongoing symptoms or treatments
8	Decreased production due to symptoms among existing herd and losses of replacement stock (e.g., abortions, neonatal mortality, sterility, inability to breed)
10	Total production loss due to culling and/or acute mortality with no replacement stock available
Status of Immunity (7) – The extent to which the population has immunity to the disease due to previous exposure or vaccination:	
0	Close to 100%
2	Majority (>80%) of population have immunity
4	Significant portion (20-80%) of population have immunity
6	Previous vaccines may have reduced impact
8	Small subset (<5%) have immunity
10	No presumed immunity to agent in population
Acute Mortality (8) – The number of deaths from the disease per 100 diagnosed cases (case fatality rate). Deaths are based on a non-vaccinated, sensitive population and includes deaths resultant from culling practices.	
0	Close to 0%
2	1-9%
4	10-29%
6	30-39%
8	40-49%
10	50-100%

(Continued on following page)

TABLE 1 (*Continued*) Criteria scoring definitions.

CONSEQUENCES	
Transmission Farm to Farm (9) – The extent to which the disease can be transmitted from one farm to another	
0	Non-communicable and non-transmissible between farms
2	Transmission via wildlife
4	Fomite transmission and/or limited farm to farm transmission observed
6	Vector transmission
8	Fomite and vector transmission
10	Air-borne transmission
Public Health Impact (10) – The potential impact on human health from the agent	
0	Does not cause disease in humans
2	Causes mild symptoms and/or is only rarely lethal in humans
4	Causes moderate morbidity and low mortality (CFR <9%) in humans
6	Causes moderate morbidity and mortality (CFR 10-29%) in humans
8	Causes high morbidity and mortality in humans (CFR >30%)
10	Causes high morbidity and mortality in humans and is human-to-human transmissible
MITIGATION	
Availability of Vaccines (11) – The availability of vaccines and extent to which they can be rapidly deployed and administered in response to an animal health emergency to prevent disease and transmission:	
0	No vaccine required (includes already vaccinated) or unlikely to be administered
2	Widely available and easy to deploy efficiently, e.g., a single course
4	Widely available but difficult to deploy efficiently, e.g., multi-course, lengthy; or lacks efficacy
6	Approved vaccine available in limited quantities and/or vaccine approved in other countries; available in US through IND
8	Experimental, unapproved vaccine in development
10	No vaccine available
Farm Impact (12) – The potential impacts to a farm due to animal quarantine, decontamination and restoration during and after an event :	
Animal Quarantine (12a) – The duration and extent of quarantine that may be required for animals potentially exposed to the agent:	
0	None
2	1-7 days
4	8-15 days
6	16-90 days
8	91-365 days
10	>1 year or unknown
Decon and Restoration (12b) – Effort required after the outbreak to return to normal operations:	
0	No decon required
2	Low level disinfectants such as quaternary ammonium compounds are effective (e.g., gram(-) bacteria, enveloped viruses)
4	Intermediate level disinfectants such as 70% ethanol, phenolics and iodophors are effective (e.g., gram(+) bacteria, fungi)
6	High level disinfectants such as glutaraldehyde, H2O2, ClO2, peracetic acid are required (e.g., non-enveloped viruses)
8	Extensive chemical decon and restoration is required (e.g., spores, mycobacteria)
10	Highly resistant to disinfection or sterilization methods
ECONOMIC IMPACT	
Burden/ Impact on US Agriculture (13) – The potential economic impacts to US agriculture during and after an event.	
Export trade impact (13a) – The value of the industry and extent to which the commodity is exported from the US as measured by percent of total US production	
0	No impact to US industry or food industry
2	US industry size is small (<$5B/yr) and not significantly exported
4	US industry size is small (<$5B/yr) with significant exports (>10%) or expected to be minimal due to limited animal to animal or farm to farm transmission, existing treatment, and surveillance and remedial efforts
6	US Industry size is large ($5-50B/yr) and not significantly exported
8	US industry size is large ($5-50B/yr) with significant exports (>10%)
10	US industry size is very large (>$50B/yr) with significant exports (>10%)

(Continued on following page)

TABLE 1 (*Continued*) **Criteria scoring definitions.**

ECONOMIC IMPACT	
US Industry Impact (13b) – The scope and duration of impacts to US agriculture during and after an event:	
0	No impact to the Ag industry
2	Low impact to industry, as typically non-fatal and animals recover with little or no intervention
4	Short-term impact on a limited scale, due to low disease persistence and/or effective decontamination and limited farm-to-farm transmission
6	Longer-term impact on a limited scale, due to disease persistence and/or need for culling and limited farm-to-farm transmission
8	Potential for industry-wide impact due to need for culling and high farm-to-farm transmission
10	Significant industry-wide impact due to difficulty in eradication (e.g., high disease persistence, farm-to-farm transmission and need for culling)

12); and scores for 13a and 13b were averaged to give a score for Burden/Impact on US Agriculture (Criterion 13) as succinctly summarized in Figure 1.

Next, the resulting 13 factor scores, i.e., the four composite scores noted above (1, 5, 12, and 13) plus the remaining nine single-criterion scores (2, 3, 4, 6, 7, 8, 9, 10 and 11) for each biological agent were compiled in two ways: 1) a one-dimensional (1-D) ranking whereby the total unweighted or weighted sum (as defined in the next section) for each agent was tallied and the agents were ranked from lowest to highest; and 2) a two-dimensional (2-D) plot whereby the unweighted or weighted sum of the sub-scores for the "production" (1 + 2) plus "exposure" (3 + 4 + 5) branches of the hierarchy were plotted against the unweighted or weighted sum of the sub-scores for the "consequences" (6 + 7 + 8 + 9 + 10) plus "mitigation" (11 + 12 + 13) branches of the hierarchy (see Figures 4, 6).

Criteria weighting

Weights were assigned to each criterion to account for factors that may carry more significance for the goals of the select agent program. SMEs ranked each of the 13 criteria collectively, from one to three, where one described the least important criteria and three described the most important criteria. To demonstrate the MCDA methodology, two weighting schemes were tested: equal weighting, i.e., unweighted and the weighting scheme derived from the SME's inputs, as shown in Table 3. In the latter case, seven criteria (Ease of Production, Ease of Introduction, Farm Production, Status of Immunity, Acute Mortality, Transmission farm-to-farm and Burden/Impact on U.S. Agriculture) were given a 3x weight, two criteria (Availability of Vaccines and Farm Impact) a 2x weight, and the last four criteria (Ability to Genetically Manipulate, Susceptible Host, Environmental Stability and Public Health Impact) a 1x weight. For both cases, criteria and weights were combined into a single score A) by summing all the weighted numerical values (a_{ij},w_i), where a_{ij} represents a criteria score and w_j is the criteria weighting value:

$$A = \sum_{j=1}^{n} aij \bullet wj$$

To enable comparison of results using different weighting values, normalized scores were used, whereby the total or sub-total scores were normalized to those of a hypothetical agent that received 10s for all 21 criteria scores.

Agent fact sheets

To document the data used for scoring pathogens against the 21 criteria noted above, we developed agent fact sheets for 41 pathogens (Table 2). The list includes 24 USDA select agents, of which 11 are also HHS Select Agents (i.e., overlap agents), and 17 non-select agents, of which 4 aquaculture pathogens were included in the analysis based on SME input.

Development of the agent fact sheets used peer-reviewed open literature such as Medline, PubMed, Google Scholar and other unclassified data followed by extensive review by SMEs who work with the specific pathogen. In situations where there were data gaps, SME judgment provided a basis for scoring, referencing data for similar organisms or relevant models as appropriate (e.g., laboratory challenge experiments for infectious dose). In circumstances where a range of values was found (e.g., production yields, infectious dose), the worst reasonable case (i.e., leading to the largest "bad" outcome) was typically used for scoring. In all cases, SME judgement was relied upon to provide concurrence on the best available data or basis for scoring. SMEs identified by the USDA DASAT were asked to review the data provided on the fact sheets for accuracy and relevance, as well as the scores assigned to each data category. Comments received from SMEs were verified through literature search, review of unpublished data and corroboration with other SMEs and incorporated into the agent fact sheets and scoring adjusted, as necessary.

Decision support framework (DSF)

The DSF approach applies key criteria using a logic tree format to identify pathogens which may be of sufficiently low concern that they can be ruled out from consideration as a select agent. The DSF is complementary to the MCDA approach and avoids the possible unintended numerical equivalences that may occur using weighted, or unweighted, sums. Additionally, the DSF considers the potential impact associated with regulating an agent versus the agricultural implications and animal health practices. Using the DSF approach as shown in Figure 2, if a pathogen does not meet a threshold value for any one of the criteria set, it is deemed of low concern and thus is not considered for select agent status. Those pathogens that exceed all criteria thresholds are considered for select agent status. Criteria include Agent Qualification, Pathogenicity/Severity of Illness, Production/Introduction/Stability/Route of Infection, Vulnerable

Population/Susceptible Host, Immunity/Morbidity, Zoonosis, Transmission, Farm Impact, Medical Countermeasures, Case Fatality Rate (animals and humans if zoonotic)/Culling of animals, and Economic and Animal Health Impact. SME judgment based on data captured in the agent fact sheets provided the basis for scoring. In general, criteria which received a score of zero, two or four in some cases typically served as a basis for a "low concern" qualitative assessment. In contrast to the MCDA approach, which uses a graded scoring system for ranking agents, the DSF approach can rule out an agent from select agent consideration using a single (low scoring) criterion. Many of the criteria overlap between the MCDA and DSF approaches.

Results

Data gaps and quality

When considering many micro-organisms across a broad range of attributes, data gaps and variability in data quality are inevitable. Data availability in the open literature tended to parallel scientific inquiry for the organism; for example, aerosol studies were more prevalent for pathogens known or suspected to be infectious by the aerosol route, and surface stability data were generally more available for pathogens where fomite transmission is a concern. Overall, we found the most prominent data gaps were in aerosol stability and animal infectious dose by inhalation and ingestion routes. For aerosol stability data, we typically used data for similar organisms (e.g., same virus family) as proxies, and infectious dose data from animal models where available to address data gaps.

Unweighted rankings

To facilitate comparison of the analytical results with current assignments as Tier one select agents, select agents, and non-select agents, the three classes of agents were color coded red, blue and green, respectively, in Figures 3–6.

Initial inspection of the 1-D results, whereby the total summated scores for all 41 pathogens are compared (Figure 3) indicated that, in general, the Tier 1 select agents were found at the top of the rank-ordered list, other select agents fell in the middle section, and non-select agents comprised the bottom section; however, there were exceptions. Similarly, for the 2-D plots, whereby summated sub-scores for all 41 pathogens are plotted against each other (Figure 4), Tier 1 select agents and other select agents were generally found in the upper right quadrant of the plot, while non-select agents generally fell outside that area; however, there were exceptions.

Analysis of both the 1-D and 2-D plots indicated that, although there were general trends in the data that were consistent with current classifications, there were no sharp breaks or gaps in scoring that would serve as a basis or threshold for classifying an agent as a select agent. Instead, the plots represented a continuum of scores. Additionally, any designation of a minimal score—whether the total score in the 1-D plot, or sub-scores corresponding to the x- and y-values in the 2-D plots—resulted in some exceptions to current classifications. While the current Select Agent List is not absolute nor the definitive source for which agents should be considered select agents, it provides a useful reference point for evaluating the impact of setting minimum scoring thresholds as the basis for classifying pathogens as select agents.

For example, in the 2-D plot, if the threshold for the x-axis and y-axis scores for a select agent were designated as 0.53 and 0.54,

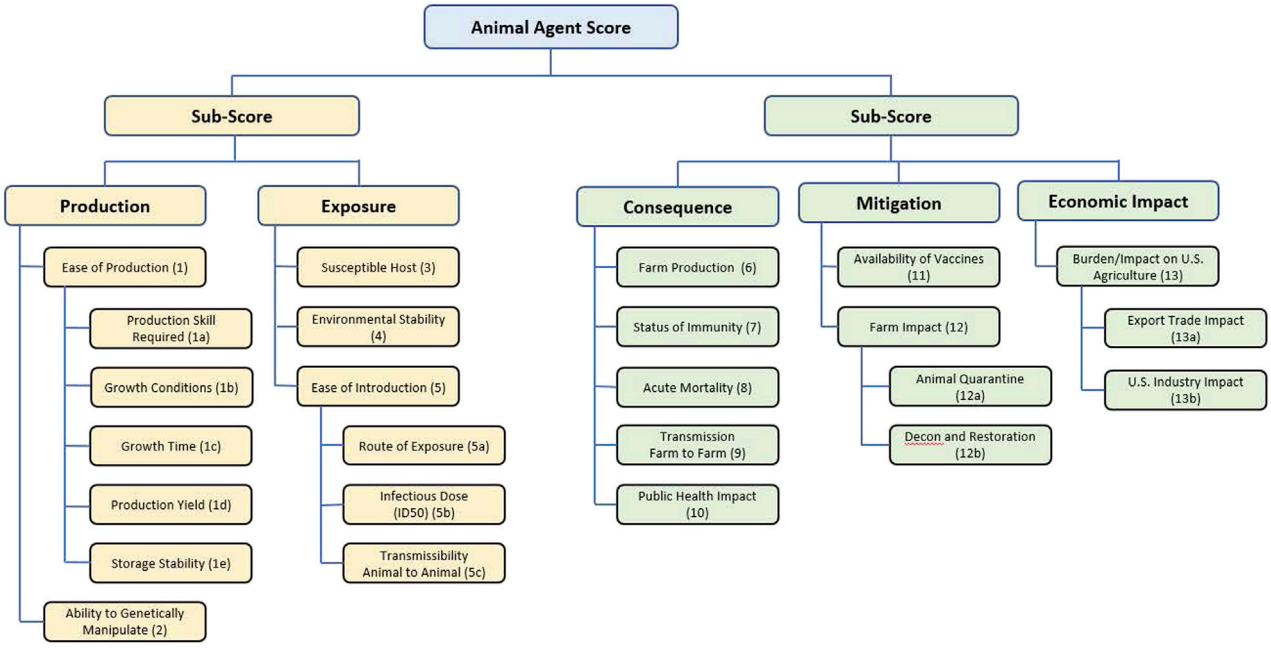

FIGURE 1
Summary of the criteria and hierarchy captured in the MCDA tool and fact sheets for animal select agent tiering.

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TABLE 2 List of animal and aquaculture select, and non-select agents considered in this analysis.

Tier 1 Select Agents	Non-Select Agents
• *Bacillus anthracis*[a]	• Avian Influenza virus (low path) (LPAI)
• *Burkholderia mallei*[a]	• Bluetongue virus
• *Burkholderia pseudomallei*[a]	• Camel Pox virus
• Foot and Mouth Disease virus (FMD)[b]	• Getah virus
• Rinderpest Virus	• Japanese Encephalititis virus (JEV)
Select Agents	• Louping Ill virus (LIV)
• African Horse Sickness virus (AHSV)	• Malignant Catarrhal Fever virus (MCFV)
• African Swine Fever virus (ASFV)	• Menangle virus
• Avian Influenza virus (hi path) (HPAI)	• Nairobi Sheep Disease (NSDV)
• *Bacillus anthracis* Pasteur[a]	• Orf virus
• *Brucella abortus*[a]	• Rabies virus
• *Brucella melitensis*[a]	• Suid Herpesvirus 1 (SHV1)
• *Brucella suis*[a]	• Vesicular Stomatitis virus (VSV)
• Classical Swine Fever virus (CSFV)	• Infectious Hematopoietic Necrosis virus[c] (IHNV)
• Hendra virus[a]	• Infectious Salmon Anemia virus[c] (ISAV)
• Lumpy Skin Disease virus (LSDV)	• Spring Viremia of Carp virus[c] (SVCV)
• *Mycoplasma capricolum*	• Viral Hemorrhagic Septicemia virus[c] (VHSV)
• *Mycoplasma mycoides*	
• Newcastle virus	
• Nipah virus[a]	
• Peste des Petite Ruminants virus (PPR)	
• Rift Valley Fever virus[a] (RVFV)	
• Sheep and Goatpox virus (S&G Pox)	
• Swine Vesicular Disease virus (SVDV)	
• Venezuelan Equine Encephalitis virus[a] (VEEV)	

[a]Overlap Select Agents.
[b]Abbreviations used in Figures.
[c]Aquaculture pathogens.

respectively, based on SME input, this led to the notional threshold for classification as shown in Figure 4. Using this basis for classification, we found that all current select agents reclassified as select agents except African Horse Sickness virus, *B. anthracis* Pasteur, *B. abortus*, *B. suis*, and Venezuelan Equine Encephalitis virus. All non-select agents reclassified as non-select agents except Japanese Encephalitis virus, Louping Ill virus, Malignant Catarrhal Fever virus, and Rabies virus.

Weighted rankings

The data using the proposed weighting scheme in Table 3 for 1-D and 2-D formats are shown in Figures 5, 6, respectively. As observed with the unweighted data, the general trend in the data was consistent with current classifications; however, any designation of a minimal score as a basis for classification—whether the total score in the 1-D plot, or sub-scores corresponding to x- and y-axes values in the 2-D plots—resulted in some exceptions to current classifications. For example, in the 2-D plot, if we designated the lowest *x*-axis and y-axis scores allowed for classification as a select agent to be 0.59 and 0.58, respectively, based on SME input, as illustrated in Figure 6, we found that all select agents reclassified as select agents except African Horse Sickness virus, *B. anthracis* Pasteur, *B. abortus*, *B. melitensis*, *B. suis*, and Venezuelan Equine Encephalitis virus. All non-select agents reclassified as non-select agents.

Decision support framework

To evaluate the 41 select and non-select agents using the DSF approach, we leveraged the agent fact sheets developed for this analysis.

TABLE 3 Proposed weighting schemes explored for animal select agent tiering.

Criteria	SME assigned weight
1) Ease of production	3
2) Ability to genetically manipulate	1
3) Susceptible hosts	1
4) Environmental stability	1
5) Ease of introduction	3
6) Farm production	3
7) Status of immunity	3
8) Acute mortality	3
9) Transmission farm-to-farm	3
10) Public health impact	1
11) Availability of vaccines	2
12) Farm impact	2
13) Burden/Impact on US agriculture	3

For the factor of Pathogenicity/Severity of Illness, the score for Farm Production was used as it incorporates the clinical information affecting diseased animals, with a score of 2 or below used to determine low level of concern. A score of 4 or below for Ease of Production was used to determine low level of concern for production. Ease of Introduction was used to determine Introduction, Stability, and Route of Infection with a score of 4 or below to determine agents of low concern. A score of 0 for Vulnerable Population and Susceptible Host was used to determine an agent was of low concern. A score of 0 for Immunity and Morbidity was used to determine an agent was of low concern. A score of 0 for animal-to-animal transmission was used to determine the low level of concern. A score of 0 for Transmission Farm-to-Farm was used to determine an agent was of low concern. A score of 2 or below was used for Farm Impact to determine an agent was of low concern. A score of 0 for availability and effectiveness of medical countermeasures was used to determine an agent was of low concern. A score of 4 or below for Case Fatality Rate and Culling of Animals by leveraging Acute Mortality data to determine an agent was of low concern. A score of 2 or below for Economic, and Animal Health Impact was used to determine an agent was of low concern. The results (Figure 2) showed that all select agents were identified for consideration as select agents except African Horse Sickness virus, *B. anthracis* Pasteur, *B. abortus*, *B. melitensis*, *B. suis*, and Venezuelan Equine Encephalitis virus. All non-select agents were ruled out from select agent consideration.

Discussion

Pathogen selection and prioritization for a specific intended use could be carried out using a formalized risk ranking process with weighted criteria that were selected to meet a required objective (McFadden et al., 2016). Similar processes have been previously used in both public health and veterinary health spheres (Caroden et al., 2009; Havelaar et al., 2010; Ciliberti et al., 2015; McFadden et al., 2016; Roelandt et al., 2017) to support prevention, early warning surveillance and control measures for disease incursion. Although there is no universal methodology for risk ranking, it is important that risk ranking exercises use a structured approach, which is transparent and consistently documented to be reproducible. MCDA- and DSF-based risk assessments are already recognized as useful tools to support select agent and toxin designations (Pillai et al., 2022; Pillai et al., 2022).

Here we investigated using MCDA and DSF as a structured approach to inform the designation of select agents of agricultural significance. The approach was flexible with the ability to adjust both the criteria and their weighting based on SME input and contribution.

The criteria we employed in this analysis are based on those identified in the Agricultural Bioterrorism Protection Act of 2002 Part B (Public Law 107-188, 2002), which directs the USDA Secretary to establish and maintain a list of biological agents and toxins that he/she determined have the potential to pose a severe threat to animal health or products. In addition, Title 7 U.S. Code 8401 requires the evaluation of whether such inclusion would have a substantial negative impact on the research and development of solutions for the animal and plant disease caused by the agent or toxin; and whether the negative impact would substantially outweigh the risk posed by the agent or toxin to animal or plant health if it is not included on the list. Comparison of these criteria with other published methods shows that many of them overlap, such as morbidity and mortality, route of exposure, environmental stability, transmissibility, ease of production, availability of Medical Countermeasure (MCMs), etc. We also include the Public Health Impact based on SME input to capture potential zoonotic impacts. Note that while it is considered an additional risk factor, zoonotic potential in and of itself would not be enough to push an otherwise low-scoring animal pathogen above thresholds for consideration as an agricultural select agent. Criteria we did not consider include

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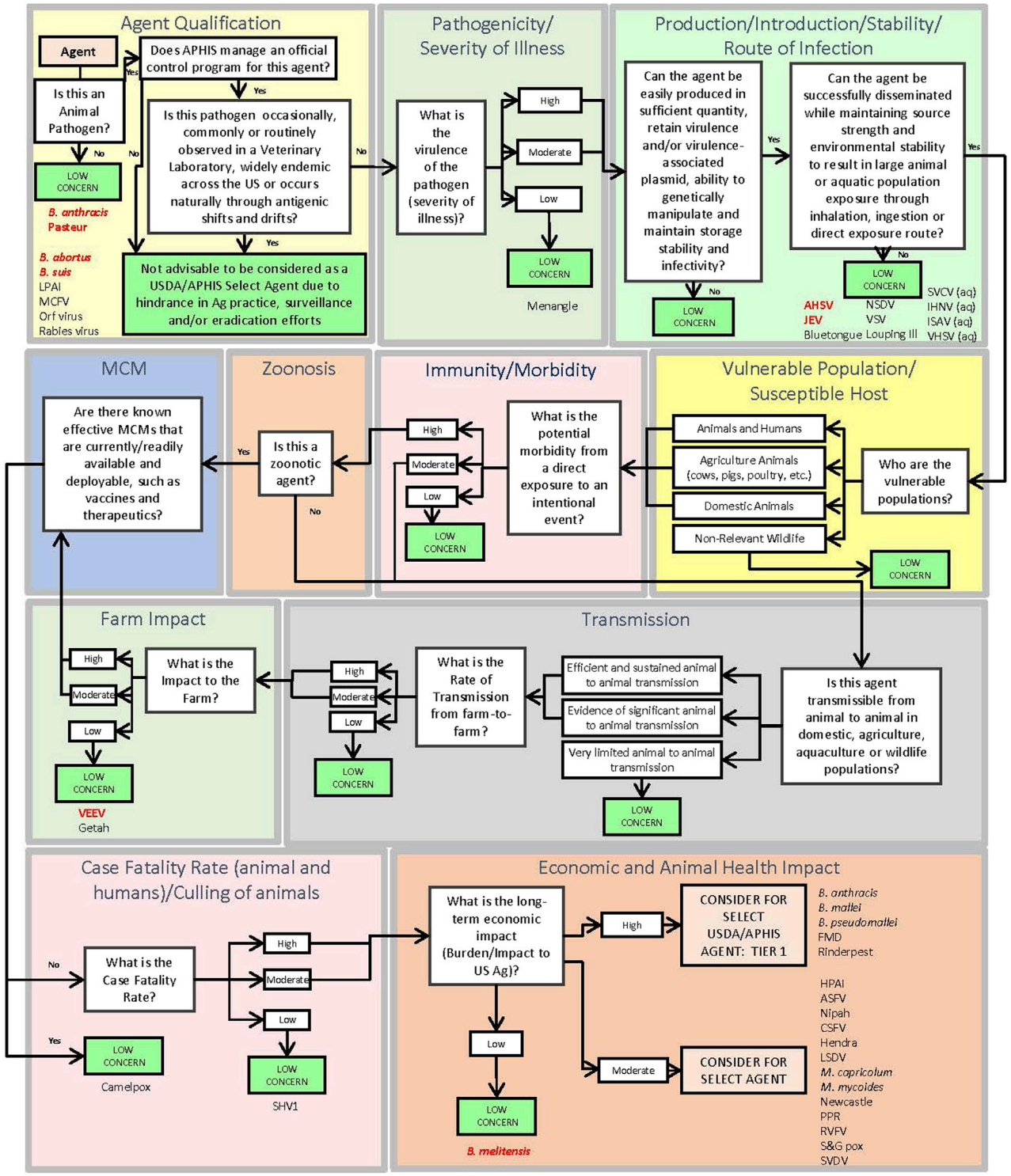

FIGURE 2
Schematic of the Decision Support Framework logic tree showing assignment of animal select and non-select agents (Abbreviations as in Table 2).

public perception or terror factor, accessibility of agent and ease of detection, surveillance and laboratory diagnosis.

In addition to the choice of criteria, the focus on agroterrorism (i.e., aerosol or food-based introduction through animal feed) attacks affecting a large segment of the agricultural animal population is embodied in the scoring scales. Common pathogens causing mild illness and where there are treatments readily available may be unlikely to require a large-scale agricultural health response.

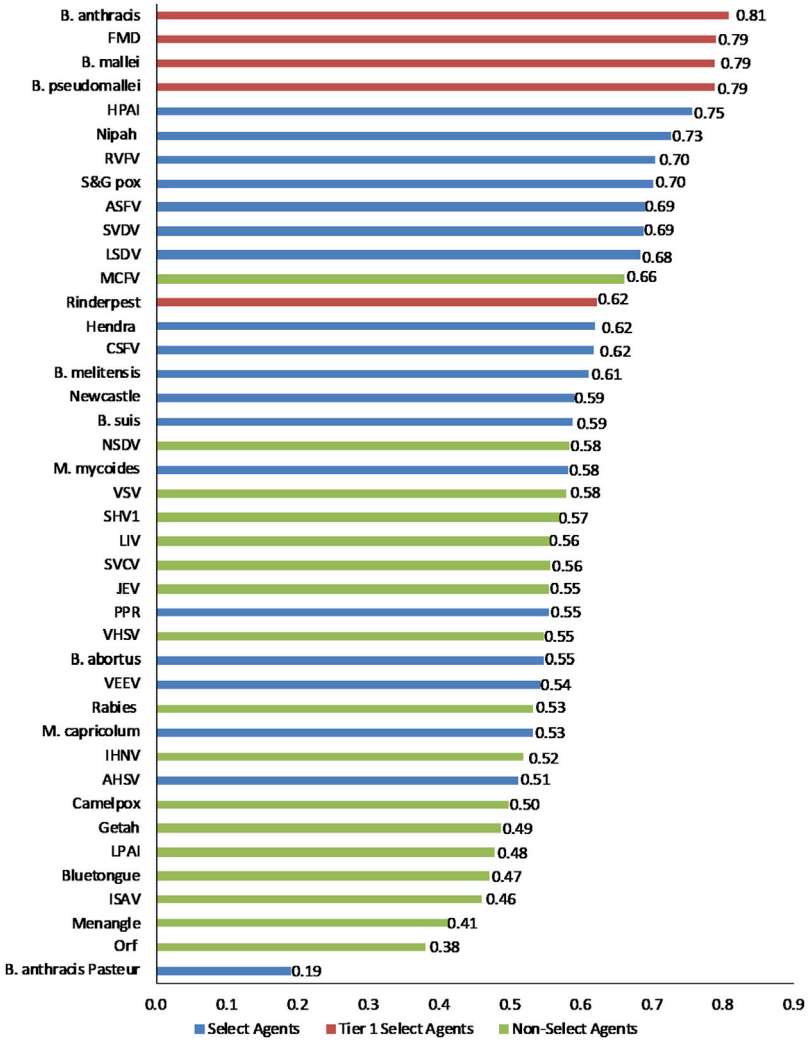

FIGURE 3
1-D plot of unweighted scoring results for animal select agent tiering (Abbreviations as in Table 2).

We evaluated two methods, MCDA and DSF, for their individual merits and to provide confirmation of the observed results. While both methods enabled a risk-informed comparison of a diverse set of pathogens in a structured way, the MCDA results were challenged by a continuum of scores that did not suggest natural thresholds for classification of select agents. Potential pitfalls of MCDA techniques are described in Cox et al., 2005, and while alternative treatments of the data may be of future interest (see for example, Pillai et al., 2022), this analysis highlights some of the challenges that can arise when considering a large, diverse set of pathogens. Alternatively, the DSF employs a series of criteria thresholds to identify pathogens for consideration as a select agent and provides clear classification assignments.

The finding that both approaches arrived at a consistent set of pathogens for consideration as select agents supported their usefulness. Interestingly both approaches also arrived at a consistent set of current select agents that should not be considered as select agents. The MCDA and DSF methodologies supported all current DASAT animal select agent designations and all non-select agents (Table 2) except for *B. anthracis* Pasteur, *B. abortus, B. melitensis, B. suis,* African horse sickness virus and Venezuelan equine encephalitis virus, which are currently select agents but failed to meet the criteria established for MCDA and DSF methods.

With African Horse Sickness Virus, the DSF factors related to the production and dissemination of the virus resulted in USDA SMEs concurrence that difficulties exist in the successful dissemination and effective transmission of the virus that will result in a large animal population exposure. MCDA factors that contributed to the outcome were the existence of an efficacious vaccine along with low to moderate environmental stability and difficulties associated with the introduction to an animal population and to maintain sustained transmission.

With *B. anthracis* Pasteur, the primary DSF criteria that it is not an animal pathogen indicated this agent does not qualify as a USDA select agent. MCDA factors similarly showed the agent was of no risk to farm production, mortality, farm to farm transmission, economic impact, and low risk to farm impact. In addition, the low virulence of the agent provided additional supporting data for supporting removal of the agent from the Select Agent List.

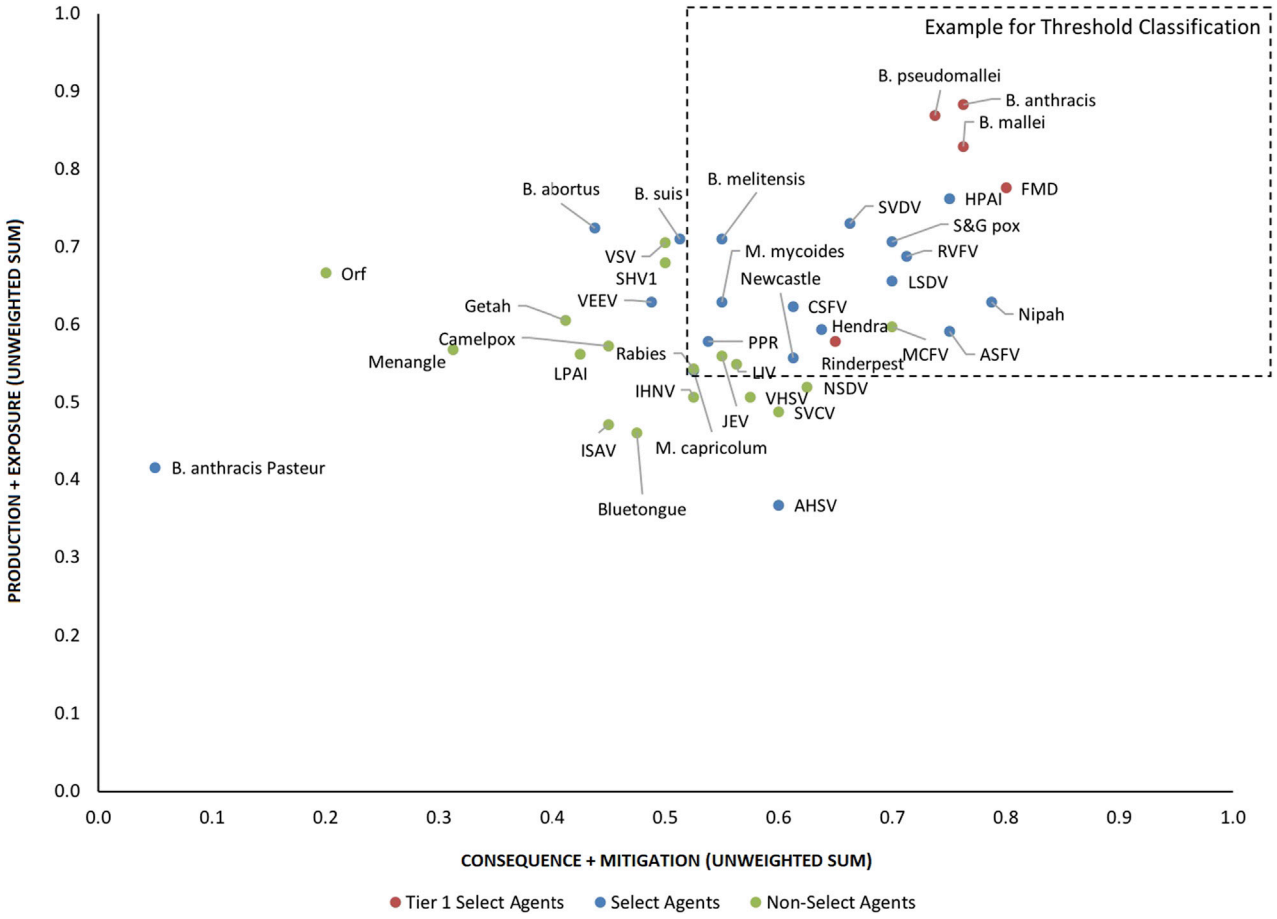

FIGURE 4
2-D plot of unweighted scoring results for animal select agent tiering (Abbreviations as in Table 2).

During the analysis of *B. abortus* using the DSF, it was recognized that the agent is occasionally observed in veterinary diagnostic laboratories in endemic areas, is widely distributed in wildlife hosts such as the Bison and Elk populations in Yellowstone National Park and continues to increase in prevalence and distribution. As such, inclusion of the agent on the Select Agent and Toxin list would have a substantial negative impact on the research and development of solutions for the animal disease. MCDA factors that contributed to the outcome were the existence of an efficacious vaccine, moderate immunity status of vulnerable population, limited Farm-to Farm transmission risk and moderate farm impact, and moderate risk due to difficulty related to large-scale introduction to an animal population. Economic impact was considered to have low risk from a domestic and international trade perspective due to limited Farm-to-Farm transmission, and factors that would be more regional or local to an infected premises. Public health impact was considered as a low risk with efficient treatment methods available and very low untreated mortality rates which can range from 0.5%–5% with an average of <2% (WHO guidance, 2004) and treated mortality rate is <1% (Castano et al., 2017); and in the U.S. is close to 0% (CDC, personal communication).

B. suis was ruled out for consideration as a select agent using the DSF because the agent is occasionally observed in veterinary diagnostic laboratories and is widely endemic in animal populations, such as feral swine population in more that 40 U.S. states, and continues to pose a significant threat to domestic swine population across the U.S. As such, inclusion of the agent on the Select Agents and Toxins list would have a substantial negative impact on the research and development of solutions for the animal disease. MCDA factors that supported removal as a select agent were the limited Farm-to-Farm transmission risk and moderate farm impact, and moderate risk due to difficulty of large-scale introduction to an animal population. Economic impact was considered to have low domestic and intentional trade risk due to limited Farm-to-Farm transmission, and factors would be more regional or local to an infected premises. Public health impact was considered as a low risk with efficient treatment availability and very low untreated mortality rate which can range from 0.5%–5% with an average of <2% (WHO guidance, 2004) and treated mortality rate is <1% (Castano et al., 2017); and in the U.S. is close to 0% (CDC personal communication).

During the analysis of *B. melitensis* using the DSF, the agent was ruled out for consideration as a select agent due to low concern associated with long-term economic and animal health impact. The effect upon agricultural economic factors was low based on the size of the domestic goat and sheep industry. MCDA factors that supported

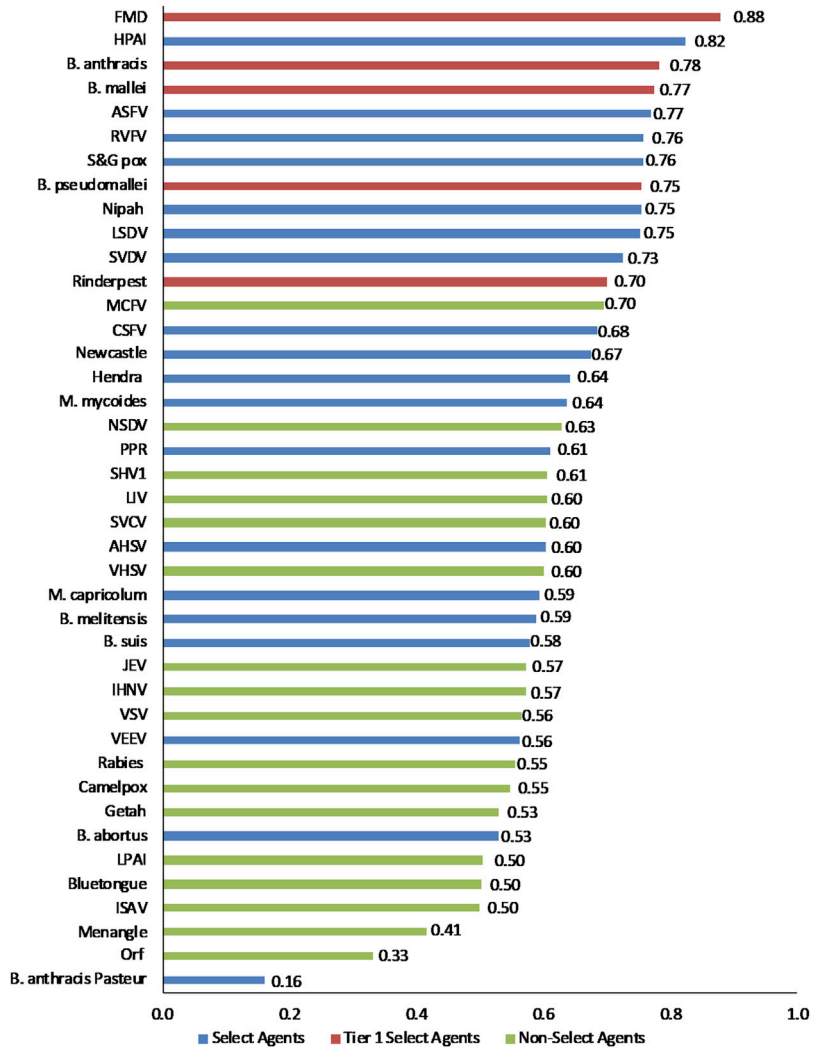

FIGURE 5
1-D results for the proposed weighting scheme for animal select agent tiering (Abbreviations as in Table 2).

removal as a select agent were the limited Farm-to-Farm transmission risk and moderate farm impact, and moderate risk due to difficulty of large-scale introduction to an animal population. Economic impact was considered to be a low risk from the perspective of domestic and international trade, and factors would be more regional or local to an infected premises. Human infections could readily be treated with antibiotics administration with a case fatality rate close to 0% (CDC, personal communication) in the U.S.

In the case of Venezuelan Equine Encephalitis, during the DSF analysis, the agent made it through the decision tree until Farm Impact where it was recognized that an efficacious vaccine existed for this agent. Based upon the vaccine contributing to a high population immunity, the agent was considered a low concern within this category. MCDA factors that supported removal as a select agent were difficulties in large-scale production and efficient dissemination due to low environmental stability of the agent. Farm-to-Farm transmission risk was considered moderate with Farm Impact considered a low risk due to the availability of an efficacious vaccine.

Both the DSF and MCDA provide support for the recommendation to remove these agents from the USDA Select Agent List and are consistent with the 2020 proposal by the DASAT to delist *B. abortus*, *B. melitensis*, *B. suis*, *B. anthracis* Pasteur strain, Venezuelan equine encephalitis virus and African horse sickness virus (APHIS, USDA, 2020 (Federal Register Vol. 85, No. 52, 2020).

Interestingly, when equal weighting was applied across the board for all criteria, *B. melitensis* scored above the threshold for a select agent, as did JEV, Louping Ill virus, Malignant Catarrhal Fever virus, and Rabies virus. However, those agents were below thresholds using the SME-proposed weighting scheme and thresholds, and were ruled out using the DSF approach, suggesting the value of employing the two analytical approaches to add robustness for decision making.

Application of the methodology across a large and diverse pathogen set, while helping to demonstrate the robustness of the approach, highlighted the challenge of how to handle data gaps for many pathogens. At times, the use of proxies and other assumptions artificially elevated some pathogens, requiring SME review of the data and discussions on how to account for the uncertainties in the

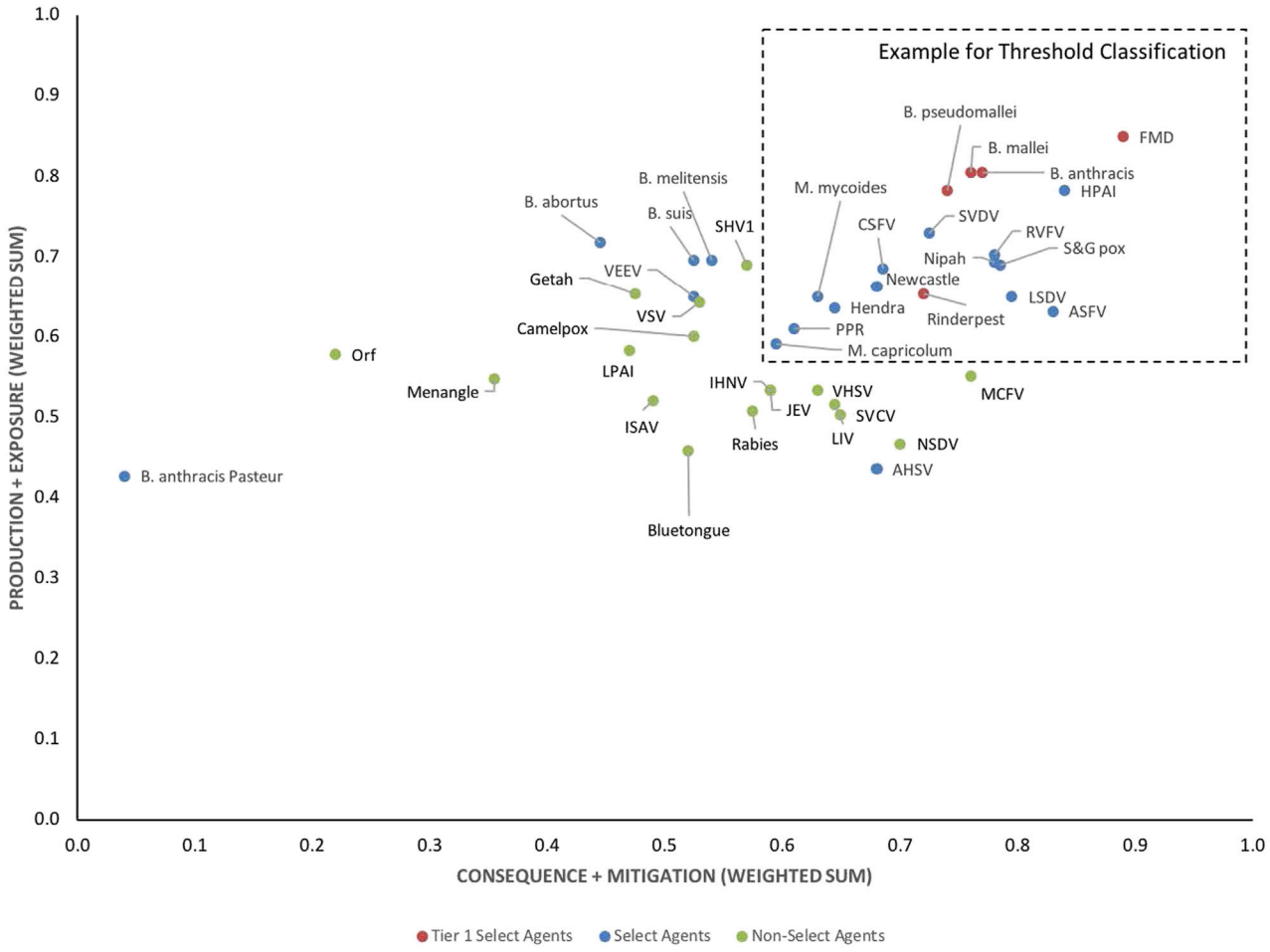

FIGURE 6
2-D results for the proposed weighting scheme for animal select agent tiering (Abbreviations as in Table 2).

data. Thus, we found that the methodology was also useful for identifying those parameters and pathogens where more data are needed, to help with prioritizing future research studies.

Conclusion

The goal of this effort was to explore the use of MCDA and DSF logic tree approaches for supporting the USDA DASAT biennial review. We found the use of two methods with different approaches for identifying pathogens for consideration as select agents provided robustness and benefit to support their intended use and application. The two-dimensional MCDA approach provided a risk-informed assessment that implemented the DASAT's decision criteria and its focus on bioterrorism scenarios with the potential for large-scale agricultural health and economic consequences. The DSF is a complementary approach to identifying select agents and provided additional insight into the factors that influence decision making. The two methods represent different ends of a spectrum for using criteria thresholding to identify select agents: the MCDA approach applies thresholds after considering 21 criteria, while the DSF approach applies thresholds at the single criterion level for nine criteria. Applying weights using the MCDA approach can be used to fine-tune the effective number of criteria used to identify a threshold.

Comparison of the analytical results with the current Select Agent List provided a useful reference point for evaluating these approaches and their potential impact on decision making. Weighted data performed better at reclassifying agents with current designations than did the unweighted data. The 2-D approach most closely replicated current designations. However, the closeness of some agents to the notional threshold suggested that the results were sensitive to where the threshold line was drawn and may be sensitive to how the weights were chosen.

Overall, almost 75% of the agents evaluated classified consistently with their current designations (either select agent or non-select agent), regardless of the method chosen. Both approaches reclassified African Horse Sickness virus, *B. anthracis* Pasteur, *B. abortus*, *B. suis*, and Venezuelan Equine Encephalitis virus as non-select agents. Furthermore, the regulation described in Title 7 U.S. Code 8401, requires that the cost of continued listing and the impact to scientific advancement in research and solutions be considered. *Brucella* species create a financial burden on the federal government,

States and livestock producers as we continue to mitigate the disease risk to livestock. Montana spends over 7.5 million dollars of State and Federal funds each year on *Brucella* risk mitigation (A Report to the Montana Legislature, 2017). Cost associated with the effective eradication of swine and bovine brucellosis in the U. S. between 1934 and 1998 are conservatively estimated to be over $3.5 billion (Roberts et al., 2012). Removal of *Brucella* species, from the Select Agents and Toxins list will allow for more scientists and entities to engage in the necessary research to develop tools (better vaccines, therapeutics, diagnostics, surveillance tools, containment measures etc.) needed to stop the spread and contain the disease. It is conceivable that without these tools, *B. abortus* could 1 day be found in wild elk and bison in every habitat in nearly every Western State, which is a risk to the domestic cattle population across the U.S. Similarly, *B. suis* could eventually spread through every state in the U.S. and spill over into the domestic swine population. The public health impact of *B. suis* was considered low risk because Human-to-Human transmission is very rare (Brucellosis- World Health Organization, 2020), infected wildlife in the U. S. often come in contact with humans without significant transmission (WHO guidance, 2004, and Mantur et al., 1996), effective treatment is available (such as combinations of rifampicin, streptomycin, trimethoprim-sulfamethoxazole, doxycycline, tetracycline, gentamycin, ofloxacin or ciprofloxacin) (Pappas et al., 2006), it has a long incubation period (ranging from 5 days to 6 months with an average onset of 2–4 weeks) (CDC- Brucellosis Reference Guide, 2017) and also has a long window of opportunity to treat brucellosis for a positive outcome after presentation of clinical symptoms (unlike anthrax and plague), and has a very low untreated mortality rate, which can range from 0.5%–5% with an average of <2% (WHO guidance, 2004) and treated mortality rate with <1% (Castano et al., 2017) and in the U.S. is close to 0% (CDC, personal communication). Also, *B. suis* was weaponized by the U. S. in the 1950s as an incapacitating agent and not as a lethal agent (Pappas et al., 2006). As such, removing *Brucella* species from the select agents and toxins list would pose no more risk to the Nation than that currently existing with *Brucella* species being endemic in many animal populations and being widely distributed across the U.S. with the potential for spill over to domestic cattle and swine population and secondary risk to farmers.

Throughout this process the members of Agricultural Intragovernmental Select Agents and Toxins Technical Advisory Committee and the SMEs they identified were key to providing input on the methodology and associated agent fact sheets. There are still some data gaps in the agent fact sheets, such as relevant quarantine data for some agents, that represent opportunities for further research. Regardless of these gaps, it should be noted that these agent fact sheets are meant to evolve as new data become available, from research or additional outbreaks. The MCDA and DSF represent a data driven approach for pathogen prioritization. However, it should also be noted that this methodology should not be used in a vacuum but as one component of a larger regulatory and policy decision framework.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Cyberbiosecurity in high-containment laboratories

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High-containment laboratories (HCLs) conduct critical research on infectious diseases, provide diagnostic services, and produce vaccines for the world's most dangerous pathogens, often called high-consequence pathogens (HCPs). The modernization of HCLs has led to an increasingly cyber-connected laboratory infrastructure. The unique cyberphysical elements of these laboratories and the critical data they generate pose cybersecurity concerns specific to these laboratories. Cyberbiosecurity, the discipline devoted to the study of cybersecurity risks in conjunction with biological risks, is a relatively new field for which few approaches have been developed to identify, assess, and mitigate cyber risks in biological research and diagnostic environments. This study provides a novel approach for cybersecurity risk assessment and identification of risk mitigation measures by applying an asset-impact analysis to the unique environment of HCLs. First, we identified the common cyber and cyberphysical systems in HCLs, summarizing the typical cyber-workflow. We then analyzed the potential adverse outcomes arising from a compromise of these cyber and cyberphysical systems, broadly categorizing potential consequences as relevant to scientific advancement, public health, worker safety, security, and the financial wellbeing of these laboratories. Finally, we discussed potential risk mitigation strategies, leaning heavily on the cybersecurity materials produced by the Center for Internet Security (CIS), including the CIS Controls®, that can serve as a guide for HCL operators to begin the process of implementing risk mitigation measures to reduce their cyberbiorisk and considering the integration of cyber risk management into existing biorisk management practices. This paper provides a discussion to raise awareness among laboratory decision-makers of these critical risks to safety and security within HCLs. Furthermore, this paper can serve as a guide for evaluating cyberbiorisks specific to a laboratory by identifying cyber-connected assets and the impacts associated with a compromise of those assets.

KEYWORDS

cyberbiosecurity, cybersecurity, biosecurity, biosafety, risk assessment, high-containment laboratories (HCLs)

Introduction

In the life sciences, the digitalization of research and development has enabled the creation of new techniques and tools, increasing the efficiency of project design and implementation (Peters, 2012; Krüger et al., 2020). In particular, biological laboratories benefit from the automation and digitalization of laboratory infrastructure, including elements such as the instruments used for data collection and analysis or electronic laboratory notebooks and data

storage (Perkel, 2017). For example, in diagnostic laboratories and healthcare institutions, increased automation of laboratory instruments has expedited the diagnostic process, increasing the throughput capabilities of these facilities, and providing patients with their test results faster (Lippi and Da Rin, 2019). The potential for new innovation resulting from integrating technological advancements in biological laboratories could significantly improve people's health and lives. However, with the increased digitalization and technological advances in the biological sciences comes the emergence of new security risks and their related consequences. In the context of laboratories, the increased cyber-connectedness of biological laboratories has resulted in an increased risk from cyber attacks, and the emergence of additional potential consequences resulting from such attacks. This issue remains underappreciated and poorly addressed in the scientific community.

Cyber attacks have increased in frequency over the last few years, with most organizations worldwide experiencing regular attacks, severely affecting the global economy (AAG Digital, 2019). These attacks have resulted in a greater focus on cybersecurity, defined in the National Institute of Standards and Technology (NIST) Cybersecurity Framework as the "process of protecting information by preventing, detecting, and responding to (cyber) attacks." The growing number of cyber attacks on institutions in the life sciences has increased awareness and led to the emergence of a new area of study termed cyberbiosecurity (Check Point Research, 2022). Cyberbiosecurity is the process of identifying and assessing the risks within or at the interfaces of cybersecurity, cyberphysical security, biosecurity, and biosafety and developing and implementing mitigation measures to prevent, detect, respond, and recover from incidents (Murch et al., 2018). Understanding the implications of cyberbiosecurity requires an understanding of the relevant disciplines from which it converges: cybersecurity and biorisk management. Biorisk management comprises two related but distinct concepts, biosecurity and biosafety. Biosecurity is an evolving concept in the life sciences community; this paper defines biosecurity as the measures used to prevent the "unauthorized access, loss, theft, misuse, diversion, or release" of biological or related materials (WHO, 2020a). Biosafety relates to the measures used to prevent the "unintentional exposure to biological agents or their inadvertent release." (WHO, 2020a). Evaluating and subsequently addressing cyber risks in biological laboratories requires understanding the risks considered in each discipline, such as safety, security, and public health.

Biological laboratories that work with dangerous pathogens have increased biosafety and biosecurity risks compared to other laboratories. While there are unique nuances concerning the classification of pathogens utilized at the individual laboratory level, generally, pathogens are defined by Risk Group, where pathogens belonging to Risk Groups 3 and 4 are often called high-consequence pathogens (HCPs) and require the most extensive containment precautions (WHO, 2020a). These groups include pathogens that cause severe or lethal diseases such as Ebola, tuberculosis, or plague. Laboratories working with HCPs are usually designated as Biosafety level (BSL)-3 or BSL-4 and are collectively referred to as high-containment laboratories (HCLs) (Yeh et al., 2021). These laboratories perform critical and timely research on infectious diseases, provide diagnostic services, and produce vaccines for HCPs; these services are essential to society, and many HCLs are considered critical infrastructure (Reed and Dunaway, 2019). Because HCLs house HCPs and their associated data and may function as part of critical infrastructure, these laboratories must have enhanced safety and security measures under the norms promulgated by international standards (WHO, 2020b). However, the increased safety and security measures currently outlined in most open source biorisk management guidance do not extend to include cyberbiosecurity considerations associated with HCLs.

Research into the threats, risks, vulnerabilities, and consequences associated with cyberbiosecurity is relatively new, and much of the threat landscape remains to be characterized. Reed and Dunaway, (2019) introduced discourse on cyberbiosecurity in laboratories, generally addressing additional risks in BSL-2, BSL-3, and BSL-4 laboratories by identifying trends that could lead to added vulnerabilities and threats in the future (Reed and Dunaway, 2019). Here, we expound upon this foundation, providing an in-depth assessment of vulnerabilities and risks for each type of HCL and identifying both cyber and physical measures to mitigate these risks. Specifically, we 1) explore examples of historical incidents that highlight the relevance of cybersecurity to HCLs, 2) identify key assets in HCLs that contribute to their risks and vulnerabilities, an exercise foundational to performing an asset-impact analysis (see methods); 3) analyze and categorize risks and consequences that may result from a cyber incident, categorized broadly as financial, public health, worker safety, security, and scientific advancement impacts; and 4) discuss the need for cyber risk management as part of a biorisk management program.

Methods

Identifying historical events

We conducted a literature review of historical incidents of cyber attacks to understand the known cyber vulnerabilities and contextualize the current threat environment in the context of cyberbiosecurity in HCLs. This literature review included news sources, government reports, grey literature, and peer-reviewed literature, all of which were searched using keywords to identify any recent high-consequence cyber attack. The keywords focused on laboratories, the life sciences, and cyberphysical systems. Examples were included in this paper if they highlighted vulnerabilities relevant to the cyberbiosecurity of HCLs The results from the literature are included in Supplementary Table S1. While the examples provided demonstrate known vulnerabilities and potential consequences of successful cyber attacks in HCLs, they do not provide a comprehensive description of historical events as many cyber attacks are not disclosed in the public domain.

Asset-impact analysis

To characterize risks in the context of cyberbiosecurity in HCLs, we applied a qualitative, asset-impact risk analysis approach described in the NIST Guide for Conducting Risk Assessments

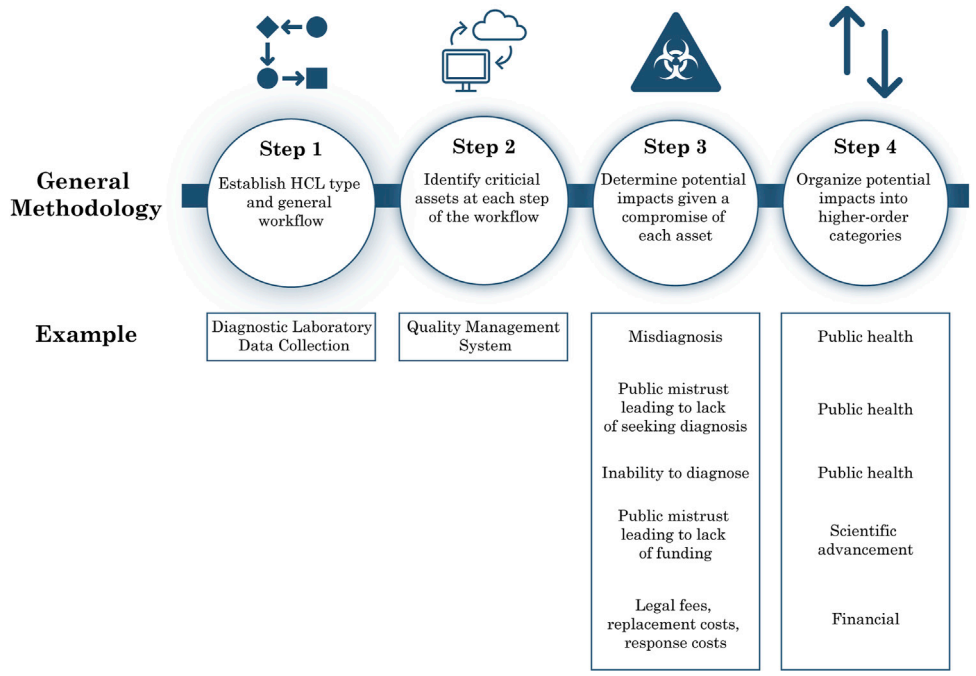

FIGURE 1
Asset-impact analysis methodology summary. Graphic showing methodology used for asset-impact analysis applied to HCLs.

(Ross, 2012). An asset-impact analysis includes identifying existing cyber or cyberphysical systems, determining the value of these assets within the organization, assessing the associated vulnerabilities due to these assets, and analyzing the impacts which would stem from compromise of the assets. To tailor this analysis approach to the context of cyberbiosecurity in HCLs, we first performed a nonsystematic literature review to determine the general cyber-workflows and common cyber and cyber-physical assets of research, diagnostic, and biomanufacturing HCLs. We then systematically identified the potential adverse outcomes that could result from the compromise of each asset, considering consequences due to a loss of confidentiality, integrity, or availability, summarized as unauthorized access, unauthorized alteration, or prevention of the use of the asset, respectively. To evaluate potential impacts due to compromise of each asset, we: 1) determined the cyber-connectivity that is possible for each asset type (e.g., we considered the storage systems with advanced options for connectivity including temperature monitoring and sample inventory rather than a basic freezer); 2) estimated the value provided by each asset that could be lost due to a cyber incident, including value lost to the organization, scientific advancement, and the public; 3) determined potential down-stream consequences from cyber-incidents that could occur due to the nature of the work done in an HCL (e.g., we considered biosafety and biosecurity risks of HCPs and incorporated those risks into our evaluations). The resulting dataset of workflows, assets, and adverse outcomes was further evaluated to identify larger areas of impact associated with cyber incidents in HCLs. The steps included in the asset-impact analysis are summarized in Figure 1. References used for determining the workflow and performing the asset-impact analysis are found in Supplementary Table S1.

Cyberbiorisk management

We performed a literature review to identify common risk management practices for cybersecurity, biosecurity, and biosafety, as well as existing literature on cyberbiosecurity. To inform our discussion, we analyzed similarities and differences in risk management practices within these fields. References which identify relevant risk management practices are found in Supplementary Table S1.

Known cyber vulnerabilities and previous cyber incidents in laboratories

Cyber attacks have been increasing in frequency and sophistication in recent years (Check Point Research, 2022). In a cybersecurity survey conducted by McAfee, only 4% of 1,500 companies reported that they did not experience a cyber incident in 2019 (Smith and Lostri, 2021). According to Check Point Research, the "Education/Research" sector was the most targeted, with an average of 1,605 weekly attacks per organization in 2021, increasing 75% from 2020 (Check Point Research, 2022). The consequences of cybercrimes take many forms and can have impacts reaching beyond the organization directly affected. Examples include but are not limited to opportunity costs,

remediation costs, losses from productivity, system downtime, data loss, shortages of critical medical supplies, and loss of public trust. The total economic cost of global cybercrime was estimated to be over $1 trillion dollars as of 2020, according to estimates by McAfee (Smith and Lostri, 2021).

Historical incidents can provide real-world examples of the consequences of cyber attacks, including those targeted at specific organizations or untargeted and sent out indiscriminately to many organizations (Biju et al., 2019). We note that while targeted attacks are less common than untargeted attacks, certain industries, including education, research, manufacturing, and healthcare, among others, experience targeted attacks more frequently than others (Kessem, 2021). Some recent examples are included in the following discussion.

Biological laboratories, including HCLs, perform critical diagnostic functions and producing essential vaccines and therapeutics. Cyber attacks compromising essential laboratory and biomanufacturing functions can have significant consequences, such as shortages of essential drugs and vaccines. For example, the pharmaceutical company Merck was hit by the NotPetya attack in 2017 (MDL, 2017). This attack temporarily shut down several essential operations throughout the company for several months, including the production of several drugs and vaccines (Henriquez, 2022). In this case, the United States Center for Disease Control (CDC) stockpiles and other manufacturers were able to meet the consumer demand for HPV and Hepatitis vaccines despite the loss of production capacity (Henriquez, 2022). However, the incident illustrates how future cyber attacks could result in shortages of essential vaccines and therapeutics. Downtime of critical research or diagnostic laboratories could be similarly disruptive, particularly in laboratories with unique capabilities for their geographic region.

Many HCLs produce data relevant to public health, such as data that informs the manufacture of essential vaccines and therapeutics. Maintaining the confidentiality and integrity of these data is critical for the data to be trusted by regulators and the public. Laboratories are also often ethically and legally required to maintain confidentiality of critical data. Cyber attacks that compromise critical data could undermine public trust in the institution or its products. In 2021, the European Medicines Agency (EMA), a regulatory agency responsible for overseeing and approving the development of COVID-19 vaccines in Europe, suffered a targeted attack suspected to be a misinformation campaign involving COVID-19 vaccines (Cerulus, 2021). Data stored on an EMA server included email screenshots, EMA peer review comments, technical documents, and presentations relating to the regulatory submission for Pfizer and BioNTech's COVID-19 vaccine candidate BNT162b2 (Cerulus, 2021). These data were accessed, manipulated, and leaked by hackers (Cerulus, 2021). Future leaks of manipulated data could similarly result in a loss of public trust in vaccines.

HCLs may also use and produce data of strategic financial value, including intellectual property (IP) or trade secrets. Cyber attacks resulting in unauthorized access to this information could result in significant financial impacts. A cyber attack campaign known as Epic Turla or Uroboros was discovered in 2014 (Global Research and Analysis Team, Kaspersky Lab, 2014). Among the targeted institutions were research and pharmaceutical production facilities located primarily in Europe and the Middle East (Global Research and Analysis Team, Kaspersky Lab, 2014). This attack successfully stole IP from pharmaceutical and research organizations, demonstrating the risks to IP and other important research data posed by cyber incidents (Global Research and Analysis Team, Kaspersky Lab, 2014).

HCLs also rely on cyberphysical systems (CPSs) for a variety of functions. CPSs integrate cyber-based control mechanisms into physical infrastructure; CPSs in many industries often pose a significant risk due to cyber attacks. In HCLs, examples of CPSs include the building automation system (BAS) and certain types of data collection and analysis instruments. A cyber attack resulting in the compromise of CPSs within HCLs could lead to a multitude of adverse outcomes, including laboratory downtime, breach of containment, or diagnostic errors, depending on the context. In 2021, hackers targeted the University of Oxford's Division of Structural Biology research laboratory, gained access to several CPSs, and demonstrated the ability to control pumps and pressure, including disabling a pressure alarm (Brewster, 2021; Osborne, 2021). Although this incident did not occur in an HCL, it demonstrates the ability of malicious actors to tamper with cyber-connected laboratory equipment and cyberphysical systems remotely.

These real-world examples demonstrate known vulnerabilities and their associated negative impacts and can provide insights into the potential risks that HCLs may encounter. The realization of such risks in these examples supports the importance of assessing the entire spectrum of cyber risks in HCLs and proactively applying appropriate risk mitigation strategies to reduce both the likelihood and severity of a cyber attack.

Cyber considerations in HCLs

These historical incidents highlight many potential impacts of cyber attacks on HCLs. Understanding potential cyber risks in HCLs requires a foundational understanding of the existing cyber and cyberphysical systems contained within the lab. Working with HCPs requires the implementation of enhanced containment precautions and additional security measures, measures which are often controlled by or connected to CPSs within the laboratory (Gao et al., 2021). Although the cyber-workflow of each individual laboratory is distinct, some general types exist with similar workflows and purposes. Most HCLs worldwide, including government, academic, and private institutions, fit within one of three groups: research laboratories, diagnostic laboratories, and biomanufacturing facilities. In this paper, we focus our initial work on analyzing workflows and risks in laboratories studying human pathogens without the use of experimental animal work. Although many of these findings might be generalizable to animal facilities (ABSL and BSL Ag facilities) and to those handling pathogens with agricultural impact, this paper only assesses the cyber biorisks associated with HCLs working with human pathogens and that do not work with live animals. Additional work would be required to account for these unique workflows and potential cyber risks.

The section below describes common cyber and cyberphysical systems found in HCLs and discusses their use within the laboratory. We first focus on commonalities between the three overarching

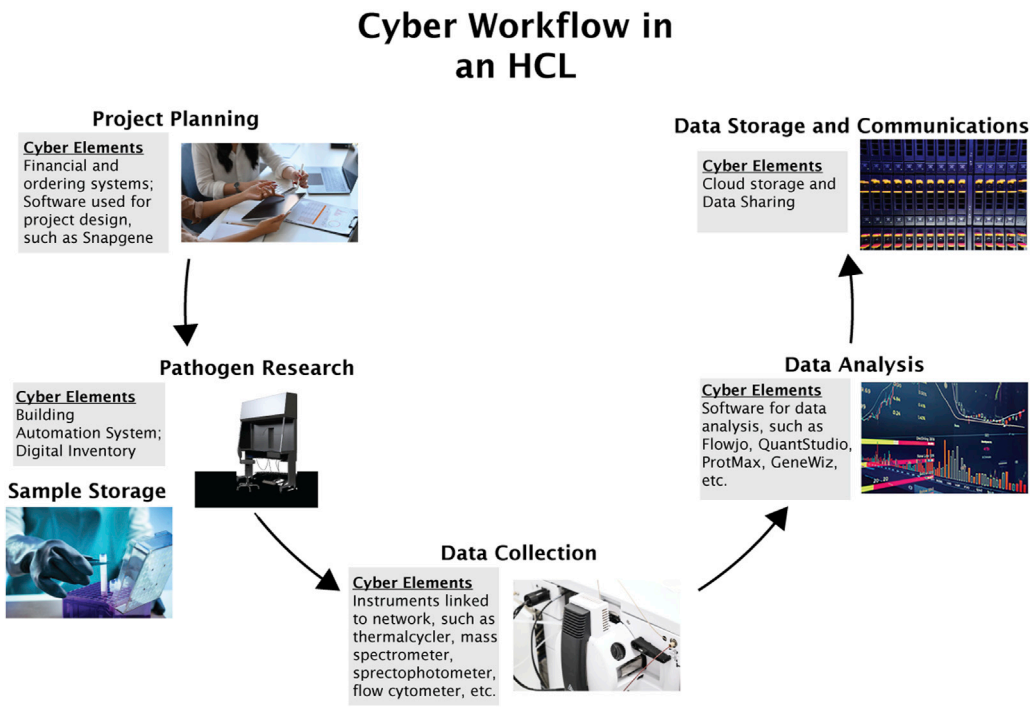

FIGURE 2
General cyber-workflow of an HCL. The figure describes six processes essential to HCL functioning: project planning, pathogen research, sample storage, data collection, data analysis, and data storage and communications.

types of HCLs, then briefly describe the unique considerations of research, diagnostic, and biomanufacturing laboratories specifically. This section describes the typical cyber-connected assets and the points of entry or attack pathways introduced because of the connection of these assets to computer networks. The following section uses this foundational identification of assets to analyze the potential impacts of cyber incidents in HCLs.

Cyber elements of high-containment laboratories

The specific workflow and assets of research labs are tailored to their subject matter area and experimental design but can generally be summarized into the following steps: project planning, pathogen research, data collection, data analysis, and data storage and communications.

Each step of the research process is associated with a unique set of cyber and cyberphysical elements, as shown in Figure 2.

Project planning

The first process in the workflow is a project planning phase. For research and biomanufacturing HCLs, this phase can include experimental design, a process which can be aided and expedited by using any number of potential software tools. For example, the software tools Snapgene and Geneious assist in the design of genetic materials for experiments (Geneious, 2022; SnapGene, 2022). In each of the types of HCLs, electronic budgets and ordering systems can assist in planning and acquiring needed materials, such as assays, personal protective equipment (PPE), genetic materials, or pathogenic samples. While simple, these systems are critical to the functioning of a laboratory. Because these systems are cyber-based, they are vulnerable to a cyber attack; furthermore, the regular downloading of various software and using online vendors may create additional entry points that malicious actors may exploit (Sarder and Haschak, 2019).

Pathogen research

The second process we considered is pathogen research. While some cyber and cyberphysical elements related to this step are specific to particular types of laboratories, several assets related to the handling and containment of pathogens during the research process were similar across HCL types. For example, most HCLs utilize building automation systems (BASs) to control various environmental and containment functions in addition to systems required to maintain normal operations of the laboratory. The most sophisticated BAS can control, monitor, and log data for the ventilation, pressurization parameters, temperature, containment functions, and power, all of which are important to preventing pathogen release and protecting laboratory personnel from accidental exposure (Coogan and Siemens, 2021). A BAS may also be able to monitor who enters and exits the building, ensuring the safety and security of workers by preventing unauthorized personnel from entering the facility (Siemens, 2021). These systems can have a built-in quality management function, logging data to determine the operationality of each part of the system (Siemens, 2021). While a more sophisticated BAS provides greater control over specific parameters within the

laboratory and can provide increased awareness of laboratory systems by logging relevant data, the more systems in a laboratory that are connected to the BAS, the greater the attack surface and the greater the scope of potential consequences should a successful cyber attack occur.

Laboratory BASs can also control certain aspects of airflow as it pertains to biological safety cabinets (BSCs), depending on the type and class of cabinet used in the facility (Siemens, 2021). Class II/III BSCs, which are used for handling the HCPs worked with in HCLs, perform three main functions: to protect the samples from contamination, the workers from accidental exposure, and the environment from accidental contamination (MIT EHS, 2019). This is achieved through High-Efficiency Particulate Air (HEPA) filtering both intake and exhaust air and creating a negative pressure airflow under the hood of the cabinet, simultaneously preventing contaminated laboratory air from entering the workspace, preventing infectious material from flowing out of the cabinet, and preventing the exhaust of contaminated air from the BSC (WHO, 2020b). Disruptions to the airflow can occur through direct tampering with the settings on the BSC, a loss of power to the BSC, or by altering the conditions of the airflow within the laboratory or the exhaust by compromising the integrity or availability of the BAS. Even minor airflow disturbances can significantly impact the protective functions of the BSC, which are essential to preventing worker exposure, environmental contamination, and inaccurate experimental results due to sample contamination (Parks et al., 2022). While most BSCs currently in use are not connected to the internet, advances in the CPSs of laboratory equipment, including BSCs, has facilitated increased networking and internet connectivity options. Thermo Fisher recently announced the release of the Herasafe 2030i Biological Safety Cabinet, which can connect to Wi-Fi and be monitored remotely through the Thermo Fisher app (Thermo Fisher, 2021a). A BSC like this one, which is connected to the internet, is therefore also vulnerable to a direct cyber attack.

Sample storage

The third process we considered was sample storage and inventory management. Samples stored in HCLs naturally include HCPs. Inventory of pathogenic samples can be managed differently depending on the available resources of a laboratory, ranging from manual logs and written labels to integrated laboratory information management systems (LIMS) equipped with sample tracking software that can monitor samples and reagents throughout the workflow (Aguirre et al., 2013; Hashim and Arifin, 2013). In storage, many samples are sensitive to changes in the environment and require specific conditions to maintain the quality of the samples (Theron et al., 2003). Sample storage devices, such as freezers and incubators, must therefore maintain consistent environmental conditions such as temperature and humidity to ensure the desired growth rates and prevent contamination (Thermo Fisher, 2019). In many laboratories, sample storage devices do not connect to the internet and are managed in the laboratory. However, remote monitoring and internet-connected laboratory instruments and equipment are increasing in availability (Perkel, 2017). In the case of some storage devices, this allows personnel to set up alerts if certain environmental conditions are not within set parameters and monitor when storage is accessed, or to remotely change environmental conditions as necessary (PHC Corporation of North America, 2021). Some sample storage devices use digital security measures such as a passcode or some form of identification to access the samples and reagents, in which case the physical security of samples includes a dependence on the cybersecurity of the system (Darwin Chambers, 2022).

Data collection

The next process we considered was data collection, a process which is also becoming increasingly internet-connected, allowing for more sophisticated laboratory automation systems and workflows (Perkel, 2017). Depending on a given laboratory's capabilities, certain groups of instruments can be fully automated, semi-automated, or completely nonautomated (Lippi and Da Rin, 2019). CPSs which automate data collection are increasingly common in research and diagnostic laboratories (Lippi and Da Rin, 2019). Laboratories with fully automated, cyber-connected groups of analysis instruments allow for efficient and complete analysis of samples, capable of doing several different types of tests and working with different sample types in parallel (Lippi and Da Rin, 2019). In a semi-automated laboratory, several types of tests can be run automatically, but the cyberphysical system is generally limited to one type of sample (Lippi and Da Rin, 2019). Even if workflows are not automated through sophisticated systems, individual instruments may still be cyber-connected as many instruments contain a cyber-physical element where data collection is controlled through a connected computer. Because the data collection workflow is critical to the functioning of an HCL, understanding which assets are cyber-connected and how these cyber-connected assets are networked is foundational to assessing cyber risks in an HCL.

In recent years, the rapid advancements in laboratory automation have led to unique cyberphysical systems such as a "mobile robot chemist" and other similar advances where automated robots may work with materials, chemicals, or even pathogens (Burger et al., 2020). Similar robotic aids are being used in hospitals, and it is reasonable to expect they will become more common in HCLs, especially if robots are designed to safely handle dangerous pathogens (Sashin, 2019). As these technologies are integrated into HCLs, they will bring their own cybersecurity implications because of their vulnerability to compromise due to a cyber incident.

Data analysis

While we distinguish data analysis and data collection as two individual processes, they are often intertwined in the laboratory as data analysis may occur directly within the programs that control instrumentation for data collection. To perform data analysis, it is common for laboratories to utilize software and third-party platforms. These programs are highly dependent on the specific type of work being performed. Still, there are countless examples of software packages for data analysis, such as Flowjo or QuantStudio, which perform analysis of flow cytometry and Polymerase Chain Reaction (PCR) experiments, respectively (FlowJo, 2022; Thermo Fisher, 2022). These tools, including an abundance of open-source tools, are cyber assets and, therefore, may be directly affected by a cyber attack.

Data storage and communications

The final step we considered is data storage and communications. HCLs store data relevant to significant research findings, intellectual property, or diagnostic information. For many laboratories, this stored data is of significant value to the laboratories themselves and the scientific community and can be considered the key information asset possessed by laboratories. To store this data, laboratories may utilize data storage platforms, such as GitHub or Google Drive, or their own on-premises or cloud-based data storage solution (GitHub, 2022; Google, 2023). Each of these solutions has different levels of cybersecurity and could introduce an additional attack vector through which a cyber attack could occur (Voas and Hurlburt, 2015).

As an extension of data security considerations, data sharing and communications can also introduce new vulnerabilities into the cyber-workflow of research laboratories (University of Cambridge, 2022). Research partnerships and data sharing have considerable benefits but can introduce additional vulnerabilities. Like many workplaces, communication among laboratory personnel and collaborators is often conducted via email, one of the most common attack vectors used in cyber attacks (Trend Micro, 2022). HCLs could experience a cyber incident through a compromise of one of their assets, a corrupted email sent by an unwitting colleague, or a targeted attack by a malicious actor pretending to be a colleague. Data and information sharing between partners also increases the number of devices storing valuable data, thereby increasing the attack surface and creating a potential for interception of communications.

Cyber elements of research laboratories

Of the types of HCLs, research laboratories map most directly to the general considerations outlined above. Unique priorities within research laboratories may ascribe extra value to certain assets. For example, research data may be particularly valuable, especially if the lab possesses unique and hard-to-reproduce data sets or research findings. Compared to other types of HCLs, research data is more likely to have dual use potential, posing a greater target for a malicious actor. Research labs may also possess legacy samples and biorepositories of pathogen samples which are impossible to recreate. This inventory may be managed through cyber-connected systems. Finally, research HCLs are likely to be part of universities of other larger institutions, where these laboratories may operate within a larger institutional cyber-infrastructure. If cyber systems are connected within the broader institution, a cyber incident anywhere in the institution could impact the laboratory.

Cyber elements of diagnostic laboratories

Diagnostic HCLs function as part of a laboratory system that requires coordination and communication between hospitals and clinics, other laboratories, and public health entities within the diagnostic network to conduct disease surveillance operations and facilitate sharing of information, samples, and resources between laboratories (Naidoo and Ihekweazu, 2020; Pabbaraju et al., 2020). The workflow of a diagnostic HCL can be summarized as receiving data and samples, storing and handling samples, collecting and analyzing sample data, and reporting results. Like research laboratories, diagnostic laboratories rely on inventory and sample storage for operations and may utilize BASs, BSCs, and third-party platforms for data management and utilize laboratory automation. While automation in research laboratories is becoming increasingly common, many diagnostic laboratories have already achieved some level of automation and therefore have more cyber-connected assets (Lippi and Da Rin, 2019). The importance of these common assets and their cybersecurity considerations are discussed in the previous section.

Cybersecurity considerations specific to the diagnostic laboratory begin when a laboratory receives a sample and accompanying metadata. Metadata can include sensitive information such as patient data [e.g., personally identifiable information (PII), protected health information (PHI)], type of sample, tests to be performed, or the location of the patient (Viswanadham, 2021). While policies and regulations differ between countries, the information obtained and used by the diagnostic laboratory is considered highly sensitive information in most countries (Bellman et al., 2004). Due to the sensitive and personal nature of the information, ensuring confidentiality is a high priority for diagnostic laboratories.

Cyber elements of high-containment biomanufacturing facilities

A small subset of biomanufacturing facilities requires the advanced containment precautions found in HCLs to produce live-attenuated vaccines (LAVs) for pathogens such as SARS-CoV-2, *Bacillus anthracis*, and *Yersinia pestis*, the causative agents of COVID-19, anthrax, and plague, respectively (Feodorova et al., 2014; Ditchburn and Hodgkins, 2019; Goswami, 2020). A live-attenuated vaccine (LAV) is created using a live pathogen that has undergone a process reducing its ability to cause disease in a specific host (Pöyhönen et al., 2019). Thus, LAVs are created from viable pathogens and, in the case of LAVs for HCPs, may require high-containment precautions. For a review of more general cyber risks of biomanufacturing facilities, see Mantle et al. (2019) and Guttieres et al. (2019).

Like other HCLs, high-containment biomanufacturing facilities rely on inventory and sample storage for operations. They may also utilize a BAS, BSCs, third-party data platforms, and laboratory automation to increase efficiency, safety, and security within the laboratory. However, several components and unique systems within high-containment biomanufacturing facilities have special cyberbiosecurity considerations that differ from diagnostic and research laboratories.

During the upstream production process of LAVs, biomanufacturing facilities employ a number of CPSs to carry out and control processes (Arenas and Maria, 2022). Bioreactors are common CPSs used in the propagation of LAVs and are programmed with certain parameters that control conditions such as nutrient concentrations, oxygen concentrations, and dilution rate (Sha, 2021). These systems ensure proper growth rate, retention of attenuation, and prevention of contamination of the LAV stock, all of which are essential to the overall safety of the product and the safety of the workers interacting

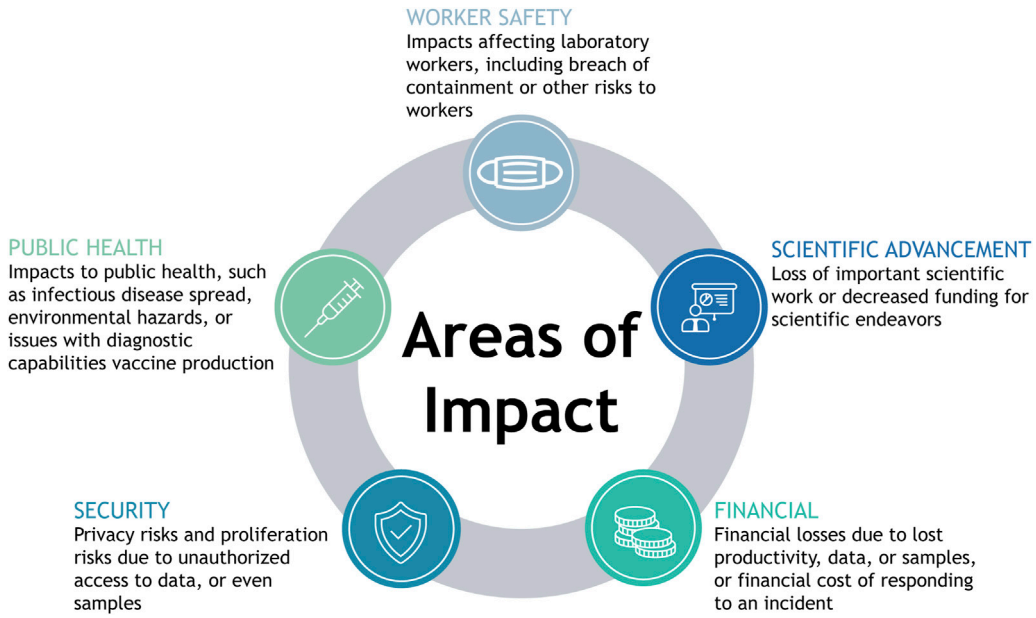

FIGURE 3
Identified areas of impact. Graphic showing areas of impact including public health, worker safety, security, scientific advancement, and financial.

with the vaccine stock (FDA, 2017). Certain bioreactors allow for internet connection and remote monitoring, providing a potential point of entry to deliver a cyber attack (Lab Owl, 2020). Downstream processing may similarly utilize CPSs such as chromatography systems to purify the strain, removing contaminants from the vaccine stock (Arenas and Maria, 2022). Chromatographs can connect to and be monitored by networked systems, making these instruments vulnerable to cyber attacks (Thermo Fisher, 2021b).

Maintaining the integrity and availability of the production process is essential to ensure the safety and efficacy of the distributed LAV. During each step of the production process, data is routinely collected and reviewed for both quality control and research and development purposes as a part of the quality management system (QMS) (Mantle et al., 2019). Quality control management is essential to ensure the desired product is safe, free from contaminants and meets regulatory standards. Understanding the cyber-connectedness of the manufacturing and quality control systems within biomanufacturing laboratories is foundational to understanding the associated impacts.

Identified Areas of Impact

The discussion above highlights the critical functions of many cyber and cyberphysical elements within HCLs. Given the critical functions of the cyber and cyberphysical systems in HCLs, a cyber incident could lead to a range of negative consequences. This section analyzes the mapped workflows in diagnostic, research, and biomanufacturing laboratories to identify the potential impacts that could occur due to a cyber incident. We first connected each asset to related potential impacts, considering losses of confidentiality, integrity, or availability of each asset due to any form of cyber attack. Upon identifying potential impacts due to the compromise of cyber and cyberphysical systems in an HCL, we found five overarching categories under which all of the identified impacts fell: worker safety impacts, public health impacts, security impacts, impacts affecting scientific advancement, and financial impacts (Figure 3). In the following section, we present the range of potential consequences due to a cyber incident in an HCL, referring to the abovementioned assets. Examples of potential forms of loss, the types of HCLs that could experience such losses, and the assets through which a cyber attack leading to each form of loss could occur are outlined in Table 1.

Worker safety

An analysis of impacts due to the compromise of a variety of assets in an HCL revealed worker safety to be a primary area of concern in the event of a cyber incident. Worker safety considerations include consequences associated with the exposure of laboratory personnel to infectious material and consequences resulting from the physical endangerment of laboratory personnel. There are several potential attack vectors through which laboratory personnel could be exposed to infectious material. For example, a cyber incident could compromise the integrity or availability of the BAS, potentially leading to altered pressure differentials between high-hazard areas and low-hazard areas or altered airflow, which could result in the exposure of personnel to infectious material. In addition to potential exposure to infectious materials, a cyber attack on a HCL could cause other worker safety risks. For example, for laboratories with electronic locks controlled by a BAS, a cyber attack resulting in a loss of availability of the BAS when personnel are physically inside of the laboratory could result in the locking of the external electronic doors, trapping personnel inside. Another

TABLE 1 Examples of Potential Forms of Loss in HCLs. The table shows selected forms of loss in HCLs within each area of impact and outlines the type(s) of HCL(s) and workflow stage(s) affected and the assets that could be compromised to result in each form of loss.

	Example loss	Lab type	Workflow stage	Asset(s)
Worker Safety	Exposure of laboratory personnel to infectious material	All	Pathogen research	BAS (containment functions), inventory management system
	Non-pathogen related worker safety risks	All	All	BAS (security and environmental functions)
Public Health	Community spread of pathogens	All	Pathogen research	BAS (containment functions)
	Loss of critical manufacturing functions	Biomanufacturing	All	Any asset that is critical to biomanufacturing facility functioning
	Misdiagnosis, or inability to diagnose	Diagnostic	Data collection, data analysis, data storage and communications	Servers/cloud-based data storage (diagnostic data), instruments, QMS
	Distribution of ineffective or unsafe materials	Biomanufacturing	Data collection, data analysis	Servers/cloud-based data storage (experimental data), instruments, QMS
	Public mistrust similar to EMA example*	Research, Biomanufacturing	Data storage and communications	Servers/cloud-based data storage, communications
Security	Unauthorized acquisition of dangerous samples from facility	All	Sample storage	BAS (security function), inventory management system, sample storage
	Unauthorized acquisition of dangerous samples during transport	All	Project planning	Financial and ordering systems
	Unauthorized acquisition of sensitive data	All	Data storage and communications	Servers/cloud-based data storage (pathogen data), communications
Scientific Advancement	Loss or corruption of large or unique datasets	Research	Data storage and communications	Servers/cloud-based data storage (large or unique datasets)
	Loss or corruption of large or unique sample sets	Research	Sample storage	BAS (security function), inventory management system, sample storage
	Public mistrust leading to loss of funding	All	All	BAS, QMS, Servers/cloud-based data storage (experimental data, diagnostic data)

*See section on previous cyber incidents in laboratories.

potential consequence is unauthorized access to the facility by an intentional actor or an unaware individual. This presents a physical danger to laboratory personnel and a risk to the unauthorized individual if they are unfamiliar with HCL safety procedures.

Worker safety risks may also stem from cyber incidents affecting the LIMS. An incident that compromised inventory data could leave workers unable to identify and unknowingly access dangerous samples without the appropriate protective equipment. Although no incidents of inventory corruption due to a cyber attack in an HCL are documented in the public domain, mislabeled samples have posed a risk to workers in past laboratory incidents and near-misses (Sun, 2014).

Rapid advances in robotics in the laboratory could impact worker safety. Researchers working towards integrating these evolving technologies in settings such as HCLs will need to assess the potential impacts. Depending on the role of such robots, they could also pose a risk in other categories, such as public health or scientific advancement, if a cyber incident compromised their integrity. As these advances continue, cybersecurity factors should be considered in order to protect workers who work with and around these robots.

Public health

A successful cyber attack on an HCL also presents significant risks to public health (Table 1). Within any HCL, a cyber attack compromising the BAS-controlled ventilation and pressurization systems as described above in the worker safety section, could result in transmission within the community either through the exposure of a laboratory worker or through pathogen release. Such laboratory leaks, which can result in potential sustained pathogen transmission in the community and cause outbreaks, are prioritized in biosafety risk assessments.

In addition to the risks of laboratory-acquired infections and pathogen release, cyber attacks on diagnostic laboratories carry additional risks due to their essential role in disease surveillance and outbreak response. A cyber attack could result in the loss of availability of diagnostic capability, thereby preventing or delaying patient diagnoses. Many types of cyber incidents could disrupt workflow, including an incident compromising computer networks, a ransomware attack, an attack preventing the functioning of the BAS, or an attack that affects any of the instruments essential to the diagnostic process. Attacks that compromise essential systems may not easily be replaced or restored and could lead to significant delays in diagnosis. This could result in delays in treatment and, in the case of an outbreak, the inability to perform disease surveillance could lead to increased community transmission of disease. In addition, to delay in diagnostic capabilities, a cyber incident could affect data integrity during the diagnostic process, potentially resulting in the misdiagnosis of patients. Given the multiple cyberphysical elements in the workflow, loss of integrity could occur during data collection,

data analysis, quality control, or data storage and communications. Misdiagnosis can have similar, and potentially worse, consequences compared to delays in diagnosis, including patients receiving incorrect treatments or continued transmission of diseases throughout the community. Again, these consequences can become more extreme in the event of an ongoing outbreak, when systems-wide laboratory capacity is already limited, or when a loss of data integrity goes undetected.

In addition to diagnostic laboratories, biomanufacturing facilities also perform functions essential to public health. The NotPetya cyber attack described earlier illustrates this concept (Mcquade, 2018). Briefly, Merck's infrastructure was hit by a non-targeted cyber attack, resulting in a months-long shutdown of critical operations relating to the production of several essential drugs and vaccines (Mcquade, 2018). High-containment biomanufacturing facilities could also become a victim of such an attack, which could reduce vaccine production and slower rollout. In the case of the NotPetya attack, CDC stockpiles and other producers were able to meet demand; however, future incidents could create shortages of a vaccine or other critical medical countermeasures, resulting in increased disease spread, morbidity, and mortality (Mcquade, 2018). Furthermore, much like potential misdiagnosis in diagnostic laboratories, a cyber incident compromising the integrity of data analysis and quality control could result in delays and ineffective or unsafe vaccines. While this would most likely require a specific targeted cyber attack, the risk to public health is considerable and should be taken seriously.

Laboratory automation brings a host of risks and benefits. Automation increases the productivity, reproducibility, and throughput of a diagnostic laboratory but also introduces far more networked devices, which increases the cyber attack surface. As described above, this increases the risk of downtime and/or misdiagnosis in the laboratory and the potential issues with quality controls described above. When exploring automation solutions, laboratories should consider implementing cyber risk mitigation strategies that help maximize the benefits of these new capabilities.

Cyber attacks on HCLs could also lead to a loss in public trust, affecting public health. Many cyber attacks, whether on laboratories or other entities, are not public knowledge, shielding organizations who are victims of cyber attacks from public fallout. A publicized cyber attack on a HCL could lead to loss of public trust in that specific institution, or a loss of public trust in the public health system as a whole. Additionally, cyber attacks on biomanufacturing facilities or research laboratories involved in producing therapeutics and vaccines could lead to the deliberate release of misinformation about these interventions, as seen in the 2021 EMA attack described earlier (Cerulus, 2021). Loss of public trust could lead to decreased vaccination rates, misuse of medicines, and lower public buy-in to public health initiatives. The substantial public health benefits of HCLs highlight the importance of building fundamental cybersecurity measures into laboratory operations.

Security risks

A common concern in pathogen research is the potential for misuse by a malicious actor, such as the generation of bioweapons. Proliferation risk may be higher for more dangerous pathogens and certain types of experiments, such as those with dual use potential. Briefly, research with dual use potential is research that is intended to benefit society but also has the potential to cause significant harm (NIH, 2014). Dual use risk may arise from materials, methods, or information. HCLs work with pathogens (materials), develop protocols to manipulate pathogens (methods), and generate data from their work (information). All of these elements may be of interest to a malicious actor seeking to misuse research and are often considered in laboratories' biorisk management programs (Table 1).

Few potential cyber attack pathways were identified that could result in the unauthorized acquisition of dangerous samples. While unlikely, the consequences associated with a malicious actor acquiring such pathogens are high enough to warrant consideration. An actor could acquire information about pathogenic samples that a laboratory possesses and use that information to target facilities of interest to steal pathogens from storage or sample shipments. As laboratories increase their cyber sophistication, they can implement additional safeguards to securely hold sample information and improve their ability to detect illicit access to inventories.

Several cyber attack pathways were identified that could result in the unauthorized acquisition of data associated with dangerous pathogens and personal data of patients and laboratory personnel. The safeguards to prevent unauthorized access or acquisition of data are completely cyber-based. Once a cyber attack defeats the cyber safeguards and controls, there are no other mitigation measures to prevent unauthorized access or alteration of the data. Different types of data pose different risks in terms of security. Data relating to dangerous pathogen research protocols or information with dual use potential such as virulence factors, mutations that increase transmission or pathogen survival, or genetic sequences of particularly pathogenic strains, could all pose a proliferation risk if exfiltrated by a malicious actor. Many laboratory databases also contain private information of laboratory workers. Diagnostic laboratories may also hold patient-related data, including PII, PHI, genetic sequences, and test results. Securing and encrypting stored data is important for all types of HCLs, especially for diagnostic laboratories.

Scientific advancement

Considering the critical role that HCLs play in human and zoonotic infectious disease and pathogen research, a cyber attack affecting these laboratories could significantly hamper scientific advancement. This includes loss or corruption of large or unique sets of samples or data and delays in significant research (Table 1).

Laboratories hold valuable datasets that have been compiled with significant time, expense, and effort. Many of these datasets can be analyzed with modern data science approaches to quickly identify promising therapeutic and vaccine research pathways (Aung et al., 2021). Compromise of the integrity or availability of these large or unique datasets would harm scientific advancement. For example, unauthorized alterations to the dataset could lead to significant inaccuracies in findings. Even if detected, such changes could delay scientific advancement and necessitate laborious and expensive investigations to identify and correct errors in the data. Datasets from specific time periods or datasets compiled during specific

outbreaks are also unique assets that can help advance scientific discovery. These datasets are one-of-a-kind. A compromise to the integrity or availability of such a dataset, without an available backup, would be a considerable and irreplaceable loss to science.

Certain sample sets, such as large biobanks or legacy collections, incur similar unique risks to scientific advancement as those observed with large or unique datasets. The availability of a biobank could be compromised if samples are held at the wrong temperature. Cold chains and incubator controls could be impacted by a cyber attack removing power to the facility or specific rooms or compromising digitally controlled freezers and incubators. This particular consequence is exacerbated in the case of sample storage of repositories and legacy sample collections as they likely contain specific strains or certain historic samples that are irreplaceable, resulting in both a loss of general scientific knowledge and potential financial losses to the laboratory.

In addition to significant delays in research arising from a cyber attack directly, a loss of public trust could delay scientific advancement. Public trust could be affected due to a public health incident resulting from a cyber incident, a data breach, or misinformation. Loss of public trust could result in decreased funding for research or could divert funds from research leading to scientific progress to other endeavors. A similar outcome was seen following the spread of misinformation about vaccines and autism as funds were diverted from autism research to disprove the claims of the link between vaccines and autism (Pellicano and Stears, 2011). Delays in significant research, either as a result of the cyber attack or a loss of public trust, prevent scientific progress.

Financial risks

While most of this study emphasizes the unique risks in an HCL in terms of biosafety, biosecurity, and other public health considerations, financial losses to an organization from a cyber incident provide a particularly quantitative mechanism for understanding cyberbiosecurity risk. A cyber incident is likely to result in costs associated with a loss of productivity, either due to laboratory downtime or staff time to respond to the cost. In addition to the loss of productivity, financial losses include the monetary costs incurred by an HCL in the aftermath of a successful cyber attack. Examples of financial costs of a cyber attack include legal fees, replacing lost samples or compromised equipment, or hiring Information Technology (IT) contractors. Research and biomanufacturing HCLs also could incur the loss of intellectual property, which can impact the laboratory's competitive advantage and have financial implications. The NotPetya attack cost an estimated USD$1.4 billion, including effects from downtime, inability to produce essential vaccines, equipment and data replacement costs, and personnel response costs (Demberger, 2022).

Cyber incidents may become publicized if they cause issues such as delays in vaccine production or a loss of privacy. In many cases, organizations also have an ethical and legal responsibility to notify those whose data was compromised or those who may be otherwise impacted by the cyber incident. These incidents can damage an organization's reputation. Academic and government research institutions generally rely on applying for grants and government funding, so a reputational loss may affect their ability to receive funding awards. While diagnostic laboratories are an essential service, a cyber incident leading to privacy issues could also cause reputational damage. A cyber incident resulting in significant publicized consequences, such as breach of containment or sample or data theft, would almost certainly lead to reputational damage, potentially affecting funding beyond the originally impacted laboratory.

Financial losses, in particular, may stem from a broad range of types of cyber attacks and a variety of different assets in the laboratory. Essentially, any cyber incident which causes a loss of productivity will result in financial loss. The severity of financial consequences is asset dependent and further depends on the value placed on each asset by the laboratory. Therefore, we did not directly relate financial losses to specific assets in Table 1 as we did in the categories above.

Cyber risk management in HCLs

In the sections above, we identified the cyber-connected assets common to HCLs and the potential negative consequences associated with a compromise of the confidentiality, integrity, or availability, of those assets. Building upon this discussion, we turn to consider the next step in the management of cyberbiorisks: mitigation.

Risk management approaches involve first identifying and assessing risks followed by evaluating and implementing mitigation measures to reduce those risks to an acceptable risk level. The iterative processes of identification, assessment, evaluation, and mitigation of biosafety and biosecurity risks constitutes biorisk management (WHO, 2020a). Laboratories, including HCLs, use existing guidance frameworks, such as the United States CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL) and WHO's Laboratory Biosafety Manual (LMB), to guide the implementation of biorisk management programs at their facilities (WHO, 2020b; CDC and NIH, 2020). However, cyber and cyberphysical risks are not explicitly included in these frameworks. Increases in the adoption of network-enabled technology in HCLs create new entry points and potential pathways for malicious actors to exploit. Therefore, biorisk management programs must adapt to account for cyber and cyberphysical risks in addition to biosafety and biosecurity risks. Risk management, laboratory safety, and security experts must come together to formally define where and how cybersecurity fits into biorisk management processes in HCLs. Here, we provide a few underlying principles to guide this conversation.

In the fields of biorisk and cyber risk management, risk is generally modeled as the product of the severity of a consequence when it occurs and the likelihood of that incident occurring (Ross, 2012). The first step in integrating cybersecurity and cyber risk mitigation in HCLs is understanding that effective control implementation reduces the likelihood of an incident or the impacts of an incident if it were to occur. Ideally, a risk mitigation program reduces both likelihood and impact. The cyber risk management process for HCLs can follow a similar approach to other areas of biorisk management. Laboratory personnel should identify existing risks and implement controls to directly reduce those risks to an acceptable level (WHO, 2020b). Using a risk-based approach, risk management programs can identify explicit linkages between controls and the elements of risk—impact and likelihood. For example, consider a ransomware attack on a

laboratory. Because passwords can be stolen or guessed, multi-factor authentication (MFA) makes it much less *likely* that an attacker can gain access to an information system through a compromised user account. Robust data backup and recovery systems would decrease the *impact* of a ransomware attack, allowing the laboratory to restore systems quickly with minimal downtime and cost.

This example also demonstrates the value of implementing a layered set of control systems, with well-defined benefits and tiers of implementation. Many cyber risk management frameworks include a tier of basic controls that provides common-sense protection that does not require extensive risk assessment to implement (CIS, 2021). These controls are sometimes collectively called "cyber hygiene" and are the first controls that an organization new to cybersecurity should implement as broadly as practical (NIST, 2018). Basic cyber hygiene can be considered comparable to basic laboratory safety practices that should be followed in virtually all situations (e.g., Standard Microbiological Practices). In many cases, cyber controls have been standardized so that implementation progress can be ordered, measured, and compared across organizations. One example of standardized cyber controls are the CIS Controls, which can be used to improve an entity's cybersecurity posture in an organized fashion (CIS, 2021). The Center for Internet Security (CIS), the organization that maintains the CIS Controls, has divided all controls into three Implementation Groups (IG) (CIS, 2021). The first, known as IG1, includes the controls that an HCL starting a cybersecurity program should focus on (CIS, 2021). Other control systems have similar ways of designating the subset of those systems that fall into that category of cyber hygiene, or basic controls for early implementation (NIST, 2018).

As the cybersecurity controls that an HCL is implementing become more sophisticated, the HCL should focus on the risk-based approach described above. Similar to decision-making in other areas of biorisk management, determining appropriate controls starts with defining risk appetites and tolerances and, depending on the selected risk management approach, developing a risk register. A risk register is a list of the potential scenarios that could cause losses stated as concrete outcomes with identified categories of loss, pathways to that loss occurring, and treatment for such risks, similar to the analysis performed in this paper (Quinn et al., 2021). It is a powerful tool for an organization to reach a consensus about the risks it faces and the path to addressing them (Barrett et al., 2020). Once a risk register is created, the organization can link implementation of cybersecurity controls to the risks on the register to communicate and explain the need for the controls. Because cybersecurity controls are published and maintained as standards for which formal and auditable measurement is possible, an HCL can implement those controls and measure the implementation against recognized benchmarks. These standards could be integrated into biorisk management programs so that identified cyber risks can be connected to a given standard of control implementation against which laboratories can measure themselves. Examples may include requiring laboratories which work with high-consequence pathogens to meet a specific tier of control implementation, or to require laboratories to address specific cyber risks, such as those related to their BAS or sensitive data.

Because many aspects of cyber control implementation require organization-wide compliance, creating both awareness and buy-in from the HCL's staff and leadership is an essential part of cyber risk management. One difficulty in creating buy-in is that when an organization effectively implements cybersecurity controls, *nothing* happens: data is *not* lost, administrative user accounts do *not* get compromised, and information systems continue to run *un*interrupted. Issues of staff buy-in stems from a lack of awareness of their personal role in the cybersecurity of the facility and a general undervaluation of risks, including biosafety, biosecurity, and cybersecurity risks, in the laboratory (Pinard and Salazar, 2010; Naseem and Conklin, 2021). Problems in leadership buy-in arise when the cost in money or convenience of implementing controls rises to a level where the organization treats cybersecurity controls purely as an unrecoverable cost center rather than measuring the value those controls return to the organization in the form of loss avoidance. For example, imposing the added inconvenience of configuring and maintaining MFA for all users may make the compromise of user accounts more difficult, but when rigorously implemented, it adds a measure of inconvenience for all the lab's workers. Cybersecurity professionals can explain that these changes lead to greater security, but the experience of putting them in place translates to more burden in an environment where the number of account compromises was already close to zero. If an HCL has not experienced this type of compromise, the experience of adding burdens because of incidents at other laboratories or industries can lead to frustration and the conclusion that cybersecurity is not delivering value. Raising awareness of the risks associated with cyber incidents can promote responsibility among staff.

Conclusion

This work has outlined the unique cyber elements of HCLs, identifying the cyber risks associated with these laboratories. Like most laboratories, HCLs generally have a cyber infrastructure that hosts software and data for the planning, analysis, and dissemination of their work. Many instruments for data collection are cyberphysical systems that include computers connected directly to the instruments to record and subsequently analyze data. HCLs are distinguished by the HCPs with which they work; most HCLs use CPSs such as the BAS and sometimes even cyber-connected biosafety cabinets that maintain both safety and security while handling these dangerous pathogens. Most cyber elements are shared between research, diagnostic, and biomanufacturing HCLs, but each is distinguished by the types of data, samples, and laboratory work involved; therefore, the risks associated with these cyber elements is unique for each type of facility.

Understanding the cyber elements in HCLs enables analysis of the potential cyber risks. While all organizations have the risk of financial losses from a cyber incident, HCLs are also concerned with managing risks to worker safety, public health, security, and scientific advancement. HCLs have critical functions; diagnostic and biomanufacturing laboratories are essential to meeting immediate public health needs for disease surveillance and vaccine production. Research HCLs have the potential to create long-lasting and far-reaching benefits for society. The cyber risks and impacts outlined in this paper highlight the critical importance of improving cybersecurity for these laboratories as part of public health and biosecurity efforts.

The unique intersection of cyberphysical systems and biological systems in HCLs highlights the growing importance of collaboration

between biorisk management and cybersecurity practitioners. Experts from both disciplines should collaboratively identify needs and work towards building norms in the field of cyberbiosecurity. For example, future efforts could create guidance, standards, and best practices necessary to integrate cyber risk management into existing biorisk management practices.

A significant and collaborative effort is required to build awareness and cyber risk mitigation capability in laboratories. Training should help laboratory workers identify opportunities to leverage the benefits of cyber-connected infrastructure while building a practical understanding of cyber risks. Cybersecurity training could include integrating foundational concepts into existing biosafety and biosecurity training for HCL personnel and additional teaching tools and certifications specific to laboratory cybersecurity. Simultaneously, awareness-raising efforts are required to secure organizational buy-in among decision-makers, policymakers, and leaders of scientific organizations who are empowered to set policy priorities and dedicate meaningful resources to cyber risk mitigation in HCLs. Taken together, these efforts would enable HCLs to continue their impactful work in an increasingly cyber-connected environment.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

EC:contributed to conception, design, data collection, data analysis, drafting, and critical review of the manuscript. AB: contributed to data analysis, drafting, and critical review of the manuscript. LS: contributed to data collection and drafting and approved the manuscript. SJ: contributed to data analysis and approved the manuscript. VV and SB: contributed to data validation and critical review of the manuscript. GV: contributed to conception, design, and critical review of the manuscript. NT: supervised the conception, design, data collection, and data analysis, and contributed drafting, and critical review of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Authors EC, LS, GV, and NT were employed by the company Gryphon Scientific. Authors AB and SJ were employed by the company Veribo Analytics. Author VV was employed by the company Praxis Biorisk Systems.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Application of multi-criteria decision analysis techniques and decision support framework for informing plant select agent designation and decision making

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The United States Department of Agriculture (USDA) Division of Agricultural Select Agents and Toxins (DASAT) established a list of biological agents (Select Agents List) that threaten crops of economic importance to the United States and regulates the procedures governing containment, incident response, and the security of entities working with them. Every 2 years the USDA DASAT reviews their select agent list, utilizing assessments by subject matter experts (SMEs) to rank the agents. We explored the applicability of multi-criteria decision analysis (MCDA) techniques and a decision support framework (DSF) to support the USDA DASAT biennial review process. The evaluation includes both current and non-select agents to provide a robust assessment. We initially conducted a literature review of 16 pathogens against 9 criteria for assessing plant health and bioterrorism risk and documented the findings to support this analysis. Technical review of published data and associated scoring recommendations by pathogen-specific SMEs was found to be critical for ensuring accuracy. Scoring criteria were adopted to ensure consistency. The MCDA supported the expectation that select agents would rank high on the relative risk scale when considering the agricultural consequences of a bioterrorism attack; however, application of analytical thresholds as a basis for designating select agents led to some exceptions to current designations. A second analytical approach used agent-specific data to designate key criteria in a DSF logic tree format to identify pathogens of low concern that can be ruled out for further consideration as select agents. Both the MCDA and DSF approaches arrived at similar conclusions, suggesting the value of employing the two analytical approaches to add robustness for decision making.

KEYWORDS

multi-criteria decision analysis (MCDA), decision support framework (DSF), plant select agents, biennial review, risk assessment tool

Introduction

More than 50,000 plant diseases have been recognized in the United States (U. S.) and there are many more that occur globally. (Nutter and Madden, 2005). Plant pathologists estimate that the majority of plant diseases are caused by fungal and oomycete pathogens (Strange, 1993; Nutter and Madden, 2005; Fletcher et al., 2011). Each year, plant diseases cost the global economy more than $220 billion and crop production loss due to pests is between 20% and 40% (NIFA, 2023).

Fletcher et al. (2011) distinguished three forms of intentional use of pathogens to infect crops: 1) biowarfare, a state-sponsored and funded activity to reduce a nation's food resources, which includes commercial or economic sabotage for trade advantage; 2) bioterrorism, which involves small groups or single individuals with a political, social, or religious agenda; and, 3) biocrimes, which are motivated by issues such as commodity price manipulation, commercial competition, revenge, or to create a dependence on a particular product. The consequences of a biological attack on the U. S. agriculture sector may be significant due to its economic importance, representing about 20% of the U. S. export market since 2000 (USDA International Markets and U. S. Trade, 2022). Additional economic consequences could occur through loss of international markets because phytosanitary restrictions on trade, which are imposed by importing countries that are free of a particular highly contagious plant disease, will ultimately affect the economy of the exporting country (Wheelis et al., 2002).

Crops as targets offer several advantages to the perpetrator(s). Agricultural crops are often described as "soft targets" because they are grown over large acreages, making continuous and effective surveillance of them nearly impossible (Nutter and Madden, 2005). One consequence of this minimal crop surveillance is a potentially long lag time between the introduction of a pathogen and its detection (Nutter and Madden, 2005). For plant pathogens with extremely high reproductive rates (R_0), successful eradication and containment of such a newly introduced pathogen is only possible if it is detected soon after introduction (Madden and Wheelis, 2003). Thus, early detection, containment, treatment if available, and other appropriate preventive measures are of utmost importance in limiting the spread of the pathogen. Likewise, recognition and control of certain pathogens is also key to preventing their accidental or intentional introduction.

Another advantage of targeting crops is the ease with which a plant pathogen can be introduced into the U. S. For example, bioterrorists could carry small amounts of inoculum (less than a Gram) across the long borders with Mexico or Canada to infect crops (Madden and Wheelis, 2003). Humans are generally not susceptible to infection by plant pathogens meaning no special safety precautions are required to collect, culture, reproduce, store, or deliver the inoculum to its target (Nutter and Madden, 2005). Once an agricultural pathogen has been introduced to a new area, forensic attribution can be extremely difficult because mutations may accumulate during the potentially long time it may take to correctly detect and identify the pathogen (Fletcher et al., 2011).

The strategic use of biological weapons (BWs) against plants by state programs was considered as a means to cause economic damage or to reduce the enemy's food supplies (Whitby, 2006). After World War II (WWII), the development of anti-crop BWs was pursued by programs in the U. S., United Kingdom (U.K.), Soviet Union, Iraq, and others. Research in the U. S. focused on fungal plant pathogens and other agents for use against rice, potatoes, tobacco, sugar beets, sweet potatoes, and cotton. Most of the research centered on the causative agents of stem rust of wheat (*Puccina graminis*), rice blast (*Piricularia oryzae*), and late blight of potatoes (*Phytophthora infestans*). Other anti-crop agents under review by the U. S. for their potential as BWs included *Puccinia striiformis* (stripe rust of wheat), Hoja Blanca virus (Hoja Blanca of rice), *Xanthomonas oryzae* (Uyeda et Ishiyama - bacterial leaf blight of rice), and *Peronospora arborescens* (downy mildew of poppy) (Whitby, 2006). At the time the U. S. program was terminated, its BW stockpile contained 158,684 pounds of *P. graminis* var. *tritici* and 1,865 pounds of *X. oryzae* (van Courtland Moon, 2006). Studies showed that the *P. graminis* var. *tritici* was very potent with an infectious dose of 0.1 g/acre or 1 pound/10 square miles with aerosolized spores remaining viable for several days (Whitby, 2006).

After WWII, the Soviet Union established the Ekologiya Program whose mission was to develop viruses, bacteria and fungi that would destroy animals and plants important to U. S. agriculture, including pathogens that attacked wheat, rye, potatoes, corn, and rice (Leitenberg and Zilinskas, 2012). Most of the developmental research was conducted at facilities under the Ministry of Agriculture including the Scientific Institute of Phytopathology in Tashkent, Uzbekistan where anti-crop weapons were researched and developed; the Scientific Institute of Phytopathology in Golitsino, Russia, which developed anti-crop weapons, including agents for the destruction of wheat, rye, corn, and rice; and the Scientific Institute and Test Site at the Otar Railway Station, Kazakhstan where anti-crop BWs were tested (Alibek and Handelman, 1999). However, unlike the U. S. program, the Soviet program did not stockpile anti-crop weapons, but rather relied on its capacity to rapidly produce them when needed (Leitenberg and Zilinskas, 2012).

Convincing evidence for prior use of anti-crop BWs by state programs is scant to non-existent (Carus, 2002; Whitby, 2006; Zilinskas, 1999). However, the government of Cuba alleged on several occasions that it was the victim of biological warfare operations conducted by the U. S. (Zilinskas, 1999). These allegations included the introduction of fungi responsible for tobacco blue mold disease (*Peronospora tabacina*) in 1979–80, and sugarcane rust disease (*Puccinia melanocephalo*) in 1979. However, no credible evidence was found supporting these claims and alternative natural explanations for these outbreaks were considered more likely (Zilinskas, 1999).

The deliberate misuse of biological agents posing a threat to the agricultural sector and the food chain has been termed agroterrorism (Ryan and Glarum, 2008). The threat of agroterrorism has led to the promulgation of regulations in the U. S. to ensure the biosafety and biosecurity of plant pathogens. A list of high threat pathogens for humans (select agents) has existed since 1997 (Morse, 2015); however, the addition of comparable pathogens for animals and plants did not occur until after the passage of the Public Health Security and Bioterrorism Preparedness and Response Act in 2002 (Public Law 107-188, 2002). Subtitle B (Agricultural Bioterrorism Protection Act of 2002) of PL 107–188 directed the Secretary of the USDA "to establish and

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TABLE 1 Changes to the USDA/APHIS Plant Select Agent List since its inception.

Pathogen	Year				
	2002 (inception)	2005	2008	2012	2018
Candidatus Liberibacter africanus	Included		Delisted		
Candidatus Liberibacter asiaticus	Included		Delisted		
Coniothyrium glycines (formally *Phoma glycinicola* and *Pyrenochaeta glycines*)			Added		
Peronosclerospora philippinensis (Peronosclerospora sacchari)	Included				
Phakopsora pachyrhizi	Included	Delisted			
Plum pox potyvirus	Included	Delisted			
Ralstonia solanacearum phylotype II sequevar 1 (Race 3, biovar 2)	Included				
Rathayibacter toxicus			Added		
Sclerophthora zeae (raysiae)	Included				
Synchytrium endobioticum	Included				
Xanthomonas oryzae	Included				
Xylella fastidiosa (citrus variegated chlorosis strain)	Included				Delisted

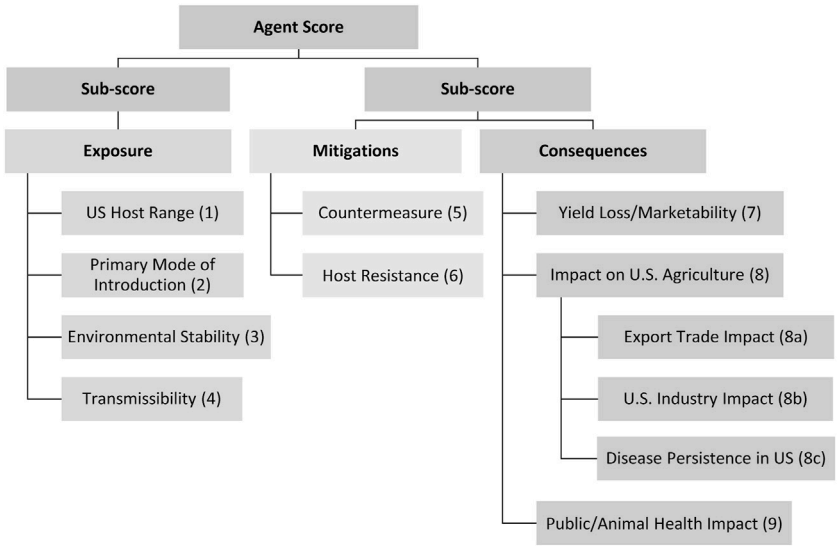

FIGURE 1
Summary of the criteria and hierarchy captured in the MCDA framework and fact sheets.

maintain a list of biological agents and toxins that he/she determined have the potential to pose a severe threat to plant health or products. The criteria for inclusion on this list included: 1) the effect of an agent or toxin on plant health or products and marketability of plant products; 2) the virulence of an agent or degree of toxicity of the toxin and the methods by which the agents or toxins are transferred to plants; 3) the availability and effectiveness of treatments (e.g., fungicides) for any illness caused by an agent or toxin; and 4) other criteria that the USDA Secretary considers appropriate to protect plant health or plant products" (CFR Title 7, Subtitle B, Chapter 3 part 331). The plant Select Agent list is reviewed and re-evaluated on a biennial basis (Table 1). However, there is a significant difference between Select Agent lists for humans and animals and that for plants. While the former lists include both endemic and exotic pathogens, plant pathogens that are established (i.e., unlikely to be eradicable) in the U. S. are excluded or delisted when they enter the U.S. and become established. For example, pathogens that were on the list at one time (e.g., *Phakopsora pachyrhizi*, plum pox potyvirus, *Xylella fastidiosa*, *Liberibacter africanus*, and *Liberibacter asiaticus*) were delisted after they entered and became established in the U. S. (Table 1).

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TABLE 2 Criteria scoring definitions.

U.S. Host Range 1)—Hosts in the U.S. that are susceptible to the disease (e.g., corn, soybeans, wheat, citrus, *etc.*)	
0	None
2	Oats
4	Rice, sorghum, citrus or barley
6	Wheat, potatoes, forage grasses consumed by livestock, sugarcane or cotton
8	Corn or soybeans
10	Large host range (e.g., multiple U.S. crops or multiple plant families that would significantly impact the U.S. economy, i.e., vegetables, fruit and tree nuts)
Primary Mode of Introduction 2)—The routes in which the pathogen is introduced to susceptible hosts	
0	None
2	Vector or contaminated seed
4	Through contaminated soil or ground water
6	Through direct exposure to pathogen via aerosols
8	2 different routes
10	3 different routes
Environmental Stability 3)—The extent to which the pathogen is stable outside the host, in the environment (e.g., in soil, water) and on surfaces/fomites	
0	Is not stable in the environment
2	Cannot survive in the environment without a host
4	Is stable only in the absence of sunlight or moisture
6	Is stable in soil, water or as an aerosol for up to 2 years
8	Is stable in soil, water or as an aerosol for 2–9 years
10	Is stable in soil, water or as an aerosol for 10 years or more
Transmissibility 4)—The extent to which the disease can be transmitted from plant to plant and farm to farm	
0	None
2	Seed-borne or via nematode
4	Transmitted through fomites (e.g., utensils, tires, farm equipment, boots, diseased plant material) or via mites
6	Transmitted through vectors other than nematode and mites
8	Transmitted short distances (e.g., within a farm) by wind, movement of soil or irrigation water
10	Can be transmitted long-distance (e.g., farm to farm) by wind, water or rain
MITIGATION	
Countermeasures 5)—The availability and effectiveness of countermeasures (e.g., pesticides, fungicides, soil fumigation) and extent to which they can be rapidly deployed and administered in an emergency	
0	No countermeasures required or countermeasures already used in routine operations are effective
2	Identification and elimination of infected crop is sufficient to mitigate disease
4	Specialty countermeasures required or chemical control methods such as fungicides, fumigants and insecticides are effective and can be rapidly deployed
6	Chemical control methods (e.g., fungicides, fumigants and insecticides) are partially effective and/or cannot be rapidly deployed
8	Destructive measures such as crop tillage, burning and/or destroying infected plants and the associated soil are effective
10	No effective countermeasures exist or are feasible

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TABLE 2 (*Continued*) Criteria scoring definitions.

Host Resistance 6)—The extent to which the affected U.S. crops have been genetically engineered or modified to resist disease	
0	All U.S. cultivars are resistant
2	Majority (>80%) of U.S. cultivars are resistant
4	Strains with partial resistance are available (e.g., do not protect against all pathotypes)
6	Resistant strains are available but not in common use in the U.S. (e.g., are available outside the U.S.)
8	<20% of U.S. cultivars are resistant
10	All U.S. cultivars are susceptible
CONSEQUENCES	
Yield Loss/Marketability 7)—The extent to which crop yield/marketability is lost due to disease or toxin production. Consider susceptible varieties	
0	None (or data not available)
2	<10%
4	11%–20%
6	21%–30%
8	30%–40%
10	>40%
Impact on U.S. Agriculture 8)—The burden to U.S. agriculture during and after an event (as measured by quarantine, export trade impacts and U.S. industry impacts)	
Export Trade Impact (8a)—The extent to which the crop is exported from the US as measured by percent of total US crop production in tons	
0	~0%
2	1%–10%, or low expected impact due to existing endemic disease
4	11%–20%
6	21%–40%
8	41%–50%
10	>50%
U.S. Industry Impact (8b)—The size of the U.S. industry for the crops susceptible to the disease and potential impacts to the food supply beyond the expected overhead	
0	None to low impact as control measures are included in overhead costs for endemic diseases
2	<$100M, or low expected impact due to existing control measures for endemic disease
4	$100–999 M
6	$1—9B
8	$10B- 50B
10	>$50B
Disease Persistence in U.S. (8c)—The means by which the pathogen can persist in the U.S. following an introductory event, through harboring in vectors, reservoir populations and/or with conducive climate conditions	
0	No persistence
2	Limited persistence due to unfavorable climate conditions (temperature extremes, rainfall, *etc.*)
4	Persistence contributed by vectors
6	Persistence contributed by alternate host such as weeds and other crops
8	Moderate persistence contributed by contaminated water
10	High persistence contributed by contaminated soil

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TABLE 2 (*Continued*) Criteria scoring definitions.

Direct Public/Animal Health Impact 9)—The potential impact on human or animal health from the agent	
0	Does not cause disease in humans and/or animals
2	Causes mild symptoms and/or is only rarely lethal in humans and/or animals
4	Causes moderate morbidity and low mortality (CFR <9%) in humans and/or animals
6	Causes moderate morbidity and mortality (CFR 10%–19%) in humans and/or animals
8	Causes moderate morbidity and mortality (CFR 20%–29%) in humans and/or animals
10	Causes high morbidity and mortality (CFR >30%) in humans and/or animals

Note: The contribution of nematodes as plant pathogen introduction and transmission factors were recognized during the assessment. Typically, a nematode role in disease transmission is associated with the concurrent movement of infested soil on plants or equipment, infected plant material, or water runoff. The nematode itself does not possess mobility properties to move to new locations and relies upon factors that were part of the transmissibility scoring criteria. CFR- Case Fatality Rate.

TABLE 3 USDA plant select and non-select agents evaluated in this study.

Select agents	**Disease**
• *Coniothyrium glycines*	Red Leaf Blotch of Soybean
• *Peronosclerospora philippinensis (P. sacchari)*	Phillippine Downy Mildew
• *Ralstonia solanacearum phylotype II sequevar 1*	Brown Rot of Potato
• *Rathayibacter toxicus*	Annual Ryegrass Toxicity
• *Sclerophthora zeae (rayssiae)*	Brown Stripe Downy Mildew
• *Synchytrium endobioticum*	Potato Wart
• *Xanthomonas oryzae*	Bacterial blight/Leaf Streak of Rice
Delisted or Non-Select Agent Pathogens	**Disease**
• *Clavibacter michiganensis subsp. Nebraskensis*	Goss' Wilt
• *Erwinia stewartii (Syn. Pantoea stewartii)*	Stewart's Wilt
• *Magnaporthe oryzae* (*syn Pyricularia oryzae*) *Triticum pathotype*	Wheat Blast
• *Phakopsora pachyrhizi*	Asian Soybean Rust
• *Phytophthora infestans*	Late Blight of Potato
• *Puccinia graminis f.*sp. *tritici 'Ug99'races* and variants	Wheat Stem Rust
• *Puccinia striiformis f. sp. Hordei*	Barley Stripe Rust
• *Tilletia indica (Mitra)*	Karnal Bunt
• Wheat Streak Mosaic Virus	Wheat Streak Mosaic Virus infection

TABLE 4 Proposed weight assignment by SMSs for the criteria.

	Criteria	SME assigned weight
Exposure	(1) U.S. host range	2
	(2) Primary Mode of introduction	2
	(3) Environmental stability	2
	(4) Transmissibility	3
Mitigation	(5) Countermeasures	1
	(6) Host resistance	3
Consequence	(7) Yield loss/marketability	3
	(8) Impact on U.S. agriculture	3
	(9) Public/animal health impact	2

Recently, we published the use of MCDA and DSF logic tree analyses to assist the CDC Division of Select Agents and Toxins (DSAT) Program's biennial review of the HHS Select Agent and Toxin list, applying the approach broadly to include non-select agents and toxins to evaluate its robustness (Pillai et al., 2022a; Pillai et al., 2022b). A description of these methodologies, their disadvantages, advantages, and prior application has been previously summarized (Pillai et al., 2022a; Pillai et al., 2022b).

In this study we evaluated whether approaches used for HHS agents would be effective in supporting deliberations and recommendations by the Agricultural Intragovernmental Select Agents and Toxins Technical Advisory Committee (Ag ISATTAC) regarding which pathogens to include on the USDA Select Agent list. Previous efforts by the Ag ISATTAC relied solely on SME assessments. In 2018, the Ag ISATTAC sought to improve upon previous approaches. Two analytical approaches were developed and evaluated for classifying plant pathogens as USDA Select Agents: an MCDA framework and a DSF logic tree. The analytical approaches we describe herein seek to provide approaches for assessing the impact on national security, and to reduce the burden on SMEs by documenting the supporting data from peer-reviewed literature in agent fact sheets to support the process. In this study the selection of agents was determined by SMEs based on their expertise focusing on high consequence exotic pathogens as required by USDA and did not include low consequence endemic pathogens.

Methods

Multi-criteria decision analytical framework

The starting point for the MCDA was a set of 9 criteria that affect bioterrorism risk assessment as set forth in Public Law 107–188, 2002. For convenience, these criteria were grouped into those relevant for agent exposure, mitigation, and consequence, which includes potential economic impact (Figure 1). Note that endemicity is not one of the nine criteria used for the MCDA. SMEs collectively scored these 9 criteria on a scale of 0–10, based on data in the agent fact sheets and using the

TABLE 5 MCDA criteria and scoring used to address questions in the DSF.

Questions	Thresholds for low concern
1. Is this plant pathogen endemic in the U.S.?[a]	Yes
2. Does this pathogen require specific conditions for propagation, infection or persistence?	Environmental Stability score of 3 or below or Disease Persistence score of 3 or below
3. Does the pathogen have the ability or potential to be transmissible from one farm, orchard, location to another by airborne or vector?	Transmissibility score of 3 or below
4. Does the pathogen have the ability to cause severe disruption of crops or marketability?	Yield Loss or Marketability Score of 3 or below
5. Are there effective mechanisms or methods to prevent or contain the spread of the disease rapidly (e.g., removal of infected trees or crops, quarantine followed by destruction, *etc.*)?	Countermeasures score of 3 or below
6. Are there potential countermeasures such as fungicides, pesticides, or genetically engineered crops that are resistant to pathogens?	Host Resistance score of 3 or below or Countermeasures score of 3 or below
7. Is there potential for significant economic impact caused by the pathogen?	Impact on U.S. Agriculture score of 3 or below

[a]Not a MCDA, criterion.

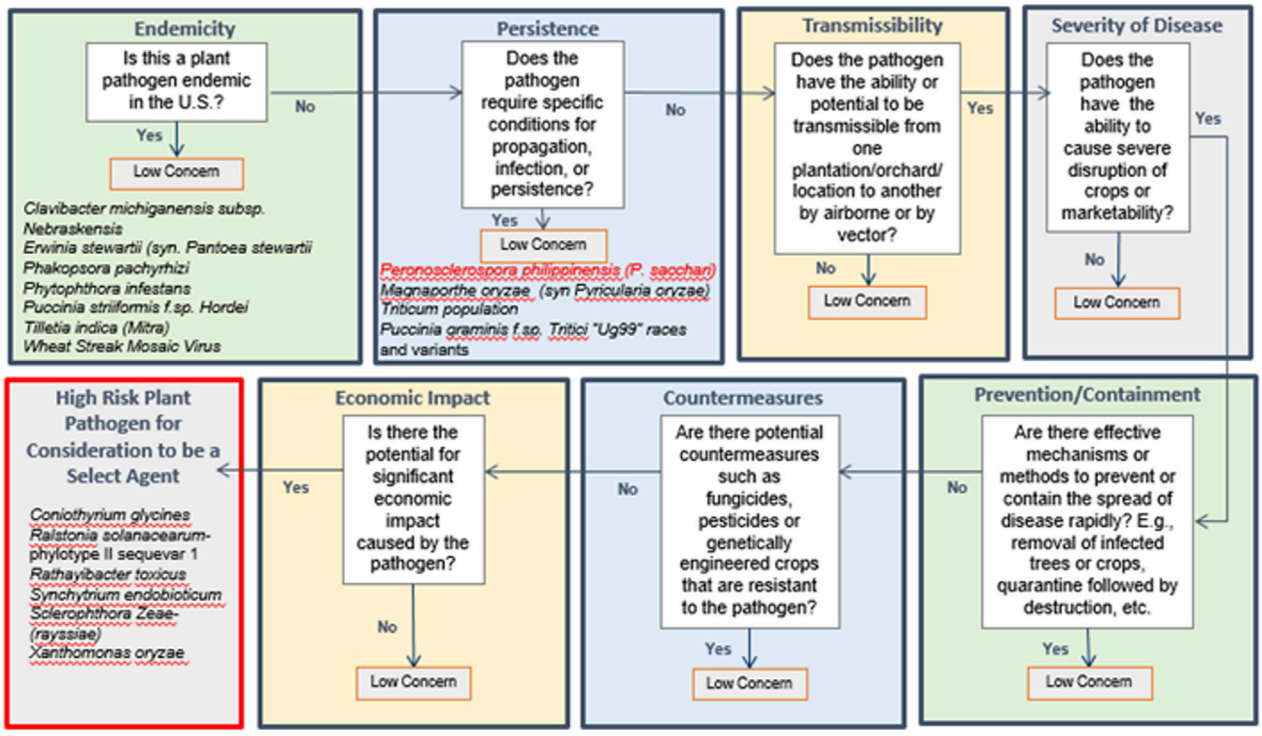

FIGURE 2
Decision Support Framework for assignments of select and non-select agents.

scoring definitions in Table 2 for each of the pathogens in Table 3. The scoring system represents the level of concern applicable to the agent's classification as a plant select agent, ranging from 0 (indicating minimal concern) to 10 (indicating maximum concern). To keep things simple, a linear scale was adopted for this assessment. During the course of the study, SME's/authors with expertise in a particular agent or taxonomic group were asked to score the criteria and lead the discussion to achieve consensus among group members for consistency. Table 2 lists the scoring definitions for each of the criteria for even-numbered scoring options (0, 2, 4, 6, 8, and 10). Odd scores (1, 3, 5, 7, and 9) were used if an SME felt that the most appropriate score fell between the provided even score options.

The 9 criteria scores (1–9) were compiled for each plant pathogen and evaluated in two ways: 1) a one-dimensional (1-D) ranking where the nine scores, either unweighted or weighted (as explained below in the **Criteria weighting** section), were added for each agent, and the agents were then ranked from the lowest to the highest.; and 2) a two-dimensional (2-D) plot where the sum of the

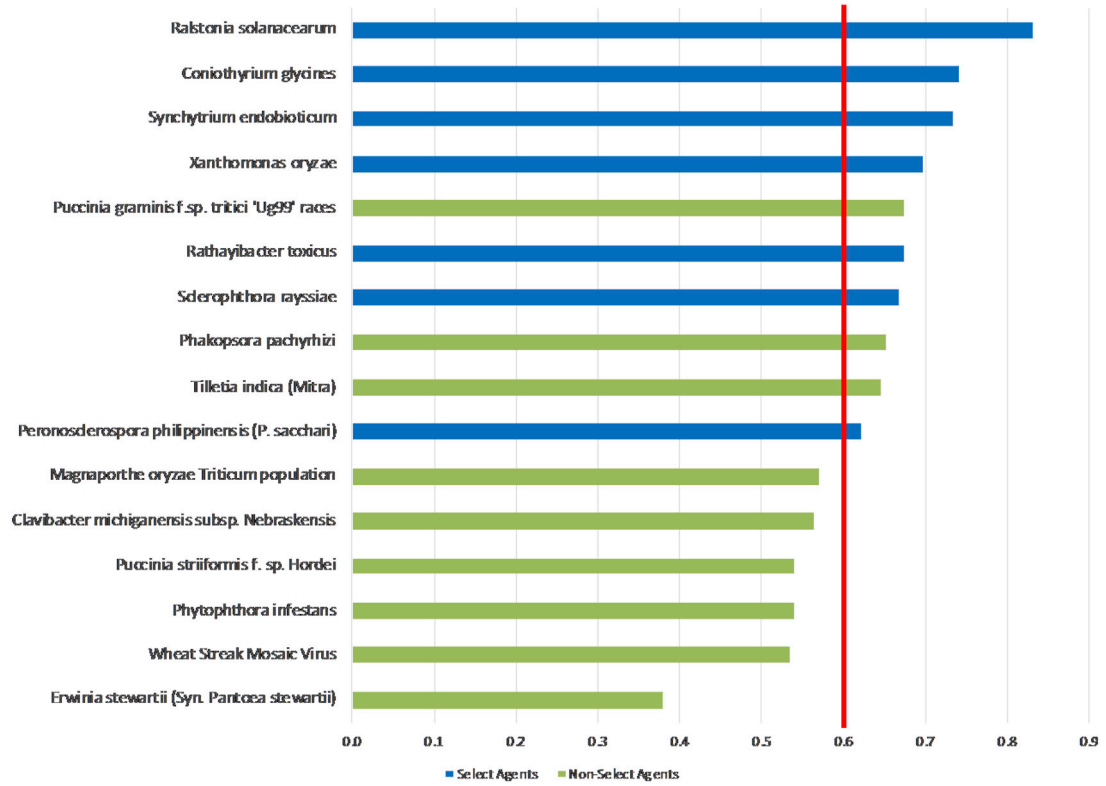

FIGURE 3
1-D plot of unweighted scoring results; select agents shown in blue and non-select agents shown in green. Score threshold, shown by red line, was chosen to be just below the lowest scoring select agent.

sub-scores for the "exposure" (1 + 2 + 3 + 4) branches of the hierarchy were plotted against the sum of the sub-scores for the "mitigation" (5 + 6) plus "consequences" (7 + 8 + 9) branches of the hierarchy. The 2-D plots, with unweighted and weighted sums, are shown in Figures 4, 6.

Agent fact sheets

To challenge the assumptions behind the methodology and provide a useful test matrix, we included pathogens not currently designated as select agents but otherwise considered high risk for other purposes; these include one former select agent that has been delisted, and emerging infectious plant diseases whose potential risks are not yet fully characterized. Agent fact sheets were developed for 16 plant pathogens (Table 3) to provide the data used for scoring pathogens. Among the 16 plant pathogens are 7 current USDA plant select agents and 9 non-select plant pathogens, including two (*Magnaporthe oryzae Triticum population* and *Puccina graminis f.* sp. *tritici 'Ug99' races* and variants) that are non-endemic in the U.S. Due to security concerns, these fact sheets are not included as part of the manuscript but can be made available upon request to the lead author.

The agent fact sheets were created using peer-reviewed open literature sources such as Medline, PubMed, Google Scholar, and other unpublished data (data provided by SMEs) followed by thorough review by SMEs specializing in the specific pathogen. If data could not be found for a particular plant pathogen, data for similar organisms or relevant plant models was used to support scoring. In circumstances where a range of values was found (e.g., Yield loss/Marketability), the worst reasonable case (i.e., leading to the largest "bad" outcome) was typically used for scoring. In every instance, the expertise and judgment of SMEs played a crucial role in ensuring agreement on the most reliable data or foundation for scoring, especially when faced with data gaps or inconsistencies. The SMEs were asked to examine the accuracy and relevance of the information captured in the fact sheets, and the assigned scores for each data category. Any feedback received from the SMEs was integrated into the fact sheets, and adjustments to the scoring were made as necessary. SMEs providing feedback were often aware of the impact of their recommended scoring changes on the results.

Criteria weighting. Since not all criteria chosen for this evaluation are equivalent in terms of risk contribution, SMEs were asked to collectively assign weights (1-, 2-, or 3-fold) to the 9 criteria based on their relative importance, impact and significance to support the risk assessment. The results are shown in Table 4. Transmissibility, Host Resistance, Yield Loss/Marketability and Impact on U.S. Agriculture were given a ×3 weight; US Host Range (by crop value and economic impact), Primary Mode of Introduction, Environmental Stability, and Public/Animal Health Impact were given a 2x weight; and Countermeasures was given a 1x weight. Countermeasures do not just include chemicals but also field burning, Integrated Pest Management, quarantine, *etc.* Therefore, the countermeasures may not control or eradicate the pathogen.

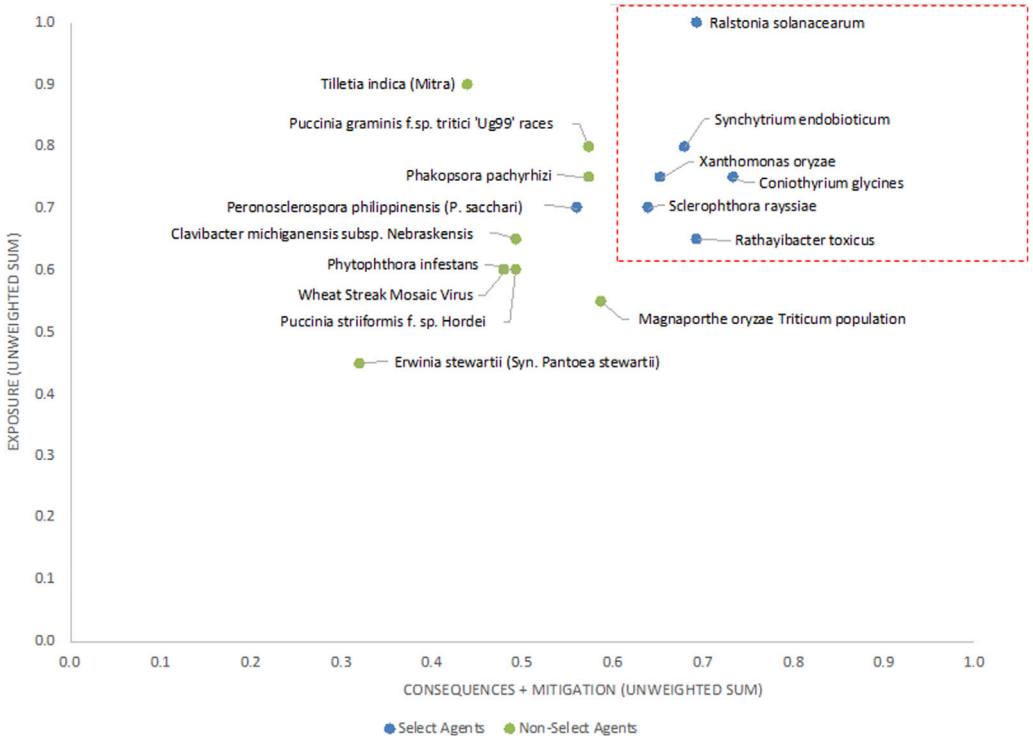

FIGURE 4
2-D plot of unweighted scoring results; select agents are shown in blue and non-select agents shown in green.

Even chemicals may only be effective for a limited time, i.e., developing resistance. Also, nothing is available that is able to prevent spread especially for downy mildews. Even *P. pachyrhizi* can be controlled but it has spread through the Southern U.S. soybean growing regions. Therefore, the SMEs gave it a ×1 weight.

Criteria and weights were combined into a single score (A) by summing all the weighted numerical values (a_i,w_i), where a_i represents a criteria score and w_i is the criteria weighting value:

$$A = \sum_{i=1}^{n} ai \bullet wi$$

To facilitate comparison of results with different weighting values—the weighted case noted above and the unweighted case where all weights are assigned as 1—normalized scores were used, where the total or sub-total scores were standardized relative to a hypothetical agent that received scores of 10 in all criteria.

Decision support framework (DSF)

The DSF methodology uses a logic tree structure with key criteria to identify pathogens that may have such a low level of concern that they can be excluded from consideration as select agents (as shown in Table 5). The DSF considers both the potential impact of regulating an agent that is already present in the U.S. and the agricultural and economic consequences of a biological attack. By employing this approach, a pathogen that fails to meet the threshold value for any of the established criteria, is considered of minimal concern and is not included as a select agent. Pathogens that surpass all the threshold criteria are considered as potential select agents. Criteria encompass elements such as Endemicity, Persistence, Transmissibility, Severity of Disease, Prevention/Containment, Countermeasures, and Economic Impact. Figure 2 provides a visual representation of the DSF logic tree. Expert judgment, based on the data in the agent fact sheets, forms the basis for scoring (as indicated in Table 2). Generally, criteria receiving a score below three are indicative of a "low concern" qualitative assessment. In contrast to the MCDA approach, which employs a graded scoring system for ranking agents, the DSF approach can exclude an agent from select agent consideration based on a single criterion with a low score. Many of the criteria overlap between the MCDA and DSF approaches, except for endemicity, which is not included in the MCDA approach.

Results

Unweighted ranking

As a reference point for comparison to historical Ag ISATTAC assessments where criteria were not mathematically weighted, we evaluated the unweighted (or, equivalently, equally weighted) data. To facilitate comparison of the results with current assignments as select agents and non-select agents, the two classes of agents are color coded blue and green, respectively, in the 1-D and 2-D plots.

The 1-D unweighted results, whereby the total summated scores for all 9 pathogens are compared (Figure 3), indicated that while four select agents received the highest scores—*R. solanacearum, C. glycines, X.*

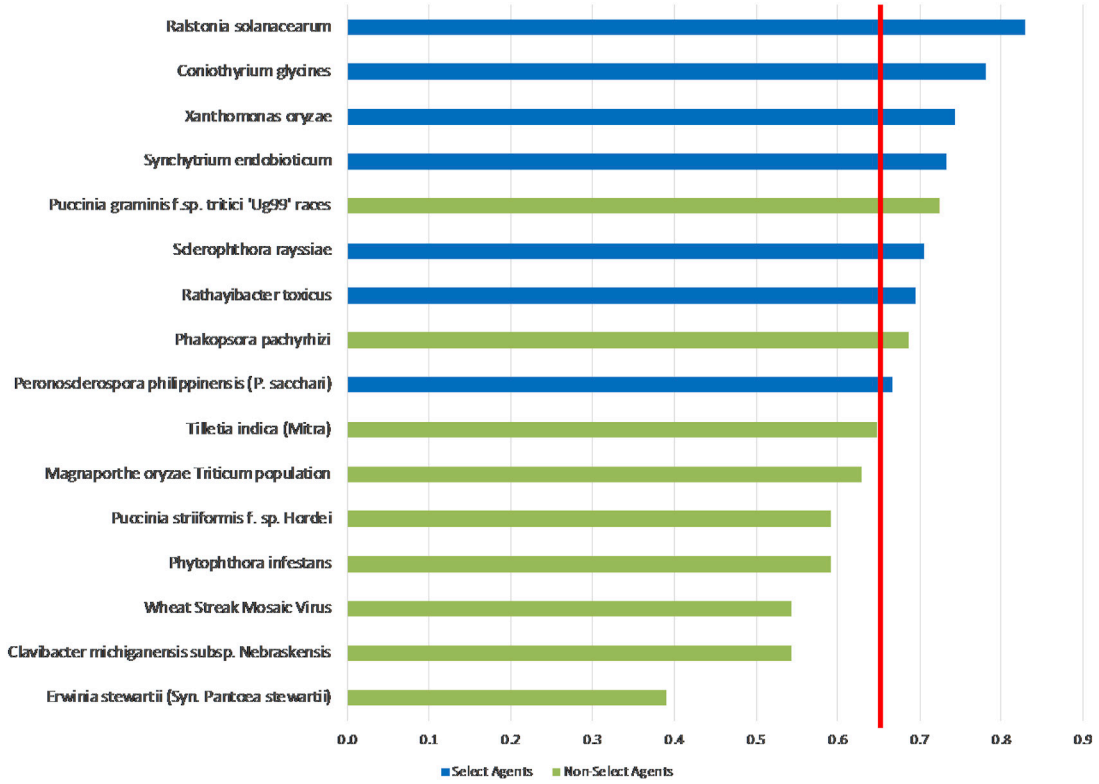

FIGURE 5
1-D plot of weighted scoring results; select agents shown in blue and non-select agents shown in green. Score threshold, shown by red line, was chosen to be just below the lowest scoring select agent.

oryzae and *S. endobioticum*—the other three select agents were further down in the ranking. There is currently no defined method for determining what constitutes a plant select agent. In this study, we have taken different approaches to determine if an arbitrary threshold can be established where there is a break in the data for use in evaluating future plant pathogens of concern. The proposed threshold score to distinguish high-risk from low-risk pathogens that would include the seven current select agents (e.g., score >=0.6, red line in Figure 3) would also include currently non-listed pathogens *P. graminis f.* sp. *tritici 'Ug99' races* and variants, *T. indica* and the delisted *P. pachyrhizi*. *Phakopsora pachyrhizi* and *P. graminis f.* sp. *tritici 'Ug99' races and variants* affect two U.S. high-value crops, soybean and wheat, respectively; have more than two modes of introduction; can be transmitted over a longer range; and could lead to large losses in yield.

The 2-D unweighted results (Figure 4) showed similar trends, with scores for current select agents placing them generally in the upper right-hand quadrant of the plot. If thresholds based on clusters of existing select agent pathogens and breaks in data were established as scores the proposed thresholds of x >=0.60 and y>= 0.63 (red lines in Figure 4) to distinguish high-risk from low-risk pathogens, then all select agents will fall into the high-risk group except for *P. philippinensis* (*P. sacchari*). In this analysis, *P. pachyrhizi, P. graminis f.* sp. *tritici 'Ug99' races* and variants and *T. indica* fall outside the high-risk group. Establishing thresholds that would include *P. philippinensis* (*P. sacchari*) in the high-risk group (for example, adjusting the threshold to $x \geq 0.53$) would also place *P. pachyrhizi* and *P. graminis f.* sp. *tritici 'Ug99' races* and variants in the high-risk group, while excluding *T. indica*.

Analysis of both the 1-D and 2-D plots indicated that, although there were general trends in the data that were consistent with current classifications, there were no sharp breaks in scoring that would serve as a basis or threshold for classifying an agent as a select agent. Instead, the plots represented a continuum of scores. Additionally, any designation of a minimal score—whether the total score in the 1-D plot, or sub-scores corresponding to the x- and y-values in the 2-D plots—resulted in some exceptions to current classifications.

Weighted rankings

The unweighted analysis described in the previous section was repeated using the criteria weighting scheme shown in Table 4. The 1-D and 2-D plots are shown in Figures 5, 6, respectively. As observed with the unweighted data, the general trend in the data was consistent with current classifications; however, any designation of a minimal score as a basis for classification—whether the total score in the 1-D plot, or sub-scores corresponding to x- and *y*-axes values in the 2-D plots—resulted in some exceptions to current classifications. In this study, we have taken different approaches to determine if an arbitrary threshold (which may differ from the previous threshold proposed for the unweighted study) can be established where there is a break in the data for use in evaluating future plant pathogens of concern. In the 1-D ranking, four select agents received the highest scores, while two select agents (*S. rayssiae* and *R. toxicus*) ranked below *P. gramminis f. sp.tritici 'Ug99' races* and variants, and one select agent (*P. philippinensis (P. sacchari)*)

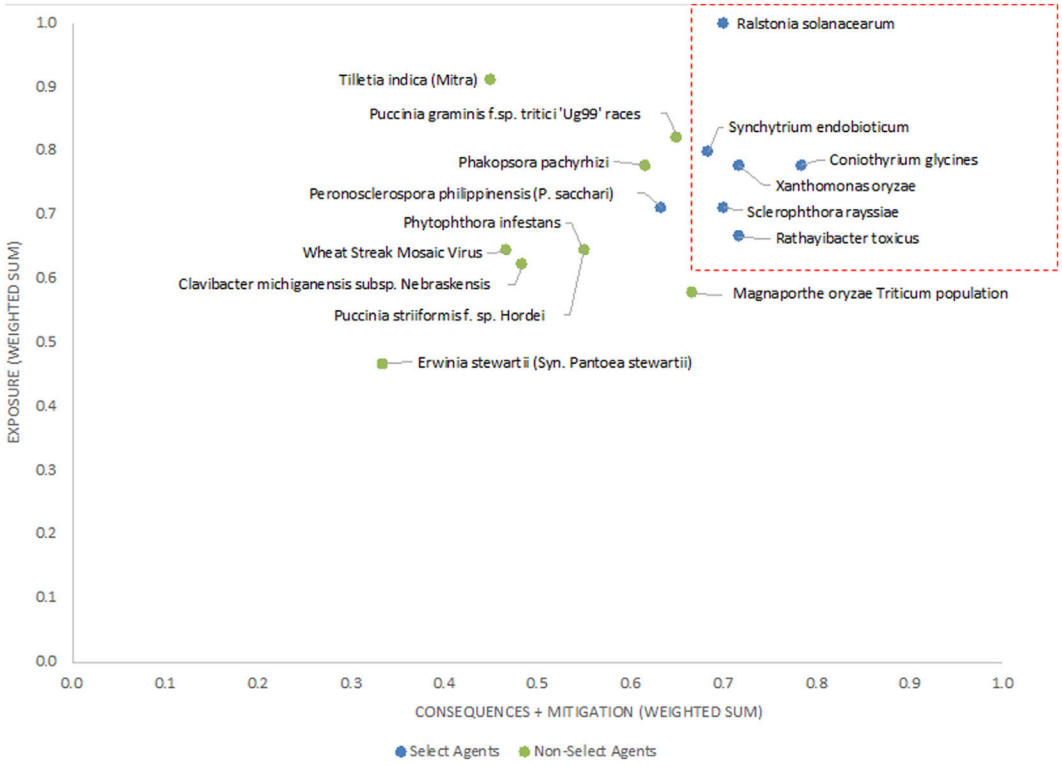

FIGURE 6
2-D plot of weighted scoring results; current select agents are shown in blue and non-select agents shown in green.

ranked below the delisted *P. pachyrhizi*. In the 2-D plot, setting thresholds to distinguish high-risk and low-risk pathogens at scores $x \geq 0.675$ and $y \geq 0.6$ (red lines in Figure 6), all select agents scored in the high-risk group except for *P. philippinensis (P. sacchari)*, placing it in the lower risk grouping along with *P. gramminis f. sp.tritici 'Ug99' races* and variants, *P. pachyrhizi* and *T. indica*. *Peronosclerospora philippinensis (P. sacchari)*, had a low score for "Environmental Stability" and a mid-level score for "Host Resistance" which were more heavily weighted. However, the unweighted 2-D analysis also placed *P. philippinensis (P. sacchari)* in the low-risk group, suggesting the application of the weighting scheme shown in Table 4 did not significantly shift the relative placements enough to allow thresholds that would include *P. philippinensis (P. sacchari)* without also including *P. pachyrhizi* (previously delisted select agent) and *P. graminis f.* sp. *tritici 'Ug99' races* and variants in the high-risk group. *Phakopsora pachyrhizi* and *T. indica* are endemic to the U.S., and thus are ruled out from consideration as select agents based on this programmatic consideration.

Decision support framework

In contrast to the MCDA approach which uses a graded scoring system for ranking agents, the DSF can rule out an agent from select agent consideration using a single low criterion score. While many of the criteria overlap between the two approaches, there are key differences such as the inclusion of "Endemicity" as the initial criterion in the DSF approach (Figure 2).

Applying the criteria for Thresholds for Low Concern listed in Table 5, seven non-select plant pathogens selected by the SMEs for inclusion in the study (which includes *T. indica* and previously delisted select agent *P. pachyrhizi*) were identified as Low Concern and removed from consideration because they are endemic in the U.S. Three additional pathogens—*P. philippinensis (P. sacchari), M. oryzae T. population* and *P. graminis f.* sp. *tritici 'Ug99'races* and variants—were identified as Low Concern and removed from consideration because of the need for specific conditions for propagation, infection or poor environmental persistence. *Peronosclerospora philippinensis (P. sacchari)*, currently listed as a select agent, was removed from consideration due to poor environmental persistence, as well as the existence of available countermeasures. Based on the DSF, the following six agents were recommended for consideration to be a select agent: *C. glycines, R. solanacearum, R. toxicus, S. rayssiae, S. endobioticum, and X. oryzae.* All of these are currently listed as select agents by the USDA (Table 1 and Figure 2).

Discussion

The overall approach employed builds on the previous Ag ISATTAC method and uses MCDA and DSF logic tree techniques. For the plant select agent tiering, we proposed initial criteria, developed fact sheets for 16 select and non-select agents using those criteria, and conducted an evaluation using the MCDA and DSF.

To our knowledge, no similar approach has been reported in the literature for assessing plant select agents across a variety of plant pathogens and U.S. crops. Criteria were selected based on 1) relevant parameters identified during the development of MCDA for human and animal health select agents, and 2) factors that addressed the statutory priorities for what constitutes a select agent for agricultural plant and plant products.

CFR Title 7, Subtitle B, Chapter 3, Part 331 currently lists the following as Plant Protection and Quarantine Select Agents based on those elements: *"Coniothyrium glycines,* (formerly *Phoma glycinicola, Pyrenochaeta glycines); Peronosclerospora philippinensis (Peronosclerospora sacchari); Ralstonia solanacearum; Rathayibacter toxicus; Sclerophthora rayssiae; Synchytrium endobioticum; and Xanthomonas oryzae"*.

The MCDA hierarchy approach breaks down the agent score into key elements of bioterrorism risk: difficulty of a successful attack (exposure), mitigation factors and consequences. While "Ease of Production" was included as a criterion for the animal and human MCDA evaluations (Pillai et al., 2022a; Pillai et al., 2023), it was weighted low and was not included here due to lack of data.

In response to plant select agent SME feedback, "Exposure" criteria were revised to better describe processes and terms specific to plants and plant pathogens. "Route of Transmission," was updated to "Primary Mode of Introduction" to more accurately describe how a pathogen is introduced to a susceptible crop. "Aerosol stability" and "aerosol" as a mode of introduction were replaced with "Environmental Stability" which focused on the stability of the pathogen once introduced into the environment and "wind", as aerosolized pathogens pertain to a mechanism of respiratory exposure in animals and people which does not apply to plants. The term was further refined to "Primary Mode of Introduction" to clarify that scores are based on the main mechanism that a pathogen infects a susceptible crop. Although some would argue that a vectored pathogen would pose similar risk as an aerially transmitted pathogen, it is important to note that not all vectored pathogens contribute to the same degree. Vectored pathogens that are localized (e.g., soil nematodes) *versus* pathogens that can be transmitted by flying vectors may have different transmissibility pattern and impact. "U.S. Host Range" was added as an exposure criterion to reflect the different U.S. crop species which could be impacted by a given agent.

The "Consequences" sub score was split into two categories: "Consequences" and "Mitigation," which included "Countermeasures" and "Host Resistance." For Host Resistance, we did not take into consideration unknown resistance simply because there is no data. For 8b, the impact associated with human health was captured under Direct Public/Animal Health Impact. Specific scoring definitions under "Countermeasures" were changed compared to human and animal criteria based on SME feedback to reflect the ways infected crop species would be addressed by industry using available chemical, physical or other measures and to incorporate whether these measures would be readily deployable.

Under "Consequences" and "Impact on U.S. Agriculture," "Quarantine" was removed, and "Disease Persistence" added. "Quarantine" focused more on regulatory policies that may vary by jurisdiction, whereas "Disease Persistence" better described the longer-range impact on a farm. "Ability to Genetically Alter Pathogen" was removed, and "Impact on Field Production" was removed as it focused solely on field crops and was difficult to score for *X. oryzae* which impacts rice. "Public and Animal Health Impacts" were added to "Consequences" to include the risk of livestock mortalities from contaminated crops, such as *R. toxicus* toxins in livestock feed. Export impacts and endemicity were captured under "Export Trade Impacts" and "U.S. Industry Impacts". While endemic pathogens may not be endemic across the entire U.S., SMEs agreed that those pathogens endemic anywhere in the U.S. should receive a lower score because of their existing persistence. Under "Export Trade Impacts," endemic agents score a "2" as the criteria was updated to include "low impact due to existing control measures for endemic diseases". Under "U.S. Industry Impacts," endemic agents score a "0" as the criterion was updated to include low impact, as control measures are included in the overhead costs for endemic diseases.

Throughout the study, a critical element was SMEs' contribution and feedback on the fact sheets and data interpretation. SMEs provided additional reference materials and data related to "Transmissibility" and "Primary Mode of Introduction," resulting in a higher score for *R. solanacearum*, *S. rayssiae, P. graminis f.* sp. *tritici 'Ug99' race* and variants, and *S. endobioticum*, and raised questions on how to address biotrophs. SMEs also provided guidance on how to score "Disease Persistence in the U.S." for *P. philippinensis (P. sacchari)*. This pathogen would most easily become persistent in weeds along the Gulf coast; however, the main crop host—corn—is not as abundant in that region. A similar concern was raised for *M. oryzae* Triticum pathotype, a pathogen which could be economically harmful, yet may be limited in spread since it favors tropical climates. Due to the high reproductive rate for *P. infestans* under ideal conditions, SMEs advised an increased score for "Yield Loss," and Ag ISATTAC members recommended increasing the "Countermeasures" score for *S. rayssiae* to reflect the fact fungicides had a time-limited efficacy.

Conclusion

We developed and evaluated two risk-based analytical approaches for classifying plant pathogens to support deliberations and recommendations by the Ag ISATTAC regarding which pathogens to include on the USDA Select Agent list. Previous efforts relied on SME assessments to rank the agents and did not apply the approach broadly to include non-select agent pathogens due to the additional burden placed on the SMEs. The analytical approaches presented here seek to provide a systematic approach for assessing bioterrorism risk, and to reduce the burden on SMEs by documenting the supporting data from the peer-reviewed literature in archivable data sheets. We applied the methodology broadly to evaluate the general applicability of the approach by including a variety of non-select agents in the assessment. The results of this assessment for classifying plant select agents offers a scientific and logical approach for supporting the biennial assessment of the country's select agent programs.

Comparison of the analytical results with the current Select Agent List provided a useful reference point for evaluating these approaches and their potential impact on decision making. Both analytical approaches suggested all the current plant select agents qualify as select agents except for *P. philippinensis (P. sacchari)*, whereas all the non-select plant pathogens we evaluated failed to qualify as a select agent. *Puccina graminis f.* sp. *tritici 'Ug99' race* and variants came the closest to the thresholds for inclusion as a select agent using the MCDA method; however, it was ruled out using the DSF framework due to the need for specific conditions for propagation, infection or persistence in the environment, the same criteria that also ruled out *P. philippinensis (P. sacchari)*. Climate change as an individual factor and its impact was not taken into consideration in this study in detail. However, evaluation of a pathogen's current host range was considered. The host range can increase or decrease based upon environmental factors. *Phakopsora pachyrhizi*, a previously listed select agent, also scored close to thresholds using the MCDA approach; however, it was ruled out using the DSF framework as it is now endemic in the U.S.

Application of the methodology using both select agent and non-select agent pathogens, while helping to demonstrate the robustness of the approach, highlighted the challenges of data gaps for many pathogens and the importance of SME input and discussions. In this study the list of plant pathogens was selected collaboratively by the SMEs from USDA, other agencies and institutions to narrow our focus to refine the methodology and assess its robustness. In addition to providing risk-based tools for informing programmatic decision-making, we found that the methodologies were also useful for identifying those parameters and pathogens where more data are needed to help with prioritizing future research studies. Our future goal is to include additional pathogens as well as performing statistical and sensitivity analysis to better understand the robustness of this tool.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Anthrax revisited: how assessing the unpredictable can improve biosecurity

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B. anthracis is one of the most often weaponized pathogens. States had it in their bioweapons programs and criminals and terrorists have used or attempted to use it. This study is motivated by the narrative that emerging and developing technologies today contribute to the amplification of danger through greater easiness, accessibility and affordability of steps in the making of an anthrax weapon. As states would have way better preconditions if they would decide for an offensive bioweapons program, we focus on bioterrorism. This paper analyzes and assesses the possible bioterrorism threat arising from advances in synthetic biology, genome editing, information availability, and other emerging, and converging sciences and enabling technologies. Methodologically we apply foresight methods to encourage the analysis of contemporary technological advances. We have developed a conceptual six-step foresight science framework approach. It represents a synthesis of various foresight methodologies including literature review, elements of horizon scanning, trend impact analysis, red team exercise, and free flow open-ended discussions. Our results show a significant shift in the threat landscape. Increasing affordability, widespread distribution, efficiency, as well as ease of use of DNA synthesis, and rapid advances in genome-editing and synthetic genomic technologies lead to an ever-growing number and types of actors who could potentially weaponize *B. anthracis*. Understanding the current and future capabilities of these technologies and their potential for misuse critically shapes the current and future threat landscape and underlines the necessary adaptation of biosecurity measures in the spheres of multi-level political decision making and in the science community.

KEYWORDS

***Bacillus anthracis*, anthrax, biosecurity, bioweapon, bioterrorism, threat evaluation, synthetic biology, converging sciences**

1 Introduction

Historically, mostly naturally occurring pathogens, such as *B. anthracis* were developed as biological weapons (BWs) due to their inherent infectious and often lethal characteristics (Frischknecht, 2003; Kaufer et al., 2020). The past decades have witnessed an immense increase in the rate of development and research related to life sciences for both industry and academia with applications in all relevant fields. Some of these technological advances and scientific techniques have an exceptional dual-use and hence misuse potential (Lentzos, 2016; Kaufer et al., 2020; Kosal, 2021; World Health Organization, 2022), and could be

adapted to develop a new class of advanced BW agents. These can be engineered to elicit enhanced or new effects and alter them to become devastating agents for biological warfare or bioterrorism (Ainscough, 2002; Paris, 2023). However, it is the combination of different technological achievements and developments that together can lower the thresholds for the development of novel biological and chemical weapons.

Multiple national and international legislative regulations such as the Biological Weapons Convention (BWC) provide legally binding measures to prevent the work with biological agents for non-peaceful purposes. Their aim is summarized in the so-called "general purpose criterion", Article I of BWC, additionally, Article IV obligates states-parties "to prohibit and prevent the development, production, stockpiling, acquisition or retention of the agents, toxins, weapons, equipment and means of delivery specified in Article I of the Convention, within the territory of such State, under its jurisdiction or under its control anywhere." (United Nations, 1972). Furthermore, multiple export regime controls, such as the Australia Group (AG) (The Australian Department of Foreign Affairs and Trade, 1958) and the Wassenaar Arrangement (Wassenaar Arrangement Secretariat, 1995) have been implemented to prevent the proliferation of dual-use goods and technologies and to promote the transparency of national export control regimes. Moreover, the United Nations Security Council Resolution 1540 (2004) (United Nations Security Council, 2004) obligates states to implement measures against terrorism with nuclear, chemical and biological weapons. With a view to BWs, however, concerns are raised that emerging technologies might serve especially bioterrorists to circumvent existing biosecurity regulations and governance raising legitimate questions about the existing biosecurity landscape (Trump et al., 2021a; DiEuliis, 2022). While such concerns have been raised before, the current threat landscape is more complex than when discussed in 1971 (United States Arms Control and DIsarmament Agency, 1971) or 2001 (Zilinskas, 2020).

Synthetic biology (SynBio) is an emerging technology with many useful applications exemplifying the technological power inherent to biotechnology like the generation of synthetic viruses, bacteria, and eukaryotic cells (Venter et al., 2022), partly synthetic chloroplasts (Miller et al., 2020), the generation of photosynthetically more efficient C_3-plants (South et al., 2019), or the by now well-known mRNA vaccines (May 2021). However, it is one of the major categories of dual-use research of concern (DURC) for pathogenic microorganisms (MacIntyre, 2015; Sun et al., 2022). With SynBio normally benign microorganisms can be engineered to secrete toxins or even hard-to-obtain regulated pathogens could be assembled in the laboratory (Singh and Kuhn, 2019; Sanz et al., 2022). Genetic modification by editing, deleting, and inserting desired sequences into targeted sites of a genome (Eisenstein, 2020; Hoose et al., 2023; Yeom et al., 2023) by harnessing the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas)9 system for genome editing (Jinek et al., 2012; Zhang et al., 2020) may increase the bio-threat potential. In addition, many important biotechnological techniques bear a dual-use and hence misuse potential such as whole-genome sequencing or oligonucleotide synthesis and DNA assembly (assembling multiple smaller fragments of oligonucleotides into the desired larger sequence). Using Golden Gate and Gibson assembly technologies, artificial DNA molecules can be synthesized with greatly reduced cost and time. In fact, the cost of oligonucleotide synthesis has dropped as low as $0.07–0.1 per base and continues to decrease (Sun et al., 2022; Hoose et al., 2023).

Furthermore, such advances do not occur in a vacuum, they are accompanied, supported, and further enhanced by converging technologies from other fields of science. Surely, one of the most influential fields is that of bioinformatics additionally boosted by the advent of artificial intelligence (AI) and machine learning, enabling all branches of omics, biomedical imaging, and signal processing (Min et al., 2016), as well as protein structure prediction (Jumper et al., 2021). Other converging technologies entail robotics relevant for manufacturing and drones, additive manufacturing leading up to 3D bioprinting (Ozbolat et al., 2016), and nanotechnology with application in physics, chemistry, biology, engineering, and medicine (Bracamonte, 2023; Malik et al., 2023; Singh and Kaur, 2023). Furthermore, meteorological data improved critically as an enabling development in biowarfare (Hemming and Macneill, 2020; Levinson, 2022). Taken together, these emerging and converging technologies pave the way for new applications for the weaponization, dissemination, and delivery of biological weapon agents (Brockmann et al., 2019; Kosal and Kosal, 2020; Favaro et al., 2022). Such new agents and BW delivery systems (e.g., drones and advanced aerosolizers) could provide an array of additional and novel use options, expanding the BW paradigm (Pethő-Kiss, 2022) innovative approaches to counterproliferation, detection, mitigation, medical countermeasures, and forensics for attribution. Consequently an adaptation or a change in the biosecurity architecture including biodefense, preparedness, and prevention is necessary (National Academies of Sciences, 2018; Trump et al., 2021b).

Indeed, thus far mostly state actors have been applying advanced technologies for weapons production, at least in past programs (Caudle et al., 1997; Riedel, 2004). Hence, traditionally, concerns over the misuse of, for example, genetic engineering have focused on state-sponsored biological warfare programs possessing the necessary high level of knowledge, skills, and resources to accomplish this challenging and multifaceted task. However, the increasing affordability, widespread distribution, as well as efficiency and ease of use of DNA synthesis and together with rapid advances in genome-editing and synthetic genomic technologies lead to an ever-growing number and types of actors who could potentially misuse existing knowledge and emerging technologies (Hoffmann et al., 2023; Paris, 2023). Therefore, as the field advances, BW are expected to become a larger concern as they could be misused by malicious non-state actors, because scientific advances will make use of biological agents more accessible (Sanz et al., 2022; Yassif, 2022). In the past, organized non-state groups and potential adversaries demonstrated they can acquire dangerous biological agents if sufficiently determined. Therefore the focus of the present manuscript is bioterrorism. In fact, there have been several confirmed cases of biological agent events between (Carus, 2001). Noteworthy, the influence of scientific progress in relevant fields on the likelihood of bioterrorists attaining and using BWs can not be quantitatively determined. Past terrorists' failures to develop and use BWs indicate that developing a BW is a highly intricate process. Thus, the impact of a single scientific breakthrough or a novel

technology on BW acquisition should not be overstated (Koblentz, 2020). Understanding the current and future technology capabilities and their misuse potential is critical for understanding the current and future threat landscape, i.e., biodefense and biosecurity. New bioagents could emerge or be developed much faster than defenses against these threats can be built.

Biological weapons do not only pose a threat through state-sponsored programs but also in bioterrorism and bio-crime incidents. (For distinction please refer to Jansen et al. (Jansen et al., 2014). This paper focuses on *B. anthracis* as a biological weapon agent. The zoonotic bacterial pathogen *B. anthracis* is the etiological agent of peracute, acute, subacute and chronic anthrax, an often fatal toxin-mediated disease primarily affecting herbivores, but also encountered in other mammals, including humans, and occasionally birds (Turnbull, 2014). Mostly in poor rural areas, up to 2,000 (WHO, 2023) cases (Hesse et al., 2022; WHO, 2023) of anthrax occur annually worldwide (Carlson et al., 2019). Although, *B. anthracis* and its persistent endospores in the soil generally do not pose a public health concern in post-industrial societies, it is one of the high-priority and most dangerous BW agents. It is thus classified by the Centers for Disease Control and Prevention (CDC) as a category A agent, posing the highest risk to the public and national security because of its widespread availability, environmental stability, easy dissemination, its morbidity and mortality, and consequently the high potential for social disruption (Rotz et al., 2002; Riedel, 2005; Cole and Bergman, 2010; Morse, 2014; Johns Hopkins Center for Health Security, 2023). Among the three major forms of human anthrax (cutaneous, gastrointestinal, and inhalational), cutaneous anthrax is the most common with a 80% survival rate even if untreated. The form most likely resulting from an aerosolized spores is inhalational anthrax. Prior to 2001, it was believed that inhalational anthrax would lead to 90% of fatal cases. However, in the anthrax attack of 2001, with prompt recognition and treatment with appropriate antibiotics, the fatality rate was reduced to 5 out of 11 anthrax victims.

Phylogenetically *B. anthracis* belongs to the *B. cereus sensu lato* group consisting of 18 closely related sporulating Gram-positive bacteria including *B. cereus* and *B. thuringiensis* (Acevedo et al., 2019). Despite their highly divergent pathogenicity, the chromosomes of these three species show very high genetic similarity, while their rRNA sequences are nearly identical, showing only varaiations expected within different species (Bazinet, 2017). *Bacillus thuringiensis* infects insect larvae, while *B. anthracis* and *B. cereus* are mammalian and human pathogens. While anthrax is often fatal, *B. cereus* is a opportunistic pathogen causing periodontitis, foodborne illness, and acute ophthalmitis in humans (Kotiranta et al., 2000; Argôlo-Filho and Loguercio, 2013; Granum, 2017; Pilo and Frey, 2018). Some bacterial strains of *B. cereus,* e.g., *B. cereus biovar anthracis*, which are ubiquitous in West Africa, cause an anthrax-like disease in a broad host range of mammals (Pilo and Frey, 2018). So far, no cases of human infections with this strain have been reported. Nonetheless, the CDC has included this pathogen in the list of Biological Select Agents and Toxins (BSAT) posing a potential risk to public health and safety (American Society for Microbiology, 2017). To the best of our knowledge, such an amendment for *B. cereus biovar anthracis* is currently lacking in the European Union (EU) regulations on dual-use items.

Using the most thoroughly studied traditional BW agent *B. anthracis* (Savcı, 2019) as a prime example, this paper analyzes and assesses the possible bioterrorism threat arising from advances in synthetic biology and other converging sciences. In addition, the possible required biosecurity adaptations in the field of biodefense are identified. Creating effective biosecurity procedures will require understanding the present state of synthetic biology and other biosecurity-relevant emerging technologies. For a realistic harm potential and threat assessment of a future *B. anthracis* BW, it is necessary to weigh and reassess identified hazards and novel threats against established mitigation measures and possible countermeasures. This includes, on the one hand, knowing the platforms and technologies available for construction or engineering *B. anthracis* or related microbes, and planning for the future when the field overcomes bottlenecks or barriers. On the other hand, effective biosecurity requires continuous technology mapping to identify possible *B. anthracis* dissemination routes, its potential targets and the ability to apply forensics for attribution after an attack. This article addresses a problem on the intersection of life sciences and security studies and is hence written from a transdisciplinary perspective.

2 Materials and methods

Our applied methodology falls under the umbrella of foresight methods defined as "a systematic, participatory, future-intelligence-gathering and medium-to-long-term vision-building process aimed at enabling present-day decisions and mobilizing joint action" (Miles et al., 2016; Foresight, 2018). Foresight methodology is applied to encourage the analysis and consideration of a range of future biosecurity hazards arising from contemporary advances in synthetic biology and other technologies to inform decision-making and public policy (OECD, 2019).

For the current study, we have developed a conceptual six-step foresight science framework approach as depicted in Figure 1. This framework was adapted from biosecurity and anti-bioterrorism studies and represents a synthesis of various foresight methodologies implementing literature review, and elements of horizon scanning, trend impact analysis, red team exercise (Zhang and Gronvall, 2020; Moran, 2021) and free flow open-ended discussions. We have chosen this framework because it builds on existing knowledge of historical anthrax attacks and analyzes the possible future implications of a changing scientific and technological environment for *B. anthracis* BW development and employment. This is a prerequisite to proactively deterring or defeating future threats by exposing vulnerabilities and allowing for corrective actions. In addition, it allows evaluation of whether advances in science and technology may enhance the possibility of malicious actors gaining access to the required knowledge and scientific infrastructure to develop and use an anthrax BW. This information is required for threat analysis, that in turn could reveal possible deficiencies in the current biosecurity management system.

By reviewing over 600 publications, the historical development of *B. anthracis* in BW programs and its potential use as a modern biological weapon agent driven partly by advances in biosciences will be traced to set the scope. Of special interest is the literature on the anthrax bioagent including methodology to genetically engineer

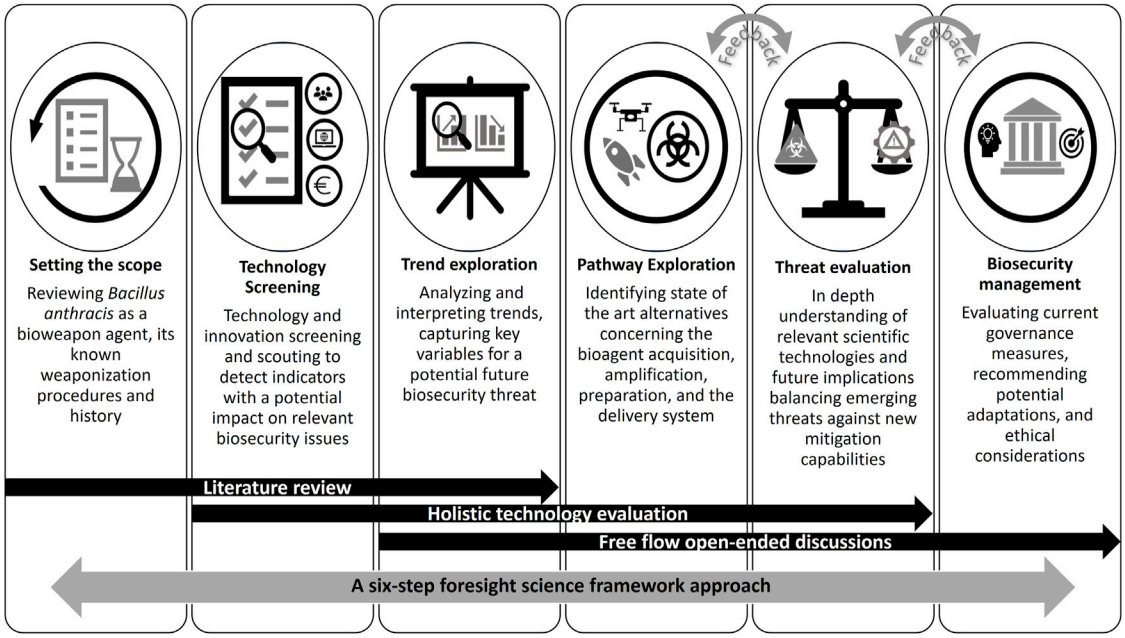

FIGURE 1
A conceptual six six-step foresight science framework approach.

B. anthracis and related strains, different delivery systems, international BW governance and mitigation strategies, but also export control laws, and agent detection methodology. Furthermore, the literature research served the purpose of feeding into steps of technology screening and qualitative trend extrapolation in identifying indicators and structural trend shifts, respectively. While indicators identified by the technology screening are hinting at potential future tendencies either in the form of basic research, patents, investments, or among others social phenomena (Amanatidou et al., 2012), trend extrapolation, on the other hand, focuses on established ongoing dynamics such as trends and driving forces. Technology screening and trend extrapolation were performed to elucidate how contemporary dynamic science developments especially in the field of synthetic biology could facilitate new biosecurity challenges (Bakhtin et al., 2017; Kohler, 2021). In the pathway exploration special emphasis was given to the current technology advancements or knowledge availability and accessibility with relevance to weaponizing. In this study, we examine the current technical obstacles and possibilities a terrorist group may encounter in the development of an anthrax bioweapon. Therefore, in this thought experiment we researched and analyzed every necessary step concerning the bioagent acquisition, amplification, sporulation and aerosolization as well as the delivery system, choosing the most economic development options and those that pose the lowest possible danger for perpetrators to be exposed to the agent during the production process (Figure 2A). Subdividing the process into the necessary labor steps (Figure 2B) helped to investigate potential loopholes and regulatory gaps. Each step (e.g., different acquisition paths to attain a virulent anthrax strain) is described in its difficulties and possibilities, potential bottlenecks, and circumventive alternatives. The goal of pathway exploration is to identify weaknesses and vulnerabilities in systems or strategies, develop more effective plans and processes, and prepare organizations to respond to unexpected challenges and threats.

Thereafter, a threat evaluation of identified hazards and novel threats was performed. The aforementioned foresight science framework steps allowed a holistic technology evaluation and hence an out balancing against established monitoring, medication and mitigation measures and possible countermeasures (Figure 2C) for a potential future *B. anthracis* BW threat evaluation. Free flow open-ended discussions led to suggesting the necessary biosecurity architecture adaptation for appropriate biosecurity management including required measures to raise awareness and preparedness. In addition, recommendations for politicians and other stakeholders were elucidated.

3 Results and discussion

For the conceptual six-step foresight science framework approach the most important findings were considered (listed in Supplementary Table S1). For the sake of convenience, a chronological-analytical representation of these findings is given below.

3.1 *Bacillus anthracis* in warfare

Not surprisingly, anthrax as a BW agent has been the focus of BW research for at least 11 decades (World Health Organization, 1970; Centers for Disease Control and Prevention, 2023). Being a traditional, non-genetically engineered BW agent, *B. anthracis* has reliable traits regarding pathogenicity and is capable of causing

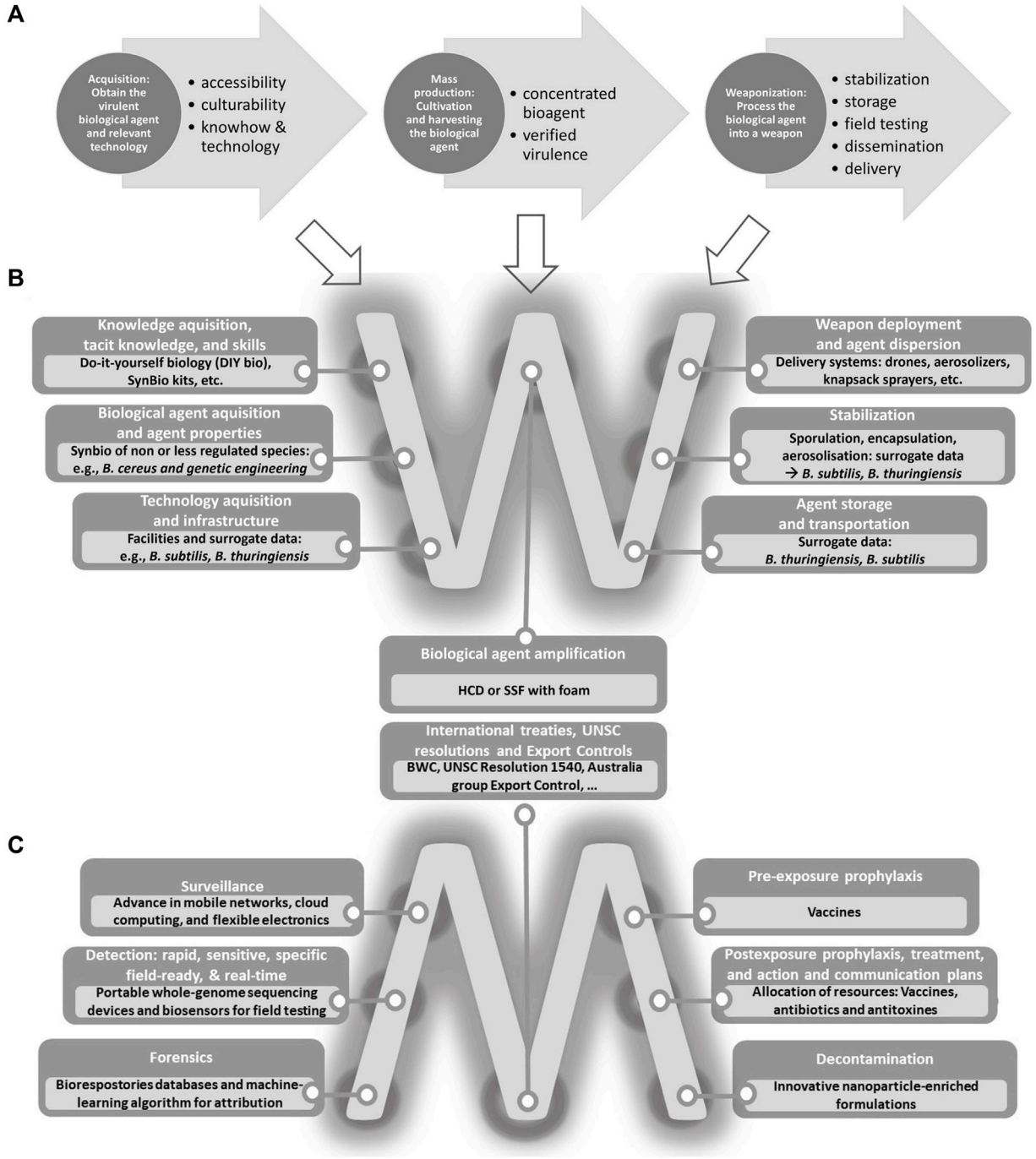

FIGURE 2
Process of the pathway exploration of a biological weapon. **(A)** Technical steps for the development of BWs. Implications of scientific developments, public accessibility of biology, knowledge, and emerging as well as enabling technologies for **(B)** clandestine development of a *Bacillus anthracis* BW; and **(C)** developing measures for monitoring, medication, and mitigation. UNSC: United Nations Security Council; HCD: High-Cell-Density; SSF: Solid-State Fermentation.

lethal inhalation anthrax. In addition, it is characterized by its agent availability; large-scale manufacturability; stability during production, storage, and transportation; ability to be efficiently disseminated including stability after dissemination limited vaccine availability; and previous research and development of the agent as a BW; with the potential of causing public panic and social disruption. Hence, it fulfills all the requirements of a BW agent except for lacking person-to-person transmission.

At least 8 nations are believed or known to have had developed offensive biological weapons programs that include *B. anthracis* until 1990s (Riedel, 2005; Carus, 2017). Indeed, already in World War I, Germany used anthrax to infect animals (Leitenberg, 2001).

During World War II, *B. anthracis* was found in most military BW arsenals. The infamous, Japanese Unit 731 tested anthrax among other BW agents on Chinese prisoners during the occupation of Manchuria (Riedel, 2004; Riedel, 2005). Until the BWC entered into force (1975), the United Kingdom (UK), the United States of America (United States), Japan, and the Soviet Union (USSR) weaponized *B. anthracis* within their military programs, even if an anthrax BW battlefield employment never happened (Bernstein, 1987; Roffey et al., 2002; Cole and Bergman, 2010; Beedham and Davies, 2020; Centers for Disease Control and Prevention, 2023).

An accidental release of anthrax spores in 1979 from the military research and production facility in Sverdlovsk, USSR exposed the deadly impact of this bioagent. At least 68 people died in the ensuing anthrax outbreak (Meselson et al., 1994). In addition, this accident showed that the USSR continued clandestine research on *B. anthracis* during the Cold War, even after the signature of the BWC In the same year, an anthrax vaccine precipitated (AVP) was licensed in the UK (Splino et al., 2005).

During this period, the scientific fields of molecular biology and microbiology, and other technologies were advancing at a very high pace. Two decades after the initiation of the BWC, the Third Review Conference in 1991 decided to establish an Ad Hoc Group of Governmental Experts (Final, 1991) that held four sessions in 1992 and 1993 to identify and examine potential verification measures from a scientific and technical standpoint. Eventually, by the end of the millennium, secret military programs including *B. anthracis* developed, e.g., by Iraq (Zilinskas, 1997; Cole and Bergman, 2010) was terminated by UNSCOM and UNMOVIC and those of USSR (Meselson et al., 1994) were allegedly terminated (Mauroni, 2022) but never veryfied. In addition, the anthrax vaccination of troops and the veterinary vaccine for livestock seemed to contain the danger of. In addition, the anthrax vaccination of troops and the veterinary vaccine for livestock seemed to contain the danger of *B. anthracis* in biological warfare.

3.2 *Bacillus anthracis* in bioterrorism

At about the same time, besides state actors and their BW programs, a new danger in the form of bioterrorist-related inhalation-anthrax attacks emerged, signaling the beginning of a new era. Foremost, terrorists tried to use anthrax as a BW. Aum Shinrikyo cult released *B. anthracis* spores in an unsuccessful biological attack in Kameido, Japan wanting to initiate an inhalation anthrax epidemic. Cult members successfully designed and built a system for pumping a bacterial suspension up eight floors of their head office building to an aerosol dispersal device on the rooftop (Keim et al., 2001). However, this and several other attempts with anthrax spores failed, due to the use of an attenuated Sterne strain, also used as a vaccine for animals (Cole and Bergman, 2010). To date, only a small fragment of the cult's program was uncovered by Japanese police and intelligence, and only parts of evidence have been made publicly available (Riedel, 2004).

In the mid-1990s al-Qaida allegedly underwent attempts to procure and weaponize anthrax bacteria, with the former USSR, Kazakhstan or East Asia as a source of these biological agents. According to United States officials in Afghanistan in late 2001, efforts to weaponize *B. anthracis* failed despite speculated assistance from Russian scientists (Cronin, 2003; Spyer, 2004; Leitenberg, 2005; Salama and Hansell, 2005).

Almost concomitantly in the fall of 2001, letters containing anthrax spores dispatched to high-profile journalists and politicians in the United States killed five non-targeted people, mostly postal workers (Quintiliani and Quintiliani, 2003). A nearly decade-long, $100 million investigation into the 2001 Amerithrax attacks, proved *B. anthracis* mass disrupting capabilities as well as the difficulty associated with investigating such incidents (Böhm and Beyer, 2003; Cole and Bergman, 2010).

3.3 Technology screening and trend extrapolation

Considering anthrax research, we identified several indicators in different pertinent fields with a dual-use potential relevant to anthrax BW development (Supplementary Table S1). The indicators most relevant to our pathway exploration were scientific achievements, advancements or discoveries that could be exploited for *B. anthracis*' BW attribute enhancement or those potentially used to circumvent biosecurity measures implemented to prevent the proliferation and development of a *B. anthracis* BW. In our qualitative approach extrapolating indicators along their trajectories, five major trends were identified as relevant (from most to least significant).

1. Increasing access to standardized biotechnology potentially reduces tacit knowledge requirements (Jackson, 2001; Revill and Jefferson, 2014)
2. Accessibility of scientific data (open access publications, online repositories, literature databanks) is continuously increasing (euroCRIS, 2016)
3. Oligonucleotide synthesis and sequencing are facilitated, readily available and steadily decreasing in costs (Hughes and Ellington, 2017; Hoose et al., 2023)
4. Converging and enabling technologies (Internet, AI, Machine Learning, Additive Manufacturing, unmanned aerial vehicles (UAV), Robotics, and advances in aerosolizing technology) expand the BW paradigm (Brockmann et al., 2019; Lentzos, 2020)
5. The Do-It-Yourself (DIY) and Frugal Science community expands and facilitates communication and protocol design and exchange (Seyfried et al., 2014; Tocchetti and Aguiton, 2015; Tennenbaum et al., 2021)

These trends project impacts that increase future threat potentials by further lowering entry obstacles for BW development, reducing the risk of being detected and uncovered, facilitating BW design and mass production, as well as employment. Potential threats are more closely examined in the pathway exploration. At the same time, these trends might also contribute to strengthening preparedness, prevention, and mitigation.

3.4 Pathway exploration

To evaluate the inherent threat posed by contemporary science and the possible new dangers arising from scientific and

technological advances, *B. anthracis* BW development stages were thoroughly analyzed. Relevant findings from technology screening and trend extrapolation feed into our pathway exploration. Generally, the development of a *B. anthracis* weapon starts with the acquisition, followed by mass production, and ends with the weaponization of *B. anthracis a*s depicted in Figure 2A.

The following part contains considerations on scientific developments, public accessibility of biology, knowledge, and emerging as well as enabling technologies for the clandestine development of a *B. anthracis* BW by terrorists (Figure 2B).

3.4.1 Knowledge acquisition

The deliberate, malicious misuse of biosciences and technology, besides a motive, requires intention and material resources including technological infrastructure, access to information as well as necessary explicit and tacit knowledge (Vogel, 2006; Nixdorff, 2020). Since the turn of the millennium, there exists an apprehension that terrorists or other state- or non-state actors might circumvent existing biosecurity regulatory systems by acquiring new tacit knowledge, expertise, or vulnerabilities to develop biological weapons (Riedel, 2005; Cole and Bergman, 2010; Mondange et al., 2022). Explicit knowledge includes standard operation procedures for producing and processing biological agents and can be acquired through open-access scientific literature. In fact, a vast amount of knowledge and information from many decades of anthrax research is available and publicly accessible (Morris and Boyack, 2005; Savcı, 2019). Undeniably, globalization and the internet have significantly diminished the barrier to acquiring explicit knowledge. Tacit knowledge on the other hand is not only acquirable through hands-on encounters but it remains a hindrance to weapon efficiency and effectiveness (Tennenbaum et al., 2021). However, the required tacit knowledge to produce risky biological products is constantly decreasing due to the combination of SynBio with AI and automation.

Principally, forums such as DIY biology classes and Journal of Visualized Experiments (JoVE) videos can transfer the necessary skill and knowledge needed to use otherwise highly sophisticated techniques such as CRISPR during the development of an anthrax BW. Moreover, available kits can help to reduce knowledge and skill requirements during such CRISPR experiments given that the actor can select the appropriate kit and troubleshoot as needed (Paris, 2023). However, it is important to keep in mind that while some terrorist groups may want to genetically engineer *B. anthracis*; others may be satisfied with the most simple way to produce spores.

3.4.2 Biological agent acquisition and agent properties

There are multiple ways to acquire the anthrax bioagent. Here, we more closely examine three, **t**he isolation from natural sources, the illegal procurement from authorized laboratories, and the modification of related organisms to an anthrax bioagent. Furthermore, we examine the possibilities to include antibiotic resistances.

3.4.2.1 Isolation

Due to its well-known danger and for biosecurity reasons, *B. anthracis* is a regulated microorganism (CDC, 2023) by national and international conventions, that cannot be easily acquired from regular sources, such as culture repositories (Sharan et al., 2007). However, being widely distributed in sub-Saharan Africa, China, Kazakhstan, North-, South- and Central America, South- and East Europe, the Caribbean, the Middle East, and Australia (Carlson et al., 2019), one possibility to acquire various starins of *B. anthracis* would be to isolate this microorganism from natural reservoirs from the soil in the reported outbreak area or infested animal carcasses according to established and publicly available protocols (Böhm and Beyer, 2003). Undergraduate microbiology skills can be used to isolate *B. anthracis* from a natural contagious source. Most of the necessary production techniques are readily available in open-access journals and textbooks. With isolated starter culture, a terrorist could grow cultures with billions of spores in a 100-L vessel in less than a week under adequate biosafety precautions. The isolates should be positive in PCR assays for pXO1 and pXO2 probes. These probes are not subjected to security screening and are in general easily attainable, even for non-authorized institutions. Drying the slurry by freeze drying, for example, for weaponization is tricky, though not impossible (Green et al., 2007).

3.4.2.2 Illegal procurement

Another possibility is the illicit acquisition from an authorized institution such as culture collections or research facilities working with dangerous pathogens. Although generally obligatory and stringent biosafety and biosecurity regulations are in place, there is always a possibility of sabotage or intentional misuse of available resources by staff members (as in the case of Amerithrax, 2001), or third parties. Therefore, the possibility of illegal procurement of *B. anthracis* from a research biosafety level (BSL-) 3 laboratory cannot be ruled out. Via relevant research publications and mapped containment laboratories (Bulletin of the Atomic Scientists, 2022) locating relevant BSL-3 laboratories has become an easy endeavor. In addition, there still exists the possibility of obtaining or thieving weaponized anthrax from a state's offensive or defensive program, however less plausible, especially given that there is no knowledge about active offensive activities anywhere and that the number of states with appropriate/suitable defensive programs is limited (Sharan et al., 2007).

3.4.3 Genetic engineering and synthetic biology

From the mid-1980s until 2003, the genome of *B. anthracis* was successfully sequenced (Read et al., 2003) and the two main megaplasmids carrying the main virulence factors pXO1 for the toxins factors edema factor (EF), protective antigen (PA), and lethal factor (LF) (110 MDa, 181 kb) (Green et al., 1985) as well as pXO2 for the capsule (60 MDa, 95 kb) encoding the three genes capB, capC, and capA (Makino et al., 1989; Okinaka et al., 1999) protecting from phagocytosis (Makino et al., 1989) were identified. Strains lacking either plasmid are either avirulent or significantly attenuated (Okinaka et al., 1999; Pilcher, 2003). In the upcoming years, knowledge about other pathogenicity factor genes increased, and "at the dawn of the 21st century, the scientific field of anthrax was perceived as a dead end" (Mondange et al., 2022).

However, as with the progress achieved with recombinant DNA in the 1970s and the rise of synthetic biology in the 2000s, the emergence of genome editing technologies, such as CRISPR in 2012, raised fears about that novel engineered strains of *B. anthracis* could become available for bioterrorism. Knowing the decoded *B. anthracis* genome with its more than 5,000 genes (Read et al., 2002; Pilcher, 2003), CRISPR made more precise editing of

multiple genes simultaneously possible. In addition, genetic engineering for a fraction of the cost of predecessor technologies became feasible. Genetic modifications were now possible that used to be too demanding, laborious, or expensive in the past (Wang et al., 2019).

A more elaborate approach to obtain an anthrax-causing agent would be to modify a related microorganism, e.g., *B. cereus*, and convey all the characteristics of *B. anthracis*. One candidate for such a method could be *B. cereus* G9241, causing anthrax-like symptoms (Marston et al., 2016; Baldwin, 2020). As of 2022, this particular strain was available for purchase in limited amounts. Working with *B. cereus* requires only BSL-2 conditions, whereas with *B. anthracis* BSL-3 conditions have to be applied for intended aerosol production (Baldwin, 2020). Access restrictions become stricter with higher biosafety levels. Therefore, it is conceivable that such an organism may be misused by an actor aiming to "reproduce" anthrax by exploiting advances in biotechnology. Based on the information on the virulence factors given above, up to 14 genes may require editing depending on the original organism to be engineered. The chromosomal engineering could be conducted using the CRISPR/Cas kits which are readily available. Wang and coworkers (Wang et al., 2019) successfully edited the genomic DNA of *B. cereus* and *B. anthracis* using CRISPR/Cas9 and showed its efficacy for genome editing in the *B. cereus* group.

There are indeed certain genetic similarities between *B. anthracis* and *B. cereus* G9241, both possess two plasmids in the bacterial cytoplasm. One of the *B. cereus* plasmids, pBCX01, has a 99.63% homology with the pXO1 plasmid of *B. anthracis*, Ames strain. However, *B. cereus* G9241 lacks the pXO2 plasmid responsible for the formation of the polyglutamic acid capsule of *B. anthracis* (Hoffmaster et al., 2006). This physiological trait allows *B. anthracis* to evade immune response-mediated phagocytosis. The pXO2 plasmid encoding the polyglutamic acid capsule can in principle be synthesized *de novo* using oligonucleotides with overlapping sequences, an approach also used for the *de novo* synthesis of the polio virus (Cello et al., 2002). Oligos can be combined using assembly PCR. Afterward, the plasmid can be transferred into *B. cereus* G9421 by applying electroporation or other established methods (Ehling-Schulz et al., 2019).

In the context of the genetic engineering of *B. anthracis*, the incident around the Aum Shinrikyo cult might be of some interest. The characterization of a *B. anthracis* strain associated with the cult's activities revealed no evidence of genetic modification (Keim et al., 2001). According to the results of the molecular genetic typing, the strain cultivated by Aum represented the Sterne vaccine strain, known to lack the pXO2 plasmid. On the other hand, Danzig and coworkers (Danzig et al., 2012) formulated a hypothesis that at some stage during their biological weapons program, one of the members of the Aum Shinrikyo cult attempted to transfer the genetic information for the capsule formation into the Sterne strain.

This would parallel the hypothetical scenario of *B. cereus* transformation discussed above. Both, *B. cereus* G9241 and *B. anthracis* Sterne do not possess the important virulence factor, the pXO2 plasmid for capsule formation. In principle, the plasmid could be transferred into the respective microorganism using the established tools of molecular biology. Without discussing the plausibility and likelihood of such an experiment performed by the Aum Shinrikyo cult, it can be insightful to compare the state of knowledge and technological advancement at that time and today.

Already 1988, at the time, when supposedly the biological weapons program of the Aum Shinrikyo cult was in progress, Makino and coworkers demonstrated the possibility of cloning the genetic region required for the encapsulation (Cap region) into *Escherichia coli* and *B. anthracis* (Cap-), which resulted in the encapsulation of both species in the presence of CO_2 (Makino et al., 1989). A year later Stepanov and coworkers performed the transduction of pXO2 plasmid into different strains of *B. anthracis* (STI-1, Sterne, KM33, KM35) and reported that a "dramatic increase of virulence for white mice has been registered for *B. anthracis* strains having acquired the pXO2 plasmid replicon" (Stepanov et al., 1996). These experiments, among others, show that genetic manipulation of non-pathogenic *B. anthracis* or other microorganisms to convey the particular characteristics of lethal wild type anthrax was already feasible at the end of the 20th century.

The *de novo* synthesis of the respective genetic material for the encapsulation and the subsequent bacterial transformation would spare the necessity of acquiring such a regulated strain in the first place. In principle, a *de novo* synthesis of a Cap region could also be performed using the solid-state phosphoramidite method developed by Caruthers (Caruthers et al., 1987). The sequence of the 3.2 kbp long Cap region was published by Makino et al., in 1989 (Makino et al., 1989). By 1995, the longest DNA segment synthesized chemically and assembled from a large number of oligonucleotides was about 2.7 kbp (Stemmer et al., 1995).

No doubt, the possibilities for misuse of *B. anthracis* by terrorists have been expanded by the advances in science and technology and the huge amount of knowledge that has accumulated around *B. anthracis*. The (mis-)use of emerging technologies to genetically modify a harmless microorganism to produce anthrax toxins has been well documented in prokaryotes as well as eukaryotes. One of the candidates is *E. coli*, for which the expression of LF, EF, and PA of *B. anthracis* and their subsequent purification from this Gram-negative bacterium have been reported (Robertson and Leppla, 1986; Sharma et al., 1996; Kumar et al., 2001). Additionally, a Gram-positive spore-forming bacterium, *B. subtilis*, was used in one of the studies to produce recombinant LF (Gholami et al., 2021). Since *E. coli* and *B. subtilis* are broadly used in biochemistry and molecular, biology the barrier for an actor with malicious intent is rather low. In addition, there are other alternative systems for the expression of anthrax toxins. For instance, the yeast species *Pichia pastoris* was used for the expression of the *de novo* synthesized toxin of *Bacillus thuringensis* (Gurkan and Ellar, 2005). The advantage of using a eukaryotic organism is the post-translational modification of the toxins produced, which is lacking in prokaryotes.

It can be argued that the respective technical challenge of genome synthesis in the laboratory is lower nowadays due to the possibility of obtaining the corresponding oligos from commercial suppliers. The cost of ordering such sequences has steadily decreased over the years (Hoose et al., 2023), making the technology more accessible for use in biolabs, but also for misuse for malicious purposes. The beginning of the synthetic biology era marks the possibility of ordering *de novo* synthesized DNA from a commercial provider at desired concentrations and 100% purity. The orders of synthetic DNA are not subjected to mandatory screening. However, a vast majority of the companies working in this field have voluntarily introduced screening procedures based on the guidelines by the United States Department of Health and

Human Services (HHS), e.g., Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA (U.S. Department of Health and Human Services, 2010). The ordered sequences are matched with databases regarding pathogen and toxin sequences. Nevertheless, it is possible to evade such control mechanisms. One novel way around this is re-coding, which can be done either, less promisingly, by changing the codons, resulting in the same amino acid sequence. Or with encryption, whereby ultimately codons code for other amino acids (iGem Team, 2017). This would be a much more promising way to circumvent such controls, although much costlier, as all the gene sequences within the agent would have to be encrypted in the same way to allow functionality. So far, large-scale "rewiring" is not feasible, but research in this field advances rapidly. These aspects show that current screening algorithms need to be redesigned according to the developments in biotechnology.

But also the Engineering Biology research Consortium (EBRC Engineering Biology Research Consortium, 2022) itself lists some gaps, for instance, sequences of 200 bp or smaller are usually not cross-checked, since the results of oligo screenings might be ambiguous and expensive, compared to the cost of the DNA synthesis itself. Such shorter sequences could be assembled into longer sequences, thus creating a backdoor for abuse. Furthermore, the guidance refers only and explicitly to double-stranded DNA (dsDNA), not to single-stranded DNA or RNA. Both can be converted to dsDNA *in vitro*. Furthermore, the working group assumes that about 80% of the world's DNA synthesis capacity is combined under these provisions, which leaves out a significant 20%. This is a major loophole in biosecurity, which can result in the synthesis and shipment of sequences of concern such as toxins.

Alternatively, the synthesis of oligos and short dsDNA can nowadays be also performed fast and at relatively low costs directly in the lab using state-of-the-art benchtop synthesizers. For more details see Carter et al. (Carter et al., 2023). Some of the devices synthesizing nucleic acids greater than 1,500 bp in length are subjected to export controls under the AG (2021) (The Australian Department of Foreign Affairs and Trade, 1958), but this does neither apply to all commercially available instruments nor national trade.

Even with all regulations in place, there remains the risk that an order may evade screening. For example, the sequence in question may be camouflaged by benign genes. Such a construct has a high probability to circumvent the screening procedure. The camouflaging genes can thereafter be removed through methods such as CRISPR/Cas9, and the deletions repaired via homology-directed repair (HDR), leaving a sequence encoding for a dangerous toxin or a virulence factor. This scenario may sound technically elaborate. However, proof of concept has been conducted by Puzis et al. (Puzis et al., 2020). No respective obfuscated DNA encoding a toxic peptide was detected by the screening algorithm, and the order was moved to production.

3.4.4 Making bioagent antibiotic-resistant

It should be pointed out that *B. anthracis* is one of the most extensively studied microorganisms. Several mitigation measures and therapeutic strategies have been established over the years, which can be efficiently applied in the case of a potential outbreak (Supplementary Table S1). However, it is possible to introduce antibiotic resistance genes into the bacterial genome to circumvent these therapeutic strategies. Multidrug-resistant bacterial strains have been successfully engineered in the past (Dassanayake et al., 2021). In certain cases, multidrug resistance can lead to loss of virulence due to pleiotropic effects, as reported for the *Francisella tularensis* strain engineered to be resistant to multiple antibiotics as part of the USSR's BW program (Leitenberg et al., 2012). There are also publications available, indicating that a *B. anthracis* strain (STI-1 vaccine strain) was engineered to resist several antibiotics (Stepanov et al., 1996). Therefore, the threat of a biological attack involving a multidrug-resistant lethal strain of *B. anthracis* cannot be understated.

3.4.5 Technology acquisition and infrastructure

No doubt, the emerging, converging and enabling technologies led to a decrease in the requirement for sophisticated equipment thereby expanding the realm of feasibility and hence the BW paradigm. In addition, tools traditionally siloed in academic and government labs are increasingly becoming accessible to a wider audience (Dunlap and Pauwels, 2017; Sanz et al., 2022). Moreover, during the process of *B. anthracis* weapon development, less dangerous and easily available surrogate microorganisms can be used. Historically, the Japanise, the United States, the UK, and Iraq used *B. anthracis* surrogates in biological warfare test studies (Balmer, 2001; United Nations, 2007; Greenberg et al., 2010). More recently, research data were generated that could be exploited for *B. anthracis* BW development with a surrogate microorganism. For example, *B. thuringiensis* was effectively used as an appropriate model for *B. anthracis* in aerosol and re-aerosolization testing allowing environmental release without pathogenicity concerns (Tufts et al., 2014). In addition, using *B. thuringiensis* as a surrogate test organism opens new possibilities even for alternative non-regulated cultivation technologies such as solid-state fermentation (SSF) application as a new production system (Lima-Pérez et al., 2019).

Furthermore, significant progress in frugal science, collectively describing the attempt to create cheap, easy-to-use low cost and low electricity-requiring scientific equipment alongside emerging technologies made for anyone, anywhere could potentially be exploited to develop biological weapons (Tennenbaum et al., 2021).

3.4.6 Biological agent amplification

Novel developments and contemporary lab practices make the cultivation and scale-up of *B. anthracis* more feasible. For large-scale cultivation, *B. anthracis* could be grown in submerged high-cell-density fermenters, as shown for the comparable *B. subtilis* (Grossman and Losick, 1988; Riesenberg and Guthke, 1999). Further upscaling would usually require large liquid-state fermenters which are subject to export controls under the AG (The Australia Group, 2021). To circumvent this restriction, a novel, unrestricted alternative SSF with polyurethane foams could be performed. This method was developed for *B. thuringensis* but is in principle applicable to *B. anthracis* (Lima-Pérez et al., 2019). SSF would therefore represent an unrestricted alternative method. All these aspects must be considered when discussing the imminent threat of an anthrax attack.

3.4.7 Agent storage and transportation

There is also a large body of literature available on anthrax sporulation. It can be induced by a lack of nutrients in a freely available sporulation medium (Chen et al., 2020). Common

histological stains (Moeller or Schaeffer-Fulton) are suitable to verify sporulation. Once obtained, the spores can be stored for extended timespans and disseminated by aerosolization. Not only can *B. anthracis* spores be stored for a long time, since they remain viable for decades, but they can also be easily transported in sealed containers and survive exposure to the Sun, air, rain, and violent dissemination methods. The spores are even so heat-resistant, they could be disseminated using explosives (Fetter, 1991).

3.4.8 Stabilization and weapon-grade spore preparations

Historical indices show that several state and non-state actors transformed cultivated *B. anthracis* spores into a powder form as a part of anthrax weaponization. The purpose of this step is to improve the dissemination and aerosolization of this bioagent. It was reported that the United States (Matsumoto, 2003) and USSR (Zilinskas, 2014) produced dried anthrax spores in the scope of their BW programs. Also, the Aum Shinrikyo cult attempted to obtain anthrax in powder form (Danzig et al., 2012), while Iraq experimented with lypholization (Mondange et al., 2022). The best-known case of deploying anthrax as a powder is the 2001 anthrax attack (v. s. Amerithrax), where the spores were dried to the concentration of 2.0×10^{12} colony-forming units per Gram (USDOJ, 2010). Tufts and coworkers (Tufts et al., 2014) showed that *B. thuringensis* can be used as a surrogate to optimize the aerosolization of anthrax spores. Such a procedure requires sufficient technical expertise and special freeze- or spray-drying equipment. This category of dual-use equipment with a water evaporation capacity between 0.4 and 400 kg/h, and the ability to reach particle sizes below 10 μm or to sterilize or disinfect *in situ* is subjected to export controls under the EU regulations (European Union, 2021), implementing the AG-control lists (The Australia Group, 2021). Additional safety precautions such as a glovebox with negative pressure and protective clothing with an external oxygen supply are also highly recommended when working with aerosols containing pathogens. These items are also included in the AG control list of dual-use equipment and technologies. However, it can still be purchased within the country, second-hand, or crudely manufactured, albeit with technical challenges. Thus, the Aum Shinrikyo cult, although unsuccessfully employed a rudimentary self-made drier. Thus, aerosolization may be considered a bottleneck in the production of an anthrax BW. Probably the safest way to circumvent the regulations and controls in this regard would be to build the necessary equipment oneself, which would mean a significant increase in the necessary know-how in the field of engineering and infrastructure in the form of corresponding clandestine production facilities.

Another aspect in the context of anthrax weaponization is the encapsulation of the purified and dried spores. The encapsulation would impart additional stability and prevent aggregation (Matsumoto, 2003). It was suspected that the spores disseminated in the Amerithrax case were coated, based on the high silica level determined during the investigation. However, this hypothesis could not be verified experimentally. According to the results of the transmission electron microscopy, the silica was localized to the spore coat within the exosporium, and not on the surface. Therefore, it was concluded that silica was incorporated into the cells as a natural part of cell formation, rather than by a deliberate attempt to coat the spores. Despite this experimental evidence, the controversial debate on the spore coating in the Amerithrax case is still ongoing (Rosenberg, 2002; Bernstein, 2010; Epstein, 2010; National Research Council, 2011a).

From the perspective of this manuscript, it is of interest to evaluate the state of technology, which could in principle be applied to encapsulate the anthrax spores. As previously mentioned, *B. subtilis* can be used as a model organism for *B. anthracis*. Therefore, the encapsulation procedure described by Balkundi and coworkers (Balkundi et al., 2009) for *B. subtilis* has to be considered in the discussion on the advances in knowledge and technology, which might be misused for the weaponization of anthrax.

3.4.9 Weapon deployment and agent dispersion

The method of BW deployment depends on the agent, its preparation, its stability, and the route of infection. In 1970, a World Health Organization (WHO) expert committee estimated that "an aircraft release of 50 kg of anthrax over an urban, developed population of 5 million would result in 250,000 casualties". Medical resource limitation and capacity strain in such a scenario is enormous, ultimately requiring 13,000 hospital beds, 60-day of antibiotics for 125,000 patients leaving 95,000 dead. This would undoubtedly result in a quick and complete collapse of medical resources and civilian infrastructure. More recent estimations have confirmed the original WHO data (Congress, 1993). The CDC has developed an economic model that puts forward costs of $26.2 billion per 100,000 people exposed to an anthrax attack (Kaufmann et al., 1997). Fetter (Fetter, 1991) estimated that a missile armed with 30 kg of anthrax spores would affect an area of 6–80 square kilometers, delivering doses greater than 0.1 mg/min/m^3 (the estimated ECt_{50} for anthrax) depending on the weather conditions and kill an estimated 20,000–80,000 people if a large, sparsely populated city was attacked. Alternatively, bioterrorists may disperse *B. anthracis* spores through aerosols using knapsack sprayers or a crop-spraying light aircraft to disseminate the biological agent (Durrant, 2002; Haas, 2002; Aduojo et al., 2022). The intimidating scenario of an attack with a UAV, commonly known as a drone, on a vulnerable target delivering weaponized anthrax can be considered increasingly realistic. In the Sverdlovsk incident, 1 g of wind driven anthrax spores killed sheep at a distance of up to 50 km (Durrant, 2002). Despite international regulations such as well-established import-export control regimes, up-to-date drones offer terrorists the convenience of anonymity and bypass traditional security measures (Pethő-Kiss, 2022).

3.5 Monitoring, medication, and mitigation

Fortunately, technological advances not only serve the development of bioweapons but also the development of mitigation strategies. Before an attack, surveillance through efficient bio-detection systems for environmental monitoring informing early warning systems, and preventative measures through vaccination as part of pre-exposure prophylaxis (PrEP) can be implemented.

To respond to an anthrax BW attack and mitigate its effects, multiple countermeasures including rapid detection, a

comprehensive investigation and an effective response including post-exposure prophylaxis (PEP) must be considered. Designing rapid and reliable diagnostic systems by classical microbiology, immunoassays, and nucleic acid-based methods, including molecular forensics to identify *B. anthracis* or a related bacterial strain as the biological anthrax threat agent is a prerequisite to improve the response efforts (Blatny and Green, 2007) and to start with the therapeutic countermeasures. The treatment of infected people and animals with disease-specific interventions with antibiotics and antitoxins for PEP is necessary to decrease morbidity and mortality as much as possible (Honein and Hoffmaster, 2022; Rathish et al., 2022). In addition, panic and fear among the public must be managed by an interagency, intersectoral and international cooperation (Beeching et al., 2002) and proper public communication to minimize the disruptive impact of an anthrax attack (Cameron et al., 2019). Finally, containment and decontamination efforts after an anthrax event are necessary. In the following section, we discuss the most important of these aspects to draw conclusions considering biosecurity management.

3.5.1 Detection

Shortly after the most recent bioterrorist Amerithrax attack and the complete genome sequencing of *B. anthracis* (Read et al., 2002), remarkable innovations and advances in the realm of anthrax detection and the newly initiated field of microbial forensics (Keim et al., 2001; Rasko et al., 2011) were made (Schmedes et al., 2019; Revill et al., 2022).

Conventionally, samples are assessed via microbiological growth analysis, Gram-, spore-, and capsule staining, microscopic analysis, hemolysis tests and phage susceptibility (Zasada, 2020). These methods require highly trained laboratory personnel, BSL-3 facilities, and practices. Novel detection methods are based on diverse targets, from detection based on DNA (Pal and Alocilja, 2010; Hao et al., 2011; Kaittanis et al., 2011; Chen et al., 2020), chemical reactions (Boyer et al., 2007; Duriez et al., 2009; Čapek et al., 2010; Kuklenyik et al., 2011), antibodies (De et al., 2002; Biagini et al., 2006; Campbell and Mutharasan, 2006; McGovern et al., 2007; Hao et al., 2009; Mwilu et al., 2009; Tang et al., 2009; Zahavy et al., 2010; Wang, 2013; Atabakhshi-Kashi et al., 2020), phages (Schuch et al., 2002; Fujinami et al., 2007), peptides (Acharya et al., 2007; Park et al., 2009), aptamers (Alibek and Handelman, 1999; Huan et al., 2009; Cella et al., 2010; Oh et al., 2011; Kim et al., 2013) or even DNA-peptide chimeras (Zhang and Appella, 2007; Kim et al., 2015; Wang D-B. et al., 2021). To date, a variety of detection methods for environmental or clinical anthrax samples emerged, each with distinct advantages over conventional culture and PCR-based detection. Xu and coworkers (Xu et al., 2023) developed a rapid (<40 min), easy-to-implement and accurate DNA endonuclease targeted CRISPR trans reporter (DETECTR)-based detection and identification method as a novel screening and diagnostic user-friendly portable devices for pathogenic *B. anthracis* (Xu et al., 2023). Overall, the speed, sensitivity and accuracy of modern detection methods have increased, potentially saving uncounted lives in case of an anthrax BW attack. Early detection is the prerequisite for adequate treatment and mitigation. In order to make a difference, these detection methods, must be widely available.

3.5.2 Preparedness

In addition, the Amerithrax incident led to important investments in medical funding for biodefense. In the following decade, the United States, for example, spent 5.6 billion dollars on biodefense known as the Project BioShield Act 2004 (US Congress, 2004). While many wealthy countries followed the United States in an attempt to globally improve the capacity to face an emerging outbreak, although, with budgets that were and are orders of magnitudes lower (Mondange et al., 2022).

Currently, preparedness against the intentional use of *B. anthracis* relies on increased disease as well as environmental surveillance (US BioWatch program (National Research Council, 2011b)), laboratory capacity, information and system technology, education, and workforce training as well as clinical practice that integrates all accessible countermeasures such as new antimicrobials and advances in critical care (Blatny and Green, 2007; Scales and Horney, 2023). The armamentarium for PEP and treatment of anthrax involves numerous effective antimicrobials, including alternatives for resistant strains, antitoxins, and vaccines (Kaufer et al., 2020; Honein and Hoffmaster, 2022) that must be stockpiled in adequate quantities. Protocols to deal with anticipated *B. anthracis* scenarios are developed and tested in exercises.

Considering the preparedness towards anthrax attacks, there have been efforts to adopt strategies by various countries. Some examples are listed in the following: The US CDC (2015) published a clinical framework and medical countermeasure use during an anthrax mass-casualty incident. The focus was set on the allocation of scarce resources with different treatment plans depending on whether anthrax developed meningitis. The CDC recommends additional treatment with antitoxin in meningeal anthrax cases. The European Centre for Disease Prevention and Control (ECDC), however, only monitors cases in EU/European Economic Area countries and discusses them in their weekly Communicable Disease Threat Reports (ECDC. COMMUNICABLE, 2022). In their Annual Epidemiological Report for Anthrax, the ECDC also discusses the complementary administration of antitoxins, albeit additional benefits have been contested. The Department of Public Health of the Australian Government published a Public Health response plan for Anthrax (Australian Government Department of Health, 2012). Next to the description of clinical etiology and different treatment plans, this response plan entails measures for five different response codes (threat levels), for deliberate anthrax releases, defining the main actions and communication plans to be taken by the government and jurisdiction for each threat level and the key stakeholders.

3.5.3 Pre- and postexposure treatments

Not surprisingly, the development of effective anthrax vaccines was spurred on by the potentially nefarious use of *B. anthracis* as a biological warfare agent. Already in 1953 and 1959 the USSR licensed their live spore vaccine for scarification and subcutaneous administration, respectively (Biselli et al., 2022). As new biochemistry methods in the 1950s and 1960s, paved the way for discovering and deciphering the capsule of *B. anthracis* (Smith et al., 1953; Thorne, 1960; Stanley et al., 1961), responsible for the toxin-mediated disease anthrax. In the 1970s, these breakthroughs and biodefense endeavors lead to the successful development and approval of a novel and enhanced cell-free human preparation of

aluminum hydroxide gel adsorbed protective antigen, now known as anthrax vaccine adsorbed (AVA) formulation in the 1970s (Tournier et al., 2009; Tournier and Mohamadzadeh, 2010). At the same time, the WHO declares anthrax one of high-impact bioagents (World Health Organization, 1970).

Currently, the two primarily used culture filtrate vaccines, the Europe- and US-licensed Anthrax Vaccine Adsorbed (AVA; trade name BioThrax) and the United Kingdom-licensed vaccine, AVP, contain PA and variable quantities of LF and EF. Guidelines "recommend vaccination for people at risk, such as veterinarians, abattoir workers, those working with animal hides or furs, laboratory workers and the armed forces in areas with a high risk of exposure. In addition to PrEP the anthrax vaccine is also recommended for PEP, along with antibiotics" (ECDC, 2013). For individuals 18–65 years of age, various Anthrax vaccines are licensed or in development for PEP (Wolfe et al., 2020).

In addition, the antibiotics ciprofloxacin, penicillin, and doxycycline were approved by the Food and Drug Administration (FDA) for the treatment of anthrax and may be also useful in combination with other antibiotics for the treatment of inhalation anthrax (Inglesby et al., 2002). In *B. anthracis* antibiotic resistance to, e.g., amoxicillin, penicillin G, and/or cotrimoxazole has been documented. Although drug resistance mechanisms of *B. anthracis* have not yet been fully exploited, beta-lactamases against β-lactam antibiotics and efflux-pump mediating cross-resistance to fluoroquinolone antibiotics like ciprofloxacin in *B. anthracis* have been reported. Genetic modification of *B. anthracis* (to induce resistance to vaccines or antimicrobial drugs) has not yet been achieved by terrorists. Yet, the illicit Soviet program was successful. Hence, the introduction of safer and more efficient chemotherapeutic options are required (Dassanayake et al., 2021).

Moreover, antibiotics are effective against bacteremia caused by antibiotic-susceptible strains of anthrax but not against the toxemia that drives pathogenesis. In fact, the quantities of secreted anthrax toxins in some cases lead to death despite efficient antibiotics administration. The discovery of the biochemical structure of LF and EF (Pannifer et al., 2001), of the cellular receptors of PA (Bradley et al., 2001), and description of the precise effects of LF and EF on the cell biology (Moayeri et al., 2015), therefore, were important scientific achievements in the toxin field. In 2009, the first monoclonal antibody targeting PA was finally authorized by the FDA (Migone et al., 2015). Nowadays, three anthrax antitoxins have been approved by the FDA and stockpiled by the United States: two monoclonal antibodies (raxibacumab and obiltoxaximab "Anthim"), and the human polyclonal purified IgG from vaccinated humans (intravenous anthrax immune globulin AIG-IV, also referred as Anthrasil) (Huang et al., 2015; Avril et al., 2022), regardless of uncertainties associated with the clinical effectiveness of antibodies. Hence, Anthim and Anthrasil can be administered solo or in combination with antibiotics for a more effective anthrax therapy. According to the CDC, the administration of both antibiotics and antibodies is recommended, regardless of recent studies doubting the efficiency of antibodies (Tournier et al., 2019; Avril et al., 2022).

3.5.4 Decontamination

Generally, remediation following a *B. anthracis* BW attack requires decontamination, confirmatory sampling, and testing. The decontamination strategy should include the decontamination of surfaces and affected areas (space), as well as the proper disposal of any decontamination wastewater (Urban-Sorensen, 2018). In the aftermath of the Amerithrax attack, both private and government facilities were affected, and their cleaning up was an unexpected challenge. The decontamination work was not only high-profile but also very time-consuming and expensive. A complete renovation of all facilities required over 3 years and cost about $320 million (Urban-Sorensen, 2018). Meanwhile, specific advances in nanotechnology and material sciences led to the improvement of decontamination and decontamination capabilities even against spore-forming bacilli. For decontamination applications against *B. anthracis* with up to 100% efficacy after 10–15 min, Ginghina (Ginghina et al., 2022) demonstrated the antimicrobial activity of organic solutions enriched with ZnO, TiO_2, and zeolite nanoparticles. Another effective strategy is to incorporate different semiconductors to enhance their bactericidal synergistic effects for water disinfection. A maximum antimicrobial activity against *B. subtilis* was shown by $CuWO_4$/CuS CuS nanopowder (Dong et al., 2022). Moreover, Nakonieczna (Nakonieczna et al., 2022) recently identified three new siphophages that can specifically infect and lyse siphophages that can specifically infect and lyse *B. anthracis* and have applications as decontaminants or disinfectants (of skin, surface, or clothes).

3.6 Threat evaluation

To assess vulnerability, a threat evaluation is necessary. The prevention of unwanted events from occurring and/or protection, the ability to react during an event, and the ability to mitigate its subsequent impact are the goals of any good security measure (Tennenbaum et al., 2021). Given i) the potentially very high death toll due to an anthrax attack and the societal and economic disruption in the aftermath of an attack, ii) the demonstrated relative feasibility of acquisition, mass production and weaponization of anthrax, partly by circumventing existing regulations and governance measures, iii) the existence of disseminating technology, iv) and the difficulty of effective emergency response including the sufficient stockpiled antibiotics, antitoxins and vaccines, it is crucial to strengthen preparedness, prevention, and mitigation measures.

For an evaluation of the posed threat considering anthrax and BW research, we identified many indicators in different fields with a dual-use potential relevant to anthrax BW development (Supplementary Table S1). As can be seen from the prominent examples of Amerithrax and Aum Shinrikyo, foremost terrorists try to use anthrax as a bioweapon. Based on identified indicators in different relevant fields with a dual-use potential relevant to an anthrax BW development, our analysis clearly showed the rapid speed at which scientific achievements in the field of SynBio and other emerging and converging technologies are taking place (Supplementary Table S1), thereby paving the way for potential novel and high consequence BW threats.

On the one hand, key technologies that could support efforts to engineer a novel anthrax BW were identified. The indicators most relevant to our pathway exploration were scientific achievements,

advancements or discoveries that could be exploited to increase the BW threat potential or to circumvent biosecurity measures aiming at preventing BW proliferation and development. The essential anthrax virulence factors are located on just two plasmids, allowing their transfer from one bacterium to another (Makino et al., 1988; Stepanov et al., 1996), as was already proven in *E. coli* (Robertson and Leppla, 1986; Sharma et al., 1996; Kumar et al., 2001). Together with the existence of phylogenetic closely related and less dangerous surrogate species such as *B. subtilis* (Zhang et al., 2019; Gholami et al., 2021), *B. thuringiensis* (Lima-Pérez et al., 2019) and *B. cereus* (Manoharan et al., 2023), this presents a major possibility for exploitation and potential for safer, low-cost and undercover BW research. Furthermore, the advent of CRISPR made genetic modification easier, quicker and cheaper, while toxin sequences (GenBank, 1995) and protocols for the isolation of *B. anthracis* from contaminated soil (Chikerema et al., 2012), high-cell density cultivation (Zhang et al., 2019), sporulation (Chen et al., 2020) and other techniques necessary for BW development are readily available on the internet. To top this all off, there are commercial suppliers for mail-order nucleic acid sequences, which are not bound to perform mandatory screenings. And even if they were mandated to perform screenings, there would be ways to circumvent them (Atkins and Baranov, 2010; Engineering Biology Research Consortium Security Working Group, 2022). In addition, potential hazardous modifications include antibiotic resistance, or heightened pathogenesis, an easier disseminatable and enhanced aerosolization of the BW agent. Using recombinant DNA technology even a non-regulated *B. cereus* strain could be turned into an anthrax BW that could escape the established bio-detection and biomedical defense strategies. In addition, many of the identified technological advances are explicitly designed to decrease the technical expertise required to produce sufficient quantities of biological agents for a bioterrorist group with nefarious intentions. Importantly, this can fundamentally change signatures used to identify suspicious and illegal activity by intelligence analysts and law enforcement professionals.

On the other hand, technological advances also led to an improvement in the realm of counterproliferation, detection, and development of medical countermeasures, thereby raising the PrEP and PEP targeted to counter and reduce threats. The chemotherapeutic management of anthrax has become challenging due to the global emergence of antibiotic-resistant strains. However, a plethora of bioactive phytochemicals with an antibiotic-potentiating ability and reversing antibiotic resistance in *B. anthracis* have been identified (Dassanayake et al., 2021). In addition, the discovery of potent new antibiotics such as anthracimycin with a novel mechanism of action (inhibiting DNA/RNA synthesis) and low toxicity to human cells represents a major advance in the field of antibiotics against *B. anthracis* helping to counter existing or future antibiotic resistance problems (Tian et al., 2022). Moreover, three new siphophages that can specifically infect and lyse *B. anthracis* were recently isolated. Beside finding potential use in *B. anthracis* identification and detection assays, the siphophages, after removing the genomic modules essential for lysogeny, can be applied to treat human or animal anthrax (likewise their endolysins), or as surface or skin decontaminants or disinfectants (Nakonieczna et al., 2022).

However, in a large-scale bioterrorist anthrax incident, it is especially critical to meet the need for anthrax vaccines and antitoxins (Dassanayake et al., 2021; Hesse et al., 2022). Representing a bottleneck for mitigation in case of an anthrax attack, vaccines and antimicrobics have to be stockpiled for rapid mobilization and distributed to large numbers of people (Beeching et al., 2002).

The combination of these findings draws a sobering picture implying a low-entry and potentially high-threat situation. However, these advancements simultaneously also offer new opportunities to address them.

3.7 Biosecurity measures

All of the BWC Review Conferences since the 1990s have failed to take decisions that would help shaping biosecurity measures on the international as well as national level or at public or private biotechnology facilities. The here discussed measures were hence developed through other mechanisms. The current technological possibilities to weaponize *B. anthracis* discussed here highlight several aspects which are of importance in the context of risks posed by dual-use research (see Introduction).

- Misuse of results published openly in literature (e.g., creating multidrug-resistant strains of *B. anthracis*, expressing anthrax toxins in other microorganisms).
- Conducting gain-of-function experiments (GOF) for malicious intent (e.g., genetic engineering of *B. cereus* to convey the characteristics of wild type *B. anthracis*).
- Exploiting recent and emerging advances in technology for malicious purposes (e.g., UAVs, modern aerosolizers or ordering DNA sequences encoding for *B. anthracis* toxins or virulence factors from a commercial provider).

These aspects present just a fraction of the dual-use research problems in science and industry that need to be addressed by designing and applying comprehensive ethical and legal frameworks. However, in the scientific community, there is still little awareness of the fact that research results and technological achievements can be misused by certain actors for hostile purposes.

While such efforts have no bearing on terrorists, many initiatives for the scientific codes of conduct have been recently developed to minimize biosafety and biosecurity risks. They include the Recommendations for Handling Security-Relevant Research drafted by the German Research Foundation and the National Academy of Sciences Leopoldina (Deutsche Forschungsgemeinschaft and Deutsche Akademie der Naturforscher Leopoldina, 2014) and the Global guidance framework for the responsible use of the life sciences (World Health Organization, 2022). Another prominent example is the Tianjin Biosecurity Guidelines for Codes of Conduct for Scientists (Wang L. et al., 2021), which were, however, not endorsed by the Ninth Review Conference of the BWC in December 2022. All these promising ethical tools urge that *"[m]easures should be taken to prevent the misuse and negative impacts of biological products, data, expertise, or equipment"* (WHO, 2022). This also implies a responsible publication of results in scientific

literature. Transparency and knowledge sharing are undoubtedly important driving forces in high-quality research. Nevertheless, as we illustrate in this manuscript, some published data might pose a great risk of misuse. Examples include investigating the sporulation process of *B. anthracis* while using other related organisms such as *B. thuringensis* or *B. subtilis*. A more striking example is the publication on the genetically engineered multidrug-resistant *B. anthracis* strain. The data on this experiment was published in the 90s, prior to the "Fink Report" (National Research Council, 2004). However, the open-access body of literature on some research areas of concern outlined in this report continues to grow, as demonstrated by the recent pre-print publication on the chimeric recombinant Sars-CoV-2 (Chen et al., 2023). This clearly indicates the need for a more sophisticated review mechanism for scientific journals and addresses the issue of making publications openly available in preprint repositories before they undergo a review process.

In addition to the research results published in scientific journals and preprint repositories, other sources of scientific data can be potentially subjected to misuse. This for instance applies to open-access genomic and proteomic databases. The National Institute of Health GenBank contains complete genome sequences of various microorganisms and viruses with varying data quality, including *B. anthracis* Ames, Hepatitis B virus, Influenza A (segments 1–8), *Yersinia pestis*, *etc.* The fact that this information can be misused for the *de novo* synthesis of some of the genes, or even for the recreation of an entire organism (see cases of poliovirus and horsepox virus) cannot be denied. One of the possible mitigation strategies could be more restricted access to the data banks through a licensing policy. A preregistration of research for biosecurity risk assessment earlier in the research process and eventually access-controlled repositories or application programming interfaces after completion of research has already been demanded (Smith and Sandbrink, 2022). These steps, however, require scrutiny and a solid proof-of-principle in order not to create a serious bureaucratic obstacle to peaceful science, while making a minimal contribution to biosecurity (due to the existence of possible backdoors for misuse, *etc.*).

Another important aspect is the highly controversial GOF research area. It has sparked numerous debates in the past (Kaiser, 2022). A more in-depth analysis of the matter is beyond the scope of this manuscript. Nevertheless, it is of relevance to our discussion on weaponizing anthrax. Modifying *B. cereus* in such a way that it would express anthrax toxins and important virulence factors would meet the definition of "enhancing" an agent. Recently, the United States National Science Advisory Board for Biosecurity approved a report on amending the review process of GOF experiments in the United States and abroad (in cooperation with United States research institutions) (Reardon, 2023). According to it, all studies should be subjected to a meticulous review, if they could be "reasonably anticipated" to make a pathogen more dangerous. The guidelines are still vague and will undergo several modifications before being finalized. Even if applied in a consequent manner, there remains the question, of which impact, if any, these regulations would have on other GOF experiments conducted worldwide. The current frameworks do not appear to be effective in limiting dissemination of research that could enhance the dangers posed by a future use of *B. anthracis*. This matter requires an open dialogue on a multinational level.

In the broad discussion about the ethical obligations of the scientific community, little attention is paid to the responsibility of other stakeholders, such as the private sector. As the bioeconomy grows, privately funded life science research with dual-use potential is on the rise (Epstein, 2023). A major drawback there is, for example, the lack of standardized guidelines and customer screening mechanisms to reduce the risk of misuse of advanced medical and biotechnological applications and devices supplied. Thus, companies providing dual-use equipment should implement reliable mechanisms to check their customers' and cooperation partners' backgrounds. Full-scale training in biosecurity, international norms, and ethical issues should be provided in both non-commercial research institutions and industrial biotechnology facilities.

In the pathway analysis, we focus on the case of commercial providers of synthetic DNA and indicate that the guidelines proposed by HHS are, for now, still voluntary and bear some limitations concerning biosecurity. A unified easy-applicable and low-cost mechanism for screening both the customer and the ordered DNA is of utmost importance. Several proposals for such mechanisms have been developed over the years, including the implementation of a harmonized database for the "sequences of concern" (sequences encoding for toxins and virulence factors, excluding other housekeeping genes of an organism, to make the screening less ambiguous and time-consuming). In particular, the Nuclear Threat Initiative (NTI) is making laudable progress in establishing an international common mechanism for DNA synthesis screening based on the above criteria, which should be operational soon (The Nuclear Threat Initiative, 2023). However, all guidelines will have limited effectiveness unless they are declared mandatory worldwide. To the best of our knowledge, only the California government has taken an initial step in the direction of strengthening SynBio-security, requiring scientists to develop systemwide guidance for purchasing gene synthesis equipment and products from "providers who prevent the misuse of synthetic genes" (LegiScan. California Assembly Bill, 1963, 2022). However, more stringent (international) legislation, including also legally binding regulations for the industry is still needed.

In summary, we support the overarching proposals made by the above-mentioned ethical frameworks and would like to emphasize the following.

- Comprehensive training for raising awareness in the scientific community should become a mandatory part of any curriculum at academic institutions; it should also be included in annual training of the scientific staff at non-profit research and industrial facilities;
- Biosecurity relevant research should be registered for biosecurity risk assessment;
- A background check should be considered for scientific staff members working on biosecurity-relevant research;
- Ethical and policy recommendation committees should be convened at institutions to monitor and evaluate the proposed research projects and to guide them during their progress;
- Access-controlled repositories or application programming interfaces for open science should be implied while access to genome/proteome databanks should be better supervised (through e.g., licensing);

- A broader and stricter mechanism for reviewing the submitted manuscripts and research proposals should be put in place by the funding agencies and the scientific journals (since Some journals are published by for-profit publishers and may not be as rigorous);
- Screening procedures of the synthetic DNA ordered from commercial suppliers should be unified and mandatory;
- Strict and continuous documentation, monitoring, and accountability of laboratory storage, and utilization of pathogens, toxins, biosecurity-relevant substances, and sequences
- BWC states parties using the intersessional process towards the 10th review conference in 2027 to develop sufficient multilateral activities, such as the installation of a Science and Technology advisory board, a verification system shaped to the progress in the field, adopting a significant code of conduct for the life sciences, adopting and strengthening the system of Confidence Building Measures, *etc.* Such a verification system should consider the fundament of the draft BWC verification protocol of 2001, might be conceived similarly to that of the Chemical Weapons Convention (CWC) but should but must be extended due to the different technology and stakeholder environment (i.e., mail order, DIY labs, cloud labs, *etc.*). Furthermore, transparency toward DURC and GOF research should be included in a monitoring and verification system.

These steps should be openly discussed with and accepted by the scientific community and other stakeholders. Otherwise, they might result in a patchwork-like loose implementation that hampers scientific progress, while making little contribution to biosecurity.

4 Conclusion

Biological weapons do not only pose a threat through state-sponsored programs but also in bioterrorism and bio-crime incidents. Globally, huge efforts are being made to strengthen the norm against biological weapons and to implement effective biological arms control strategies. These include binding laws prohibiting the development, production, stockpiling and use of biological weapons. In fact, many states have been historically financially and technically capable of engaging in clandestine biological warfare programs including *B. anthracis* BWs. Two decades have past since the Amerithrax attacks without any further comparable incidents. Throughout the entire time period, however, the narrative developed that the threat is constantly growing. This article, investigates the anthrax BW threat by terrorists in the here and now.

In our pathway analysis, we analyzed three different acquisition pathways, isolation, illegal procurement and various routes of genetic engineering. These pathways vary greatly in labor intensity, necessary secrecy levels and biosafety requirements, as well as costs. While it may be possible to steal already weaponized spores from legitimate facilities, isolation and genetic engineering requires much more work. Similarly, biosafety requirements may widely differ depending mostly on the level of readiness of the illegally procured bioagent. Hence, costs may scale where the highest costs would be expected for the genetic engineering pathway. We would refrain from estimating explicit cost ranges, since they mostly depend on the number of people involved and their monetary compensation, as well as the necessary infrastructure which may vary situationally and geographically. Also, it is hardly possible to determine a number of person labor hours since this kind of work, especially genetic engineering, is more breakthrough-dependent. On the other hand, secrecy would likely be least sensitive in the isolation pathway and most sensitive in the illegal procurement pathway while depending on the number of people involved and the timespan of production.

Since terrorists do not comply with the existing strong global norm that rejects development of such weapons, raising preparedness and implementing preventive measures are the only effective strategies. Despite improvements in treatment, inhalation anthrax remains a deadly infection. Prevention, therefore, foremost implies promt detection, timely diagnosis, and immediate treatment of disease, as well as providing sufficient intensive-care facilities and effective antimicrobials, to significantly reduce the morbidity and mortality of inhalational anthrax. In fact, achievements have been made in all these areas including the discovery of new and effective antibiotics and bacteriophages as well as improvements in vaccination strategies and the invention of rapid portable detection devices and sensors. However, the question remains, whether they are capable of compensating the existing elevated threat level of a potential *B. anthracis* BW development and deployment by terrorists identified through our aforementioned pathway analysis. In addition, the past failures of terrorists in pursuing anthrax BWs should not be a source of consolation, but rather a warning of an activity that, if persistently pursued with the aid of advances in emerging and converging sciences, could eventually lead to success.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

DS, AK, AR, JF, and GJ contributed to conception as well as design of the study, conducted formal analysis, investigation, methodology, visualization, writing of the first draft of the manuscript. In addition, DS curated the data and GJ was responsible for funding acquisition and project administration. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2023.1215773/full#supplementary-material

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Glossary

3D	Three dimensional
AG	Australia Group
AI	Artificial Intelligence
AIG-IV	Intrevenous Anthrax immune globulin
AVA	Anthrax vaccine adsorbed
AVP	Anthrax vacinne precipitated bp Base pairs
BSAT	Biological Select Agents and Toxin
BSL	Biosafety level
BW	Biological weapon(s)
BWC	Biological Weapons Convention
CDC	Centers for Disease Control and Prevention
CRISPR	Clustered regularly interspaced short palindromic repeats
CRISPR/cas9	Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9
DETECTR	DNA endonuclease targeted CRISPR trans reporter
DIY	Do-It-Yourself
DIY-Bio	Do-It-Yourself Biology
DNA	Desoxyribonucleic acid
dsDNA	Double-stranded desoxyribonucleic acid
DURC	Dual-Use research of concern
ECDC	European Centre for Disease Prevention and Control
EF	Edema factor
EU	European Union
FDA	Food and Drug Administration
GOF	Gain-of-function
HCD	High cell density
HDR	Homology-directed repair
HHS	United States Department of Health and Human Services
JoVE	Journal of Visualized Experiments
LF	Lethal factor
MERCs	Multi export regime controls
mRNA	Messenger ribonucleic acid
NTI	Nuclear Threat Initiative
PA	Protective antigen
PCR	Polymerase Chain Reaction
PEP	Post-exposure prophylaxis
PrEP	Pre-exposure prophylaxis
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
SSF	Solid-state fermentation
SynBio	Synthetic Biology
UAV	Unmanned aerial vehicles
UK	United Kingdom
UNSC	United Nations Security Council
US	United States of America
USSR	Union of Soviet Socialist Republics
WHO	Worls Health Organization

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Safety risks and ethical governance of biomedical applications of synthetic biology

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Background: In recent years, biomedicine has witnessed rapid advancements in applying synthetic biology. While these advancements have brought numerous benefits to patients, they have also given rise to a series of safety concerns.

Methods: This article provides a succinct overview of the current research on synthetic biology's application in biomedicine and systematically analyzes the safety risks associated with this field. Based on this analysis, the article proposes fundamental principles for addressing these issues and presents practical recommendations for ethical governance.

Results: This article contends that the primary safety risks associated with the application of synthetic biology in biomedicine include participant safety, biosafety risks, and biosecurity risks. In order to effectively address these risks, it is essential to adhere to the principles of human-centeredness, non-maleficence, sustainability, and reasonable risk control. Guided by these fundamental principles and taking into account China's specific circumstances, this article presents practical recommendations for ethical governance, which include strengthening ethical review, promoting the development and implementation of relevant policies, improving legal safeguards through top-level design, and enhancing technical capabilities for biocontainment.

Conclusion: As an emerging field of scientific technology, synthetic biology presents numerous safety risks and challenges in its application within biomedicine. In order to address these risks and challenges, it is imperative that appropriate measures be implemented. From a Chinese perspective, the solutions we propose serve not only to advance the domestic development of synthetic biology but also to contribute to its global progress.

KEYWORDS

synthetic biology, participant safety, biosafety risks, biosecurity risks, ethical governance, public policy

1 Introduction

Synthetic biology is an emerging life science field in the 21st century, and there is not universally accepted definition at present. It broadly defines as a set of enabling tools allowing the modification of existing biological systems found in nature or by constructing entirely new artificial biological systems (Endy, 2005; Singh et al., 2022). One prominent strand of work in synthetic biology aims to create a range of standardized biological parts or modules that can be tacked on to bacterial chassis to produce customized biological systems (Douglas and Savulescu, 2010). It has some unique technical characteristics that distinguish

synthetic biology from conventional biotechnology. The interdisciplinary nature, engineering design concept, and standardization of synthetic biology give it great capabilities, which greatly improve efficiency of designing and manufacturing life, provide robust technical support for cost-effective, large-scale, eco-friendly, and efficient pharmaceutical research and development, medical diagnostics, and clinical treatments. However, synthetic biology has made it easier to create life, greatly increased the accessibility of technology, made it accessible to many people without a background in biology, contributed to the rise of DIY Biology, and greatly increased the potential for misuse of the technology. In addition, synthetic biology also expands the threat of bioterrorism, potentially causes irreversible and devastating damage to human health and the environment, and poses more serious biosafety and biosecurity risks than conventional biotechnology. Consequently, the established developmental, operational and containment standards of conventional biotechnology are not adequate for synthetic biology applications, and new norms and standards need to be established urgently to promote the healthy development of synthetic biology.

2 The biomedical applications of synthetic biology

Utilizing novel biological techniques within biomedicine is not exclusive to synthetic biology. Over 3 decades ago, researchers employed genetic engineering to create an array of biopharmaceuticals, including insulin and vaccines for the human papillomavirus (Goeddel et al., 1979). The implementation of synthetic biology and its associated methodologies has further catalyzed innovation within the biomedical industry, facilitating significant advancements in pharmaceutical research, medical diagnostics, and clinical therapeutics. This has garnered the attention of a growing number of academic institutions, biotechnology firms, and pharmaceutical companies, leading to increased investment in related research endeavors.

2.1 The application of synthetic biology in advancing pharmaceutical research and development

The utilization of synthetic biology in pharmaceutical research and development has primarily been focused on drug discovery and vaccine development. In drug discovery, synthetic biology can aid in expanding the scale of drug production. By leveraging synthetic biology to alter the genomes of microorganisms, the process of drug production and development can become more cost-effective, efficient, and less vulnerable to environmental factors (Grinstein, 2021). For instance, opioid medicines such as morphine and codeine, which are used for treating severe pain, pain management, and palliative care (Childers et al., 2015), were previously only extractable from poppies. Despite the high market demand for opioid medicines, the growth conditions for poppies that extract and prepare these drugs are stringent and vulnerable to external factors such as climate change and pests, resulting in unstable yields. However, through the implementation of synthetic biology, a series of well-designed metabolic modules were introduced into eukaryotic yeast, enabling the production of opiate compounds through sugar fermentation (Galanie et al., 2015).

Additionally, artemisinin is a highly effective antimalarial drug. Due to the generally low artemisinin content in wild Artemisia annua plants, large-scale mass production has been challenging to achieve, resulting in an inability to meet medical demands. Researchers such as Keasling at the University of California Berkeley applied synthetic biology to microbial metabolic engineering to address this issue. He utilized low-cost industrial microorganisms to ferment and produce artemisinin (Ro et al., 2006). This artificial synthesis method overcomes the disadvantages of low yields and long extraction cycles associated with wild Artemisia annua plants. It provides a more efficient and environmentally friendly means of production.

The utilization of synthetic biology in vaccine research and development has the potential to expedite the vaccine development process while simultaneously providing a theoretical foundation and practical support for the prevention and control of diseases. For instance, in 2018, Chinese researchers employed synthetic biology to develop a novel vaccine for the Zika virus. This vaccine not only boasts a reduced production time but also exhibits enhanced safety, efficacy, and immunogenicity (Li et al., 2018). Synthetic biology also played a crucial role in developing the COVID-19 vaccine. In May 2020, Swiss researchers synthesized the novel coronavirus and other analogous RNA viruses via genome-wide synthesis. This comprehensive synthesis approach enables the production or modification of a substantial quantity of live SARS-CoV-2 viruses within a week for utilization by medical and research institutions, thereby accelerating the development of COVID-19 vaccines and facilitating a rapid response to the pandemic (Thi Nhu Thao et al., 2020).

2.2 The application of synthetic biology in medical diagnostics

In medical diagnostics, synthetic biology facilitates the dynamic monitoring of human health and the precise evaluation of disease severity through modifying the genomes of cells or microorganisms, imbuing them with the capacity to detect abnormal cells and identify lesions within the body. For example, the CRISPR-Cas9 system can be employed to construct biological circuits within cells that specifically recognize key protein molecules in intracellular cancer signaling pathways, providing more accurate determinations for locating cancer cells and assessing disease progression (Liu et al., 2014). Researchers at Columbia University in the United States have utilized CRISPR technology to modify *Escherichia coli*, enabling it to record and monitor changes in the human digestive tract (Sheth et al., 2017). This has yielded unprecedented insights into previously unobservable phenomena and can even be applied to environmental monitoring, ecology, and microbiology.

Synthetic biology can also be employed to detect allergic and inflammatory responses. Allergic diseases are intricate chronic conditions wherein allergens constitute the fundamental cause of allergic and inflammatory reactions (Aldakheel, 2021).

Consequently, detecting and screening allergens at their source is critical in medical diagnostics. In diagnosing allergic diseases, advancements in synthetic biology have expedited the development of cell-based biosensors for clinical applications. By sensing biomarkers associated with inflammation, immunity, and metabolic disorders via biosensors, novel diagnostic and treatment systems can be devised and established (Inda et al., 2019). Synthetic biology can further facilitate the technological transformation and upgrading of allergy detection products. For instance, an engineered mammalian cell detection system can be employed for allergy testing during new drug development (Zhao et al., 2023). The efficiency of new drug development can be significantly enhanced by conducting high-throughput screening of blood samples from high-risk allergy patients.

2.3 The application of synthetic biology in clinical treatment

The clinical treatment represents one of the most significant applications of synthetic biology within the field of biomedicine and constitutes a primary objective of synthetic biology development. Clinical treatments encompassing synthetic biology include gene therapy, cell immunotherapy, and engineered therapeutic bacteria or viruses (Caliendo et al., 2019; Chakravarti and Wong, 2015). Gene therapy is among the most advanced application domains of synthetic biology. On 27 November 2020, SyngenTech announced that its world-first gene therapy product SynOV1.1, developed utilizing synthetic biology, had received clinical trial authorization from the US FDA and had undergone phase I and II clinical studies at the world's largest private cancer research center—Memorial Sloan Kettering Cancer Center, which can be employed in the treatment of liver cancer (Liu et al., 2021). Cell immunotherapy is also an application domain of synthetic biology, with Chimeric antigen receptor (CAR)-T therapy being the most representative. CAR is a synthetically engineered receptor designed to redirect lymphocytes (most commonly T cells) to recognize and eliminate cells expressing specific target antigens (Sterner and Sterner, 2021). It exhibits characteristics such as precision, high efficiency, and rapidity and has demonstrated favorable outcomes in treating leukemia and malignant lymphoma, with high expectations for its potential to cure cancer (Zhang et al., 2023). In addition to modifying cells, synthetic biology can also be applied to modify bacteria and viruses. For instance, attenuated *Salmonella* modified via synthetic biotechnology can effectively reduce tumor volume, delay tumor growth, and enhance the capacity to kill tumor cells, offering hope and a new dawn to tens of millions of tumor patients worldwide (Chen et al., 2021).

Synthetic biology possesses the potential to address the crisis of antibiotic resistance. In 2022, a research team at Rockefeller University in the United States synthesized a novel antibiotic, Cilagicin, predicated on a computational model of bacterial gene products. This antibiotic has demonstrated favorable outcomes in mice and, owing to its innovative mechanism of targeting lethal pathogens, exhibits diminished resistance compared to traditional antibiotics (Wang et al., 2022).

3 An analysis of the safety risks pertaining to the application of synthetic biology within biomedicine

The field of biomedicine presents a vast array of opportunities for applying synthetic biology. However, these opportunities are accompanied by a series of safety risks that must be carefully considered. Clinical trials involving drugs, vaccines, diagnostics, and treatments have the potential to cause harm to Subjects. Furthermore, the unintentional release of synthetic organisms may result in a range of biosafety concerns, including health risks to laboratory personnel and threats to the safety of surrounding communities and the ecological environment. Additionally, the malicious use or abuse of synthetic organisms may give rise to biosecurity issues.

3.1 Issues pertaining to the safety of subjects

In order to ascertain the safety and effectiveness of drugs, vaccines, diagnostic methods, and treatment modalities, it is imperative to conduct clinical trials. Given the distinct nature of synthetic biology and biotechnology, it can recreate known pathogenic viruses, make biochemicals via *in situ* synthesis, make existing bacteria more dangerous (National Academies of Sciences, Engineering, and Medicine, 2018), subjects participating in the trial may be exposed to health risks such as allergies, toxicity, pathogenicity, antibiotic resistance, and even carcinogenicity. Consequently, the safety of subjects is an inescapable concern.

First and foremost, clinical trials employing synthetic biology may jeopardize the safety of subjects due to factors such as inadequate experimental design and non-compliant procedures. In October 2022, the sole global participant in a Duchenne muscular dystrophy (DMD) trial tragically passed away following the administration of CRISPR gene editing therapy via an Adeno-associated virus (AAV) vector (Dongsheng, 2023; Philippidis, 2022). The precise cause of the subject's demise remains under investigation, with some researchers positing that it was precipitated by a potent immune response to the high dosage of the AAV vector (Lek et al., 2023).

Negligent clinical trials not only inflict grave harm or even death upon subjects but also impede progress in related research. The notorious Gelsinger trial exemplifies how the death of a subject can result in a regression in gene therapy research. Jesse Gelsinger, an 18-year-old afflicted with a rare condition known as Ornithine transcarbamylase deficiency (OTCD), perished after undergoing experimental gene therapy spearheaded by James Wilson's laboratory at the University of Pennsylvania (Marshall, 1999). A subsequent inquiry by the FDA uncovered numerous instances of malpractice in the University of Pennsylvania's OTCD gene therapy clinical trial, which bore an undeniable responsibility for Gelsinger's untimely death (Marshal, 2000). Presently, synthetic biology is also being applied to gene therapy and even more intricate treatment modalities, such as cellular immunotherapy and targeted therapy using engineered bacteria. These trials are inherently fraught with uncertainty and necessitate more rigorous scientific design with paramount emphasis on subject safety.

Secondly, the development of COVID-19 vaccines utilizing synthetic biology and biotechnology also raises safety and ethical issues during human trials. DNA and RNA vaccines and adenovirus vector vaccines entail synthesizing viral genes and modifying nucleic acid sequences (Kitney et al., 2021). Synthetic biology and biotechnology have been instrumental in expediting the vaccine development process and enhancing the immunogenicity and breadth of vaccines. Nonetheless, even if we can rapidly comprehend the characteristics of the virus or design its sequence using synthetic biology and biotechnology, our grasp of the interplay between the virus and the human immune system and which type of immune response is optimal for eliciting enduring effective immunity remains limited (Zhaoling et al., 2023). The safety and efficacy of vaccines necessitate protracted clinical trials and observation. However, to expedite testing of the effectiveness of COVID-19 vaccines developed via different technological pathways, some countries have initiated Human Challenge Trials (HCT), wherein a cohort of healthy volunteers are administered different test vaccines before being deliberately exposed to the virus to assess the vaccine's immune effect, to accelerate clinical data collection and reduce the time required for vaccine approval testing (Yueyue and Yali, 2021).

In 2021, the United Kingdom became the first nation globally to conduct a human challenge trial for COVID-19, wherein subjects were initially inoculated with different COVID-19 test vaccines before being infected with a "challenge" dose of COVID-19 in a controlled setting to evaluate the vaccine's immune effect (Killingley et al., 2022; Kirby, 2020). HCT remains a contentious testing methodology to this day. Subjects are required to undergo isolation for several days during the entire trial process. Although researchers meticulously monitor the entire trial process and medical personnel are on hand to provide treatment to volunteers if required, it is still impossible to fully guarantee subject safety (Williams et al., 2022).

3.2 Biosafety issues

Apart from the safety of subjects, the utilization of synthetic biology in biomedicine also presents biosafety challenges. The World Health Organization delineates "Biosafety" in its Laboratory Biosafety Manual as "containment principles, technologies and practices that are implemented to prevent unintentional exposure to biological agents or their inadvertent release (World Health Organization, 2004)." Synthetic biology has tremendous capabilities and has greatly facilitated the development of drugs and vaccines. But at the same time, it can also produce more toxic, infectious, and dangerous pathogens. Any improper operation or accidental contact may imperil laboratory personnel, neighboring communities, and the ecological environment.

Concerns derive from the capabilities of synthetic biology can pose inherent harm. Imminent concerns include re-creating known pathogenic viruses, making existing bacteria more dangerous, and making harmful biochemicals via *in situ* synthesis. These capabilities are based on knowledge that are readily available to a wide range of participants. Medium concerns include manufacturing chemicals or biochemicals by exploiting natural metabolic pathways and the use of synthetic biology to make existing viruses more dangerous, also include manufacturing chemicals or biochemicals by creating novel metabolic pathways, efforts to modify the human microbiome to cause harm, efforts to modify the human immune system, and efforts to modify the human genome. These capabilities involve more constraints and may be limited by factors related to biology and skill. Long-term concerns include re-creating known pathogenic bacteria and creating new pathogens, these capabilities involve implementation challenges. The use of human gene drives requires a minimal level of concern, as it is impractical to rely on sexual reproduction over several generations to spread harmful traits. (National Academies of Sciences, Engineering, and Medicine, 2018).

In summary, synthetic biology has the capabilities to produce more dangerous pathogens or organisms, which may exert deleterious effects on human health and the environment if accidentally released. Based on an analysis of approximately 200 articles, Joel Hewett et al. determined that the human health risks posed by synthetic biology primarily encompass: allergies; antibiotic resistance; carcinogens; and pathogenicity or toxicity. The environmental risks posed by synthetic biology primarily encompass: change or depletion of the environment; competition with native species; horizontal gene transfer; and pathogenicity or toxicity (Hewett et al., 2016). The impact of synthetic organisms or pathogens is also contingent upon factors such as the species which is designed, the nature of the change, the site of release, and the characteristics of genetic modification, particularly when alterations transpire in the toxicity, infectivity, adaptability, and host interaction mechanisms of pathogenic organisms (Bohua et al., 2023). In certain instances, once synthetic organisms or pathogens are inadvertently released, their harm may be amplified through ecological cycles, ultimately surpassing the carrying capacity of ecosystems and inflicting greater collateral damage.

In response to these concerns and risks, the National Academies of Sciences, Engineering, and Medicine have proposed mitigation options that include: 1)Relevant government departments should continue exploring strategies to address chemical and biological defense threats. 2)Relevant government agencies should assess national military and civilian infrastructure to provide information for population-based surveillance, identification and communication of natural and purposeful health threats. 3)The government should work with the scientific community to develop strategies to manage emerging risks, rather than relying solely on current agent-based lists and access control approaches. (National Academies of Sciences, Engineering, and Medicine, 2018).These measures have been effective, but the potential problems posed by synthetic biology will remain a challenge for scientists and national defenses, and continuous efforts are also needed to promote scientific and technological progress while reducing risks.

3.3 Biosecurity issues

Apart from biosafety concerns, synthetic biology also engenders biosecurity issues. The Laboratory Biosafety Manual delineates Biosecurity as "Principles, technologies and practices that are implemented for the protection, control and accountability of

biological materials and/or the equipment, skills and data related to their handling. Biosecurity aims to prevent their unauthorized access, loss, theft, misuse, diversion or release (World Health Organization, 2004)." If synthetic organisms or pathogens are employed to conduct biological warfare or bioterrorism, the extent, duration, and magnitude of harm would be unfathomable. Moreover, the emergence of DIY biology has further exacerbated the biosecurity challenges posed by synthetic biology in the biomedical domain.

3.3.1 Bioterrorism

"Bioterrorism" entails deliberately employing microorganisms or toxins as infectious agents to induce disease or death in humans, animals, or plants and intentionally engender fear among populations (Bossi et al., 2006). Unlike other forms of terrorism, such as nuclear weapons, bioterrorism can more readily inflict widespread destruction globally and is thus dubbed the "poor man's nuclear weapon (Poor Toulabi, 2023)." The advancement of synthetic biology has further facilitated bioterrorism by augmenting the capacity of malevolent actors to generate injurious biological agents with diminished resources.

For scientific research purposes, researchers may utilize synthetic biology to "resurrect" natural pathogens or modify extant pathogens (Noyce et al., 2018), or amalgamate genetic material from multiple pathogens to explore novel avenues for vaccine development (Sanders et al., 2016). However, once biological terrorists exploit these modified pathogens, they present incalculable hazards. For instance, in October 2022, researchers at Boston University announced that they had created a novel strain of the COVID-19 virus by fusing the spike protein of the Omicron variant with the strain infecting the first confirmed COVID-19 patient in the United States, and this synthetic strain is five times more infectious than the Omicron variant and has a mortality rate of up to 80% (Chen et al., 2022). In reaction, David Livermore, a professor of microbiology at the University of East Anglia in the UK, opined that such virus modification experiments are exceedingly unwise and undesirable; Professor Shmuel Shapira, Israel's chief scientist, contended that this constitutes "playing with fire" and should be categorically prohibited (Tilley et al., 2023). They both concur that if this highly perilous synthetic virus were to leak or be malevolently exploited, it would furnish an opportunity for bioterrorism and could wreak catastrophic havoc worldwide.

Additionally, owing to the open access mechanism of synthetic biology and its development tenets of engineering, informatization, and technical simplification, while broadening the accessibility of technology, it also heightens the risk of malevolent exploitation by biological terrorists (Melin, 2021; Trump et al., 2020). Publicly accessible gene sequence information and synthetic biotechnologies furnish a "blueprint" and technical tools for developing bioterrorism and biological weapons, rendering it less expensive and more convenient to fabricate pathogens using synthetic biology.

3.3.2 DIY biology

The assembly of biological components to create new drug reagents has been simplified and accelerated through the use of standardized and engineered methods. This has led to the emergence of a large number of DIY biology practitioners, including biohackers and garage biologists (Ikemoto, 2017). The aim of DIY biology is to break down the barriers imposed by traditional laboratories, disseminate knowledge about synthetic biology, promote open access and sharing of resources, and provide opportunities for everyone to engage in scientific practice (Kuznetsov et al., 2015). Most practitioners are not motivated by profit but are dedicated to using synthetic biology to develop affordable and convenient biotechnology equipment that offers alternative solutions to medical challenges faced by humanity and benefits underprivileged or underdeveloped communities. For instance, Dutch DIY biologist Bruins and his colleagues used simple devices such as hairdryer heaters, shoeboxes, and electronic products to create "Amplino"—a low-cost, high-sensitivity mobile malaria test kit (Landrain et al., 2013). This reagent is more affordable, accessible, and user-friendly than traditional diagnostic tools. Individuals can test for malaria in their homes, thereby advancing the field of malaria test and other disease detection.

DIY biology has bridged the gap between synthetic biology and the general public, attracting many interdisciplinary practitioners to research synthetic biology and invigorating technological development with creativity and dynamism. However, caution must be exercised to mitigate the risks associated with lowering technical barriers. Most DIY practitioners are amateurs who lack formal training in laboratory safety and systematic knowledge of scientific theory. The absence of specialized laws and regulations, as well as departmental oversight, can result in the misuse of technology. Zosiah Zayner, founder of ODIN in California, United States, is a proponent of DIY biology who advocates for making gene editing accessible to more people (Guerrini et al., 2019). This has raised concerns among scholars who argue that the use of gene editing outside laboratories should be restricted (West and Gronvall, 2020). These concerns are not unfounded, as DIY biology practitioners conduct experiments based on personal interests without adhering to standard operating procedures or being able to predict and ensure experimental safety. This could potentially result in biosafety and biosecurity risks.

4 Ethical considerations in the application of synthetic biology within the biomedical field

In order to address the safety risks associated with the application of synthetic biology within the biomedical field, it is imperative to establish appropriate countermeasures. The development of these countermeasures must be grounded in and guided by fundamental ethical principles. Accordingly, we propose four fundamental principles to govern the use of synthetic biology in this domain: human-centeredness, non-maleficence, sustainability, and reasonable risk control. These principles are intended to foster the responsible and healthy advancement of synthetic biology within the biomedical field.

4.1 The principle of human-centeredness

The principle of human-centeredness underscores the importance of valuing and respecting human life, addressing human needs, health, and wellbeing, and advocating for the application of science and technology to enhance human welfare.

This principle has long been a fundamental tenet of human society. In China, the "Great Declaration I" in the Book of History (尚书·泰誓上) states that "Heaven and Earth are parents of all creatures, and of those, Man is the most highly accomplished (Li, 2022)," representing one of the earliest written affirmations of the value of humanity. Similarly, humanistic traditions also exist in other cultures; for example, the ancient Greek philosopher Protagoras famously declared that "Man is the measure of all things (Kattsoff, 1953)." In summary, the principle of human-centeredness is a crucial ethical principle that is essential for understanding the relationship between humans and nature and provides important guidance for the safe and ethical governance of synthetic biology within the biomedical field.

In applying synthetic biology within the biomedical field, it is essential to adhere to the principle of human-centeredness by respecting life, safeguarding the safety and rights of patients and research participants, honoring individual autonomy, upholding human dignity, and ensuring informed consent. Many guidelines and regulations governing clinical trials reflect this principle. For example, China's Good Clinical Practice (GCP) stipulates that the rights and safety of research participants are primary considerations that take precedence over scientific and societal benefits (Jiyin, 2021). The Declaration of Helsinki emphasizes that during human experimentation, researchers must ensure research participants' physical, psychological, and social wellbeing (World Medical Association, 2013). In 2023, China's Measures for the Ethical Review of Life Science and Biomedical Research Involving Humans provide detailed provisions for protecting the privacy rights, informed consent rights, and compensation rights of research participants, requiring researchers to protect the rights of participants in clinical trials by closely monitoring their medication use, health status, and changes in clinical data, and ethics review committees are responsible for reviewing whether research participants have been treated unfairly and for promptly addressing their concerns (National Health Commission, 2023). Adherence to the principle of human-centeredness in applying synthetic biology within the biomedical field also requires that synthetic biology and biotechnologies always strive to enhance human welfare as their ultimate goal. Ethical considerations must be integrated throughout the entire technology development process to promote benevolent technological advancement that amplifies human goodness and achieves moral development by using technology to address societal challenges and ensure that technological achievements benefit humanity.

4.2 The principle of non-maleficence

The principle of non-maleficence is the most fundamental and bottom-line ethical principle in bioethics. In today's morally diverse world, this principle serves as a "global ethic" or "universal ethic" that is widely recognized and applied worldwide (Linklater, 2006). The principle of non-maleficence does not require the complete avoidance of harm; instead, it acknowledges that the development of any technology inevitably brings some degree of harm and necessitates weighing potential harms to choose the lesser harm. For example, in human challenge trials for COVID-19 vaccines, participants who received different types of vaccines developed varying degrees of COVID-19 symptoms. Does this violate the principle of non-maleficence? The answer is no. We consider that clinical human trials are an essential stage in the development of COVID-19 vaccines and play a crucial role in testing their safety and efficacy. As long as relevant laws, regulations, and ethical norms are followed, and informed consent from participants is obtained, the application of HCT is ethically reasonable. It is commendable for volunteers to sacrifice their own health for the benefit of humanity when they are fully aware and willing of the risks of the experiment. Therefore, although HCT may cause some harm to participants, it still applies to "principle of non-maleficence". The principle of non-maleficence is not a principle of no harm but rather a principle of minimal harm, reasonable harm, or morally permissible harm (Jianbing and Chuanzhong, 2007).

4.3 The principle of sustainability

The principle of sustainability is a goal-oriented principle that aims to achieve long-term harmony between humans and nature by meeting the needs of the present generation without compromising the ability of future generations to meet their own needs (Munthe et al., 2021). This intergenerational ethical principle requires the present generation to respect future generations' rights to life and development and not deprive them of their rights simply because they do not yet exist or have no voice. Sustainability is closely related to sustainable development, but the two concepts are distinct. Sustainability is a broader concept, while sustainable development focuses primarily on human welfare (Harrington, 2016). Additionally, the two concepts have different emphases: sustainability emphasizes the long-term nature of goals, while sustainable development focuses on the processes and pathways for achieving these goals. Generally speaking, the principle of sustainability encompasses ecological, economic, and social sustainability, all interconnected and inseparable (Berg, 2020). Among these, ecological sustainability is considered the most important and directly affects the other two types of sustainability.

The research and application of synthetic biology in the biomedical field may have irreversible and severe impacts on the ecological environment. Therefore, it is essential to adhere to the principle of sustainability to avoid sacrificing the ecological environment and the welfare of future generations for technological advancement and to ensure the sustainable development of ecology, economy, and society. To achieve these goals, several action principles must be followed:

Firstly, in terms of the relationship between humans and nature, the principle of sustainability requires researchers to follow the precautionary principle by proactively taking preventive measures to reduce or avoid risks to the natural environment when harm is uncertain; Secondly, the principle of sustainability requires researchers to adhere to the prudence principle. Synthetic biology is highly complex and uncertain; researchers must adhere to the prudence principle as a core behavioral norm and be responsible for themselves, future generations, and the ecological environment.

Thirdly, regarding the relationship between humans and society, the principle of sustainability requires providing maximum compensation and support to vulnerable groups; seeking public understanding and trust; strengthening unity and cooperation; involving all stakeholders in research; enhancing policy transparency; etc.

4.4 The principle of reasonable risk control

In order to address the biosafety and biosecurity concerns associated with synthetic biology in the biomedical field, it is essential to adhere to the principle of reasonable risk control. This principle mandates that managers implement measures to reduce or eliminate the likelihood of risk occurrence or to keep risks within an acceptable range to prevent incurring unbearable losses (Aven, 2016). In March 2022, the General Office of the Central Committee of the Communist Party of China and the General Office of the State Council issued the Opinions on Strengthening the Governance of Science and Technology Ethics, which proposed five principles of science and technology ethics, including the principle of reasonable risk control. This principle stipulates that scientific activities must objectively evaluate and prudently address the uncertainty and risks associated with technology and its application; Efforts must be made to avoid and prevent potential risks, prevent the misuse or abuse of scientific achievements, and avoid endangering social security, public security, biosafety, and ecological safety (General Office of the Central Committee of the Communist Party of China and General Office of the State Council, 2022). The introduction of the principle of reasonable risk control is beneficial in addressing ethical challenges posed by emerging technologies such as synthetic biology and promoting the healthy development of science and technology.

Specifically, the application of synthetic biology in biomedicine must adhere to the natures of effectiveness, advancement, whole process, initiative, and systematicity. Firstly, the effectiveness requires scientists to effectively identify potential hazards in pharmaceutical research and development as well as disease treatment processes. Subsequently, operable management measures must be formulated for identified hazards to improve risk control effectiveness. Secondly, the advancement necessitates developing or introducing advanced risk control technologies and effectively utilizing them in conjunction with China's synthetic biology industry's characteristics. Then, the whole process mandates strict control of risks at various stages of experiments related to synthetic biology through independent risk assessment and dynamic supervision throughout the whole experiment process. Additionally, research applications must undergo strict scrutiny. Furthermore, adherence to proactive control and prior control thinking is required by the nature of initiative. In response to changing environmental conditions and emerging new situations and problems, timely response measures must be taken, and response plans adjusted. Finally, risk control is a highly systematic and comprehensive task. Especially in interdisciplinary fields such as synthetic biology where risks have complex origins and far-reaching consequences, it is necessary to formulate more risk management measures.

5 Recommendations for ethical governance of biomedical applications of synthetic biology

In order to address safety concerns associated with the application of synthetic biology within the biomedical field, it is necessary to develop practical governance measures guided by the aforementioned fundamental principles. We believe that efforts can be made in several areas, including strengthening ethical review, promoting the development and implementation of relevant policies, improving legal safeguards through top-level design, and enhancing technical capabilities for biocontainment.

5.1 Strengthening ethical review

The widespread application of synthetic biology within the biomedical field has led to a sharp increase in safety risks, necessitating the development of new legal and ethical regulations and the strengthening of ethical review to ensure the safety of research participants, biosafety, biosecurity, and the prevention of exploitation by bioterrorists. Currently, China has issued regulations such as GCP (National Health Commission, 2020), Measures for the Ethical Review of Life Science and Biomedical Research Involving Humans (National Health Commission, 2023), and Guiding Principles for Ethical Review of Drug Clinical Trials (EOCJRDU, 2010), providing institutional safeguards for strengthening ethical review of clinical trials, regulating the work of ethics review committees, and ensuring compliance with scientific and ethical requirements. However, the research and application of synthetic biology within the biomedical field have disrupted traditional ethical review paradigms for clinical trials. Its enormous technological power and influence pose a serious threat to the safety of research participants and present unprecedented challenges to biosafety and biosecurity. The existing ethical review paradigm can no longer meet the development needs of synthetic biology within the biomedical field, and there is an urgent need within academia to establish a new ethical review paradigm to ensure its healthy development.

The establishment of new ethical review paradigm depends on ethics committees. At present, ethics committees are composed mainly of biologists, medical scientists and other scientists, many of whom lack the ethical literacy, and only consider what can be done, rather than what should be done, resulting in a lack of rationality in ethical review.In this regard, we call for the participation of humanities and social scientists such as bioethicists, lawyers and sociologists to join the ethics committee, and invite the participation of stakeholders such as public representatives and religious figures. In addition to disciplinary background, the composition of the ethics committee shall take into account factors such as gender, age, education, ethnicity and geographical distribution of the members, and they shall be independent of the research/experimental unit in conducting reviews, making recommendations and making decisions. Ethics committees should review the design and implementation of research plans; the risks and benefits of trials; the recruitment and informed consent of research participants; their safety and privacy; and research involving vulnerable groups. The ethics committee should also develop standard operating procedures and systems for biotechnology to ensure consistency and standardization in ethical review work.

Additionally, the ethics committee should regularly provide professional training to researchers to raise their awareness of safety and social responsibility. As synthetic biology develops rapidly, ethics committees should continuously improve their organizational management and institutional development in response to technological needs, fulfill their responsibilities to

protect the safety, dignity, and rights of research participants, enhance public support for and trust in the application of synthetic biology, and promote it scientific and healthy development within the field of biomedicine.

5.2 Promoting the development and implementation of relevant policies

The State Council should coordinate with institutions such as the China's Center for Disease Control and Prevention, the National Health Commission, the Ministry of Agriculture and Rural Affairs, and the Ministry of Ecology and Environment to establish a safety review system for joint decision-making on safety issues related to synthetic biology. The review panel should follow safety, efficacy, and economy in conducting risk assessments of synthetic biology programs and classify them according to risk level and application type (unrestricted use, restricted use, and special use), with a focus on regulating dangerous target experiments such as synthetic viruses and bacteria. The government should also establish and improve monitoring mechanisms to regularly inspect laboratory safety management facilities and systems; supervise laboratory personnel to ensure compliance with regulations and equipment maintenance; inspect the transportation and storage of hazardous reagents; and prevent accidental harm due to negligence. In addition, the government should strictly regulate the order services of synthetic biology companies to prevent malicious exploitation by criminals.

These policies are binding on professional organizations such as research institutions and enterprises but have limited effect on private research institutions and amateur enthusiasts outside institutional arrangements. To address this issue, the government can establish formal, open community laboratories to provide DIY biology practitioners with regular research venues and regulate their research behaviour. The government can implement a registration management system in community laboratories to protect DIY biology practitioners' legitimate rights and interests while clarifying responsibilities and scope and urging them to fulfill their laboratory safety responsibilities. In addition, the government should actively guide DIY biology practitioners to establish informal standards and regulations, encourage them to develop a sense of responsibility, improve their self-governance capabilities, and guide the enormous technological potential of DIY biology groups toward legal paths that contribute to China's high-tech development.

Research in synthetic biology is closely related to the public interest, and the public has the right to be informed about research results, hold researchers accountable, and exercise oversight. The government should promote public communication and encourage public participation in relevant discussions, reviews, management, and decision-making. The government can establish various effective communication channels such as setting up dedicated communication departments (e.g., Synthetic Biology Consultation Office), dedicated communication time slots (e.g., regular meetings), or more convenient communication websites, mailboxes, public accounts, etc., to solicit public opinions on sensitive issues such as synthetic viruses and bacteria and listen to public voices.

5.3 Improving legal safeguards through top-level design

Reliance on ethical principles and guidelines alone is insufficient for governance in the application of synthetic biology within biomedicine; legal safeguards are also necessary. China has established a foundation in biosafety and biosecurity legislation, such as the Biosecurity Law implemented in 2021. This law stipulates strengthening safety management for biotechnology research, development, and application activities. Relevant activities must comply with ethical principles and are prohibited from endangering public people, such as endangering public health, damaging biological resources, or destroying ecosystems and biodiversity (Pandi W et al., 2021). The law also outlines measures to prevent bioterrorism and bioweapon threats, including prohibiting the development, manufacture, acquisition, storage, possession, and use of bioweapons. It also requires formulating a special list of organisms, biological toxins, equipment, or technologies that can be used for bioterrorism activities or manufacturing bioweapons and taking measures to prevent spreading (Haiyou, 2020).

China's Criminal Law regulates illegal and criminal acts that endanger public safety and engage in bioterrorism. It includes explicit provisions for crimes such as the illegal manufacture, sale, transportation, and storage of dangerous substances; release of toxic, radioactive or infectious disease pathogens; organization, leadership, or participation in terrorist organizations; and assistance to terrorist activities (People's Republic of China, 2020). China's Counter-Terrorism Law requires strict supervision and management of infectious disease pathogens to prevent their spread or entry into illegal channels. It stipulates that, in the event of theft, robbery, or loss of infectious disease pathogens, necessary control measures must be taken immediately and reported to the public security organs and competent authorities (People's Republic of China, 2018).

Despite China's legal foundation for biosafety and biosecurity, there remain issues such as incomplete content and lack of punitive measures. In particular, there is still a legal, regulatory gap in synthetic biology research in the biomedical field. For example, the impact on the ecological environment of synthetic organisms or pathogens accidentally released during research has not yet been included in the scope of legal regulation. Some raw materials, such as oligonucleotides, are not included in the special list. Moreover, synthetic biology is rapidly developing. Biological toxins and equipment that can be used to launch bioterrorism or manufacture bioweapons are constantly changing. Legislative bodies should timely amend and follow up technical lists to improve legal norms related to synthetic organisms and pathogens. Strengthen safety management of pathogenic organism laboratories. Clarify new standards and requirements for synthesizing bacteria, viruses, and other pathogens to prevent them from being used to manufacture bioweapons or for terrorist purposes. In addition to strengthening biosafety and biosecurity legislation, China must promote biotechnology innovation and clarify the boundaries between technology safety and innovation, not to restrict technology development and produce a "chilling effect." In summary, legislative bodies should establish a sound normative document for synthetic biology safety management so

that synthetic biology research applications in various fields have laws to follow and must follow laws.

5.4 Enhancing technical capabilities for biocontainment

In order to minimize the risks associated with the application of synthetic biology in the biomedical field, it is insufficient to rely solely on external regulatory mechanisms through ethics, policy, and law. This is because accurately predicting the risks of a new product, particularly synthetic biological products that have never existed before, is challenging. It is impossible to determine their impact on humans or the ecological environment based on experience. As a result, it is necessary to design reliable internal biocontainment measures to ensure that synthetic organisms do not cause harm even if unintentionally released or maliciously used, thereby eliminating adverse effects at their source.

Common biocontainment strategies include auxotrophic organisms, toxin-antitoxin pairs, CRISPR-based "kill switches," and xeno-nucleic acids (XNAs) (Wright et al., 2013). These methods and mechanisms can be applied to whole processes involving the development of synthetic organisms. Through these safety measures, it is possible to prevent synthetic organisms from surviving or dying under natural conditions, effectively preventing their spread to experimenters, people outside the laboratory, and the environment. Furthermore, these safety measures should be continuously updated and improved as technology advances.

6 Conclusion

The research and application of synthetic biology in the biomedical field can potentially address significant public health and hygiene issues. However, as an emerging science and technology, synthetic biology faces numerous challenges, including interdisciplinary intersections, technological innovations, and unknown risks. Its application in the biomedical field is particularly complex and uncertain, posing unprecedented threats to subject safety, biosecurity, and biosecurity. In response, China must establish related laws and regulations guided by fundamental ethical principles, implement suitable ethical review mechanisms, and clarify various departments and researchers' technical and ethical responsibilities during research and development. This will diminish or eliminate risks associated with synthetic biology at different stages.

Policy guidance should also be promoted to improve relevant management systems and operating procedures. This will regulate the production and use of synthetic biology through institutional norms and prevent biosafety and biosecurity risks. Additionally, legals and regulations related to synthetic pathogens should be refined and severely punish malicious acts such as bioterrorism. The level of biocontainment must also be improved to minimize safety risks. These measures should be revised in a timely manner as synthetic biology advances. Only then can we better regulate the research and application of synthetic biology in the biomedical field and guide its positive development.

Author contributions

YO: Conceptualization, Writing–original draft, Writing–review and editing. SG: Investigation, Validation, Writing–original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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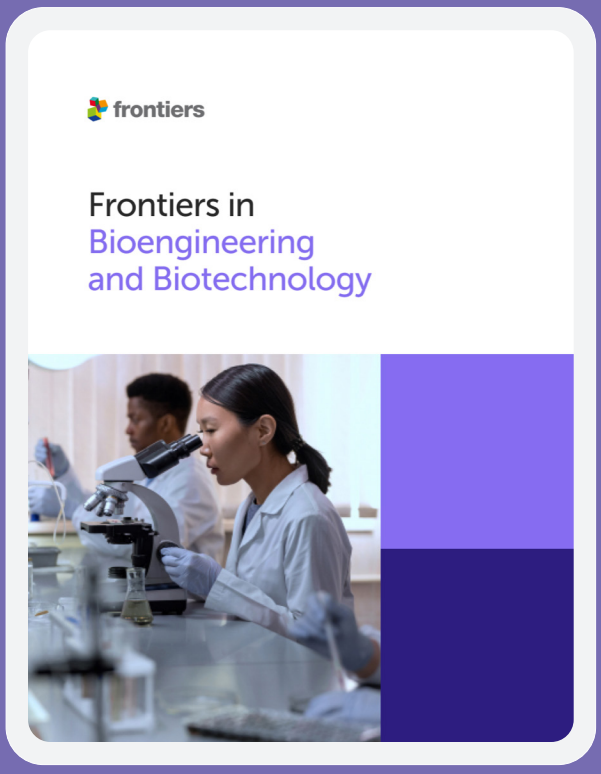

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