From Understanding to Action: Community-Based Options for Improving Safety and Security in Synthetic Biology

Stephen M. Maurer, Keith V. Lucas & Starr Terrell
Goldman School of Public Policy
University of California at Berkeley

UNIVERSITY OF CALIFORNIA, BERKELEY
RICHARD & RHODA GOLDMAN SCHOOL OF PUBLIC POLICY

Draft 1.1 April 15, 2006

Executive Summary

The vast majority of today's biosafety and biosecurity concerns predate synthethic biology and would be substantially the same even if this new field did not exist. Nevertheless synthetic biologists have an obligation to make sure that their work does not amplify earlier risks or create new ones. That discussion has been ongoing in various formal and informal venues since 2000. Today, synthetic biologists share a deep understanding of the biosafety/biosecurity problem and – in some cases – emerging consensus about what can and should be done to manage it. Many options can be implemented through community self-governance without outside intervention.

Understanding alone is not sufficient. The challenge now is action. Synthetic Biology 2.0 provides a natural forum for community self-governance. Because time is limited, however, members must come prepared. This document provides a self-contained review of previous discussions (Section I), discusses design principles for possible interventions (Section II), identifies instances where synthetic biology could potentially change earlier biosecurity/biosafety risks (Section III), and summarizes possible interventions that the community should consider at Synthetic Biology 2.0 (Section IV). Possible actions include:

> ***A.1 Insist That All Commercial Gene Synthesis Houses Adopt Current Best Practice Screening Procedures.*** While most gene synthesis companies screen orders for dangerous sequences, a few do not. This gives both community members and outsiders access to feedstocks for both wild-type and genetically-engineered bioweapons. Community members should stop doing business with any gene synthesis company that fails to implement current best-practice screening methods by January 1, 2007.
>
> ***A.2 Create and Endorse New Watch-Lists To Improve Industry Screening Programs.*** Improved watch-lists and software tools can make industry screening more accurate and efficient. Members should prepare the necessary lists and tools in time for Synthetic Biology 3.0.
>
> ***B.1. Create a Confidential Hotline For Biosafety and Biosecurity Issues.*** All experimenters contemplating "experiments of concern" should obtain independent expert advice before proceeding. The community should make such advice freely available to all experimenters, including non-members (*e.g.* hackers) who cannot otherwise obtain such advice from formal university, company, or NIH safety committees.
>
> ***B.2. Affirm Members' Ethical Obligation to Investigate and Report Dangerous Behavior.*** Members have an obligation to investigate and, if necessary, report dangerous behavior. Members should affirm this obligation by formal resolution at Synthetic Biology 2.0.

C. Create a Community-Wide Clearinghouse for Identifying and Tracking Potential Biosafety/Biosecurity Issues. Members who notice potential biosecurity issues have an obligation to share them with the broader community. A central clearinghouse will help the community to identify, track, and if necessary respond to the biosafety/biosecurity implications of a changing technology.

D. Endorse Biosecurity/Biosafety R&D Priorities. New technologies can potentially reduce current biosafety/biosecurity risks even further. Members should identify, endorse, and urge funding agencies to invest in priority technologies such as safe chasses and bar codes.

This document is part of a sustained effort by The Berkeley SynBio Policy Group to help members learn about security issues and facilitate community self-governance at Synthetic Biology 2.0. In coming weeks, we will host Town Hall Meetings at Berkeley (April 11) and MIT (April 21) to further discuss what the community can do to improve biosafety/biosecurity. Both Town Halls will be webcast to members around the world.

We expect to change this document continually between now and May to reflect ongoing community discussion and debate. This is a living document.

Berkeley SynBio Policy Group.

The Berkeley SynBio Policy Group is a joint undertaking of Lawrence Berkeley Laboratory's Keasling Lab and UC Berkeley's Goldman School of Public Policy. The Group's goal is to study and facilitate community action on issues of concern to the worldwide synthetic biology community. The Group is funded by The MacArthur Foundation and the Carnegie Corporation.

I. Introduction

A. Making Self-Governance Work.*

Community self-governance provides a realistic and potentially powerful complement or alternative to regulation, legislation, treaties, and other interventions by outside entities. Experience with Asilomar[1] and the Bermuda Protocols shows that biological research communities can and do adhere to voluntary standards. While self-governance tends to be less stringent than legislation and cannot change existing laws or institutions, it also offers significant advantages. First, self-governance is the right thing to do. In the words of the Fink Report, biologists need to "take responsibility" for "preventing potential misuses of their work."[2] Second, almost always faster than other methods. Second, it derives from consent and is therefore frequently more elegant than externally imposed solutions.[3] Finally, it is inherently international. This can be a crucial advantage in a world where science and commerce routinely span national boundaries.

The Discussion So Far. Over the past six years, synthetic biologists have devoted enormous effort to thinking about biosecurity/biosafety issues. In that time, the problem has become well-understood and many proposals – some widely admired – have emerged.

The First International Conference on Synthetic Biology 1.0 began to formalize this process in July, 2004. It hosted "moderated discussions to help begin to explore … current and future biological risk"[4] and a community-wide attempt "to be proactive about precautionary measures."[5] More recently, groups funded by the Sloan Foundation [6] and the Federation of American Scientists[7] have deepened and extended these discussions. This activity has fostered a widespread expectation that "the future is now"[8] and that Synthetic Biology 2.0 will make "significant progress" toward a "code of ethics and standards."[9]

The Berkeley SynBio Policy Group: Reducing Frictions. Average members of the synthetic biology community have relatively little time to prepare and think about biosafety/biosecurity issues. Day 3 of the Conference will offer only limited time to learn this material.[10] For this reason, success will depend on members' ability to think about and discuss these issues in advance. The Berkeley SynBio Policy Group's goal seeks to promote this discussion and ensure that members have basic information at their fingertips. Steps in this process include:

> ***This Document.*** This document provides a snapshot of current biosecurity/biosafety risks and possible interventions for managing them. The

* The authors wish to thank the MacArthur Foundation for funding this project, The Carnegie Corporation for additional support, Jay Keasling, Drew Endy, Laurie Zoloth, George Rutherford, and Raymond Zilinskas for useful suggestions, and the nearly two dozen community members who participated in interviews for this project. Any errors are ours alone.

goal is to provide a concise, easy-to-use references that members can consult in support of an informed, rational discussion and vote at Synthetic Biology 2.0. We expect to update this document repeatedly to reflect ongoing community input between now and Synthetic Biology 2.0.

Interview Program. The Berkeley SynBio Policy Group has conducted extensive interviews to learn what members believe, want, and are prepared to vote for. The current document reflects more than twenty of generous and input from twenty-one leading academic scientists, four industry representatives, and six European members.[*]

Coordination With Other Institutions and Working Groups. The Berkeley SynBio Policy Group has received extensive input from US Senate Staff, NIH's National Science Advisory Board for Biosecurity ("NSABB") and the Federation of American Scientists. The Sloan Foundation's MIT/Venter Institute/CSIS study group (hereinafter "Sloan group")[11] has been particularly generous in supplying ideas for possible interventions.

Town Halls. The Berkeley SynBio Policy Group will hold Town Hall meetings at UC Berkeley (April 11) and MIT (April 21) to discuss the various proposals outlined in this document and to elicit further suggestions from members. An additional, European-focused Town Hall is currently under discussion. Each Town Hall will feature a full discussion followed by a non-binding advisory ("straw poll") vote.

Synthetic Biology 2.0: A Unique Opportunity. Members have publicly announced that they expect Synthetic Biology 2.0 to produce "significant progress" toward a "code of ethics and standards."[12] Members participating in Day 3 deliberations will be able to call numerous nationally recognized experts, including representatives of the Sloan group and NSABB.

B. How to Use This Document.

This document is designed to help members make a rational and informed vote at Synthetic Biology 2.0. Section II ("Doing Policy") summarizes general principles for evaluating policy interventions in the biosecurity/biosafety arena. Section III reviews the traditional biosecurity/biosafety problem, focusing on the comparative handful of points where synthetic biology could have a significant impact. Finally, Section IV ("Possible Interventions") provides a menu of self-regulation interventions that the community

[*]Some categories overlap. A full list of interviewees can be found at Appendix A.

should consider adopting at Synthetic Biology 2.0. For ease of reference, we have color-coded these interventions as follows:

> Emerging Consensus Proposals. Our interviews have identified a core group of intervention proposals that (a) appear technically feasible, and b) already enjoy widespread support among members. These interventions form an obvious "short list" for discussion at Synthetic Biology 2.0. We highlight them in what follows.
>
> Resolutions. Each Emerging Consensus Proposal ends with the draft text of a resolution that members could adopt at Synthetic Biology 2.0. A complete set of draft resolutions appears at Appendix B.
>
> Other Proposals. Some novel proposals are unlikely to be refined and debated in time for Synthetic Biology 2.0. We nevertheless include them in the interest of completeness and in the hope that promising ideas will eventually be refined for discussion at Synthetic Biology 3.0 and later conferences.

As previously noted, this is a living document. We expect it to change repeatedly as a result of community input from the Town Halls and other discussions leading up to Synthetic Biology 2.0. Members are urged to contact the authors with comments and questions.

II. Doing Policy

Policymakers must address problems logically and consistently. The following principles provide a useful starting point for thinking about biosecurity/biosafety interventions.

A. Cost-Benefit.

Useful interventions should place minimal burdens on synthetic biology's ability to deliver value for society.

> ***General Benefits.*** Synthetic biology has already made making existing biotechnology programs dramatically more efficient. The value of these benefits almost certainly runs into the tens of billions of dollars.[13] In the long-term, synthetic biology stands to generate still larger gains by creating products that cannot be achieved by traditional methods.[14] Examples include drug delivery systems that detect and target tumors[15] and the development of standardized parts that let companies tailor organisms to the needs of individual users.[16] Any intervention that threatens these developments is likely to be counterproductive.

> **Biodefense Benefits.** Synthetic biology promises powerful new tools for biodefense, most notably in the area of accelerated vaccine development. In the words of one member, the biodefense problem is defined by the fact that there "are more good guys than bad guys." For this reason, regulations that handicap all researchers indiscriminately are almost always be bad for society.[17]

The expected benefits of synthetic biology are large. Proposed interventions must avoid unnecessarily stifling research.

B. Comparative Principle.

Current biosecurity/biosafety risks predate synthetic biology by many years.[18] These risks would be substantially the same if synthetic biology had never been invented. This does not mean that synthetic biology irrelevant or that members can responsibly ignore biosafety/biosecurity concerns in their work. It does, however, suggest that policy should be done "at the margin," *i.e.*, by focusing on how synthetic biology *changes* preexisting risks for better or for worse. Section III follows this prescription by focusing on areas where synthethic biology (a) potentially introduces qualitatively new pathways for accidents, or (b) potentially makes bioweapons cheaper, easier, or more effective than earlier technologies.

C. Flexibility.

It is only natural to want permanent, guaranteed solutions to policy problems. Unfortunately, this goal is seldom realistic, particularly in the biosafety/biosecurity arena. In keeping with our comparative principle, members should be prepared to consider even incomplete interventions that reduce risk.

This observation leads to three corollaries:

> ***Relationship to Existing or Future Law.*** Self-regulation will not necessarily displace traditional interventions based on regulation, legislation, and treaties. Community action should be implemented in ways that yield to more formal methods where regulation/legislation/treaties already exist or are subsequently introduced.
>
> ***Complete Solutions are Illusory.*** It is seldom, if ever possible to achieve complete security against a determined adversary. However, this may not be necessary. A complex terrorist conspiracy must daily negotiate a long chain of security hurdles. The fact that *individual* hurdles can be circumvented with high probability may not matter if the *cumulative* chance of failure is large.[19]

> ***Permanent Solutions are Illusory.*** Few, if any, security policy solutions are permanent. The best that policymakers can hope to do is to set a baseline policy and then update it in light of new developments. This suggests that members should establish permanent institutions to follow developments and update policy on a regular basis.[20]

The Romans observed long ago observed that "The better is the enemy of the good." In what follows we assume that even interventions with modest payoffs may be desirable provided that the cost is low.

III. How Does Synthetic Biology Affect Traditional Biosecurity/Biosafety Concerns?

The bioweapons/biosafety problem is only tangentially related to synthetic biology. This document is designed to help members see synthetic biology in a broader context. In keeping with our comparative principle, the focus throughout is on identifying instances where synthetic biology could potentially change pre-existing biosafety/biosecurity risks.

A. Biosecurity.

Biosecurity concerns range from assassination and psychological intimidation[21] to WMD at the nuclear weapons scale. Following most authors, we focus here on mass casualty scenarios. This benchmark case makes sense for at least three reasons. First, bioweapons offer few advantages over cheaper and more familiar technologies (*e.g.* high explosive) at scales comparable to the World Trade Center attacks (2,752 deaths[22]). According to our comparative principle, synthetic biologists can do little to reduce the danger of such attacks in any case. Second, no small scale attack is likely to erase synthetic biology's expected net value to society. Our cost benefit principle suggests that we should focus on mass casualties.[23] Finally, the interventions described in Section IV, *infra*, are broad enough to mitigate biosecurity risks across the board. Our focus on mass casualties is only illustrative.

The non-occurrence of significant biological terrorist attacks over the past fifty years[24] strongly implies that a WMD-scale bioweapons capability requires substantial investment.[*] Historically, even the smallest weapons programs required massive

[*]This does not, of course, imply that bioweapons are comparably expensive to other forms of WMD. "In comparison to nuclear and chemical weapons (CW) programs, individuals' intellectual capabilities play a far greater role in determining the success or failure of a program than the physical resources to which they may have access." UNSCOM, "Iran Survey Group Final Report," available at http://www.globalsecurity.org/wmd/library/report/2004/isg-final-report/isg-final-report_vol3_bw-01.htm.

facilities and thousands of personnel.[25] Significantly, advances in biology have done little to reduce these costs. This lesson is underscored by Soviet experience in the 1980s and early 1990s, in which genetic engineering did little or nothing to cut total program costs.[26] Iraqi and South African bioweapons programs of the late 1980s similarly document the continued need for resources and manpower.[27]

Of course, the experience of state-sponsored programs only provides a starting point. Today, the main focus is terrorism. This section asks whether and to what extent synthetic biology erodes old cost- and knowledge-based barriers to acquiring bioweapons.[*]

Identifying Candidate Organisms. Early bioweapons programs devoted enormous effort to demonstrating that candidate disease agentss could, in fact, be "weaponized."[28] The identity of many of these disease agents are now public knowledge. Iraq's decision to pursue "classical weapons agents" previously developed by the US shows that there is a powerful incentive to pursue agents that are already known to have produced successful weapons in the past.[29]

Genetic engineering is disadvantageous along this dimension. Synthetic biology does little or nothing to change this result.

Obtaining Pathogen Cultures. Obtaining pathogen strains for a potential bioweapon from Nature is difficult.[30] For this reason, weapons makers have usually started with pathogen cultures obtained from research laboratories and type collections.[31] Despite recent reforms, control of these materials remains highly imperfect. The problem is aggravated by inconsistent international standards that undercut country-by-country regulation.[32]

Synthetic biology introduces a new channel for potentially obtaining access to dangerous sequences and, ultimately, organisms. Recent experiments recreating the polio[33] and 1918 influenza[34] viruses show that this route is viable but also non-trivial. **Proposals A.1** through **A.4** seek to fill this gap by promoting and strengthening industry screening practices so that dangerous sequences are not shipped to unknown or untrustworthy purchasers.

Large Scale Manufacturing. The manufacturing requirements for a bioweapons attack are non-trivial, particularly on the scale of a conventional terrorist bombmaking plot. The earliest and still-simplest bioweapon is a concentrated liquid or "wet formulation." British military calculations from World War II suggest that an anthrax attack would require about five tons of wet agent per square kilometer.[35] Postwar advances involving aerosol sprays and more infectious diseases suggest that this figure can probably be cut to

[*]The benchmark should also be adjusted to the extent that terrorists can potentially jettison normal military requirements that (a) the proposed bioweapon exhibit dependable, well-understood effects and (b) reliable countermeasures for friendly forces operating the area.

1000 pounds per square kilometer.[36] Comparing this figure against population densities for Manhattan[37] suggests that terrorists would still need to manufacture about 200 pounds of agent in order to kill 5000 people.[*] Japanese experience with very simple manufacturing facilities in the 1930s suggests that this would require 100 fermentation tanks and 300 workers.[38] The proliferation of high performance culture equipment for biotech industry may reduced this obstacle, although terrorists would still require "persistence and experimentation to overcome process problems."[39]

Modern bioweapons programs typically process wet agents further to make so-called dry formulations or "biopowders." Reports of a Cold War-era Soviet anthrax accident[40] and American bioweapons tests[41] suggest that an attack on 5,000 people would require one to ten kilograms of material. While small in absolute terms, this would still require a thousand-fold increase over the 2-3 grams used in the 2001 anthrax attacks.[42] Iraqi experience during the 1980s suggests that extending bench- to pilot-scale production is non-trivial.[43]

Technologically, the key to more efficient wet agent production is high fermentation densities. Automated fermentation[44] and biotech manufacturing techniques[45] would be central to this effort. Improved biopowder production would require additional expertise in spray drying, milling, and other material processing technologies. Synthetic biology would add little or nothing to these efforts.

Safety. Most bioweapons manufacturing programs repeatedly infect workers. Japanese experience during World War II suggests that low tech programs can expect casualties of about one percent per year.[46] In practice, rates for a terrorist conspiracy could well be higher. The long history of terrorist bomb factory accidents since the 1870s suggests that accidents would impose substantial burdens on both morale and security.[47] Aum Shinrikyo is believed to have abandoned its efforts to manufacture nerve gas following several accidental releases in 1994.[48]

Conventional containment and manufacturing technologies are the key to achieving reasonable safety levels. Synthetic biology is largely irrelevant to this enterprise. Genetic engineering could, however, become relevant if terrorists sought to create "binary weapons" that could be safely handled prior to use.[49]

Hardiness. Wet agents have a short shelf life ranging from weeks to months.[50] This limits the value of stockpiling and makes manufacturing problems more acute. Once released, most bioweapons degrade quickly in the presence of sunlight,[51] oxidation,[52] air pollution,[53] high wind,[54] and humidity.[55] This further limits the casualties that can be inflicted in practice.

[*] This very rough estimate ignores the fact that much of the target population would be sheltered in doors. World War II-era planners routinely assumed that one-fourth of the bomb load in a bioweapons attack would be high explosive.

Traditional technologies for hardening bioweapons involve adding chemical stabilizers to wet agents or microencapsulating biopowders within a protective coating.[56] In theory, genetic engineering could also enhance environmental resistance.[57] In practice, however, genetically engineered organisms are usually less hardy than natural ones.[58] This may change as scientists learn to manipulate more factors simultaneously.[59] Learning how to insert multiple gene sequences into organisms while avoiding unintended interactions is an important focus of current synthetic biology research.

Virulence and Antibiotic Resistance. Antibiotic resistance complicates public health defense and is therefore desirable in a bioweapon.[60] Genetic engineering could similarly modify organisms to evade standard identification, detection, and diagnostic methods[61] and produce agents that are more communicable, lethal, or have a longer latency or higher mortality.[62] Russian scientists are known to have used genetic engineering to create vaccine-resistant bioweapon agents[63] and more virulent versions of anthrax and Marburg[64] during the 1980s.

Synthetic biology potentially makes these genetic engineering manipulations more accessible. **Proposals A.1** and **A.2** make it harder for terrorists to obtain gene sequences needed to build drug resistant bioweapons. Similarly, **Proposal B.1** addresses "experiments of concern" that could potentially push synthetic biology in directions that made it more useful to terrorists.

Delivery Systems. Disseminating bulk biological material is probably the single most significant obstacle to creating effective bioweapons.[65] Aerosols must be carefully controlled since (a) large droplets are seldom inhaled, and (b) very small droplets quickly dry out killing any organisms contained inside. Most commercially available aerosol generators are poorly suited to this task because they either generate large droplets or else have very low output rates.[66] For the most part, terrorists seeking to improve efficiency would turn to industrial aerosol technologies that have little or nothing to do with biology. Genetic engineering could, however, help make some wet agents easier to aerosolize.[67]

The formula and production methods for making biopowders is classified. While originally expensive to develop, the biopowder formula is reportedly simple.[68] Terrorists who do not already know the secret would presumably turn to such material handling technologies as spray drying, milling, and other methods.[69] Extensive testing would also be needed to ensure that agents remained effective. Synthetic biology adds little to these technologies.

Contagious Diseases. In principle, terrorists could obviate the need for elaborate production and delivery systems by turning to contagious diseases. During the Cold War, the Soviets concluded that the United States was such a distant "deep target" that an epidemic caused by a contagious pathogen would never reach Russia.[70] It is reasonable to think that terrorists could reach a similar judgment. That said, infectious bioweapons face their own development barriers. These include:

Public Health Defenses. Natural outbreaks of anthrax, botulism, brucellosis, cholera, and plague disease already strike the US with some frequency, though typically in numbers fewer than ten cases per year.[71] Innate characteristics of the organisms (*e.g.*, transmission from human to human and the public health and sanitation system would similarly limit the spread of organisms used as bioweapons. These barriers can be substantial. During the 1970s, European public health authorities reportedly quarantined tens of thousands and vaccinated hundreds of thousands to prevent smallpox from spreading.[72]

Stability. Natural pathogens[73] and classical weapons agents[74] both tend to become steadily less virulent over time as they adapt to human hosts. While it is reasonable to think that a genetically engineered organism would similarly lose its engineered traits to evolution, the extent and timing of this phenomenon is not known. The fact that bioremediation companies routinely choose naturally-occurring organisms over artificial ones suggests that genetically-engineered stability poses significant challenges.[75] On the other hand, genetically engineered organisms released into nutrient-rich environments (*e.g.* jungle streams) are known to be stable.[76] It is not clear where genetically engineered human pathogens fall along this continuum.

Predictive Power. Current epidemiologic models have limited ability to predict how well new infectious organism would spread if released into a complex biological system. One reason for this is that epidemic dynamics seem to be sensitive to the characteristics of small numbers of infected humans. Furthermore, many of these characteristics are either unknown or poorly understood.[77] The resulting lack of predictive power means that using synthetic biology to create a radically new organism would be an unreliable way to start epidemics. Instead, terrorists would probably find it more efficient to (a) use naturally occurring organisms that are already known to cause epidemics, or (b) modify these wild-type organisms in relatively modest ways (*e.g.* drug resistance) using traditional genetic engineering mechanisms.

We have already remarked how synthetic biology could potentially complicate public health defenses and make genetically modified organisms more stable. **Proposal B.1** addresses experiments of concern which could potentially make it easier to produce drug resistant organisms.

B. Biosafety.

Members interviewed for this project noted that there has never been a documented accident in which a genetically engineered organism escaped from a laboratory and caused harm.[78] The intuition behind this observation is that most genetically engineered organisms (and all synthetic biology experiments to date) can only survive in elaborately

artificial environments.[79] In principle, synthetic biologists should be able to design traits that reduce organisms' survival chances even further. **Proposal D.1** urges funding agencies to invest in these technologies.

At the same time, the fact that current biosafety risks are low does not mean that there is no room for improvement. The recent experiment of Jackson *et al.* [80] in which researchers trying to boost an immune system managed to turn it off instead shows how experiments can lead to unexpected results. Furthermore, even simple accidents – for example, noticing that it is possible to stick a needle through protective gloves – can provide "instructive examples" that make researchers think differently about safety.[81] Both examples suggest that better reporting and sharing of information are a good way to promote biosafety. **Proposal C.2** would create a community-wide clearinghouse for reporting and sharing safety-related information.

C. Policy Implications.

No member interviewed for this project believes that today's synthetic biology significantly changes earlier biosecurity/biosafety risks. The foregoing analysis confirms this intuition. This does not, however, mean that interventions to reduce risks are useless. To the contrary: The fact that biosecurity/biosafety problems are manageable suggests that even modest interventions can make a difference. Furthermore, synthetic biology is changing rapidly. There is no guarantee that a similar analysis five years from now would reach the same conclusion. **Proposals C.1** and **C.2** would provide institutional mechanisms for identifying and if necessary responding to new biosafety/biosecurity developments as they emerge.

IV. Possible Interventions

This section reviews four broad classes of self-regulation that members can adopt to reduce the already small biosafety/biosecurity risks posed by synthetic biology. **Part A** suggests steps that community can take to reduce the chance that commercial synthesis companies will supply dangerous genetic sequences to terrorists outside the community. **Part B** suggests new channels that would allow experimenters to obtain advice about potential "experiments of concern" and report dangerous or unsafe behavior. **Part C** proposes community-wide institutional mechanisms for tracking, publicizing, and if necessary responding to emerging biosecurity/biosafety issues over time. Finally, **Part D** urges funding agencies to fund promising technologies for enhancing biosafety and biosecurity.

A. Supporting and Extending Responsible Industry Screening Practices.

As explained in Section III, the rise of commercial biosynthesis and oligo companies creates a potential new channel for terrorists seeking to obtain feedstocks for wild-type or genetically engineered bioweapons. Current industry screening practices are (a) non-uniform within gene synthesis companies, (b) generate too many false positives for high volume oligo companies to use, (c) do not include large numbers of potentially dangerous sequences, and (d) may not be able to detect dangerous sequences that have been split into multiple orders. We found strong support among industry and academic members for steps to address the first three items. The significance of the last item remains controversial.

A.1 Insist That All Commercial Gene Synthesis Houses Adopt Current Best-Practice Screening Procedures.

Community members overwhelmingly believe that industry should screen orders whenever it is feasible to do so.*

Non-Uniform Screening Practices. In November, 2005 the journal *New Scientist* asked twelve gene synthesis companies whether they routinely screened orders for sequences that terrorists could turn into weapons. Only five said "yes." The remainder answered that they did so "usually" (1 firm), "not routinely" (3 firms) or not at all (3 firms).[82] While it is possible that some of these firms have since adopted screening, at least one large Chinese firm reportedly still does not screen.[83] The continued existence of non-screeners puts responsible companies at a competitive disadvantage and creates economic incentives to cut corners on security.[84]

Intervention. Industry members contacted for this project uniformly agreed that a community-wide pledge not to do business with companies that fail to adopt screening is worth doing and would likely persuade more companies to screen.[85] Apart from its ethical value, there is reason to think that a pledge would effect real world improvements in industry practice:

> ***Feasibility for Industry.*** Firms that adopt screening sacrifice little output or profits *provided that all firms do it.* A pledge would help to enforce this coordination. Current "best practice" screening is also straightforward to implement. The required software can be purchased commercially[86] or else built in-house using only modest expertise.[87] Companies that fail to screen can be readily detected.[88]

*Of the 21 community members consulted on this issue, two thought that the case for screening was unproven.

Feasibility for Community. Synthetic biologists account for no more than a few percent of current commercial gene synthesis purchases. However, this figure could easily reach fifty percent within five years.[89] For this reason, a community-wide pledge is likely to exert useful economic pressure on non-conforming firms. A pledge would also have moral value. Large pharmaceutical and biotechnology companies would feel pressure to follow suit. These entities account for roughly two-thirds of today's market.

Costs. The fact that most gene synthesis companies already screen suggests that costs would not rise for most community members. In a few cases, however, researchers who currently patronize non-screening companies could see costs rise. This could make research harder for underfunded scientists.[90]

A First Step. Persuading industry to adopt current "best practice" screening policies is not a panacea. For reasons discussed in below, current screening practices have significant defects. That said, universal screening would be an improvement. It is also a necessary first step toward any future progress.

Resolved: Gene synthesis companies have an ethical responsibility to screen orders consistent with "best practices" within the industry, including but not limited to the routine use of automated searches (equivalent to current Blackwatch release or higher) and hand examination of all suspect sequences by qualified scientists. Companies that practice such screening should publicly certify the fact by January 1, 2007. Thereafter, community members pledge not to place orders with any company that fails to comply with this resolution.

A.2 Create and Endorse New Watch-Lists to Improve Industry Screening.

In keeping with a recent National Research Council Report[91], members who were asked uniformly agreed[92] that current watch-lists are inadequate. These defects include:

Incompleteness. Current lists focus almost exclusively on select agents and toxins. Many other potentially dangerous sequences are not included.[93]

Overbreadth. Current organism-level lists generate a large number of false positives which must be examined by hand. This makes screening impractical for oligo houses that fill up to one thousand orders per day.[*] The number of false positives will also become a problem for gene synthesis companies as their businesses grow.[94]

[*] Recent experiments in recreating polio and 1918 influenza both used oligos.

Better software and more specific sequence lists can potentially fix these defects. Such tools would (a) make existing gene synthesis screening more accurate and sustainable, and (b) allow oligo companies to start their own screening programs.[95] In an ideal world, government would take the lead in providing such a list. For now, however, no such project exists.

Several members expressed interest in helping to create new software and watch-lists designed to make current screening programs more effective.[96] Ideally, the new tools would be available for members to review and endorse at Synthetic Biology 3.0 The proposed initiative would make current gene synthesis screening more effective and encourage oligo companies to adopt their own screening programs. It would also lay the groundwork for an eventual government-approved list.

Resolved: Better screening software and machine-readable, detailed sequence watch-lists are urgently needed to improve screening. A community-wide initiative is currently underway to create these tools on or before December, 2006. Members will have an opportunity to review and endorse these products when they meet for Synthetic Biology 3.0

A.3 Endorsing Surveillance Across Multiple Orders

In principle, terrorists can evade screening by sending multiple requests for individually innocuous sequences to a single supplier or by placing orders with multiple suppliers simultaneously. They could then assemble the sequences into a dangerous organism.[97] In practice, this strategy is highly non-trivial. Splitting orders so that they successfully evade screening would require a skilled bioinformaticist.[98] Furthermore, assembling the desired genome from multiple orders would be difficult. Current state-of-the-art methods for assembling 5kbp genomes (*e.g.* polio, 1918 influenza) fall well short of 200kbp that characterize most bioweapon pathogens.

In principle, screening could be improved by forwarding orders to a centralized screening facility.[99] However, biotechnology and pharmaceutical companies might stop purchasing services from outside suppliers if this meant compromising the confidentiality of their orders. Instead, they would demand gene synthesis kits that allowed them to perform synthesis in-house. The proliferation of such kits would pose a significant danger in its own right.[100]

A.4 Using Genetic "Signatures" or "Bar Codes" to Detect and/or Identify the Source of Organisms.

Several members suggested that special DNA sequences could be inserted into synthetic DNA for multiple purposes including:

> ***Facilitating Detection.*** Sequences could be optimized so that organisms containing selected bar codes could be readily identified using PCR.[101]
>
> ***Facilitating Deterrence.*** Demonstrating that DNA used to make a bioweapon can be traced to a particular company or transaction could deter some terrorists.
>
> ***Facilitating Authorship and Responsibility.*** Signatures potentially offer a variety of benefits beyond security. These include fostering a feeling of responsibility and authorship,[102] and potentially facilitating the enforcement of intellectual property rights.[103]

Feasibility. Several members interviewed for this project felt that bar codes were technologically feasible today.[104] However most felt that additional technical obstacles had yet to be resolved. These include:

> ***Incremental Value Compared to Natural Markers.*** The fact that existing pathogens already provide forensic clues potentially diminishes the value of bar codes.[105] Experience in the 2001 anthrax attacks – in which identification of the Ames strain did not allow authorities to trace the attacker's sub-culture to a particular individual[106] – suggests considerable room for improvement.
>
> ***Stability and Countermeasures***. Despite preliminary experiments, bar coding technologies have yet to be demonstrated. In particular, it is not clear whether bar codes would be stable against mutation.[107] Many members believe bar codes could be readily detected and removed.[108]
>
> ***Making Science Harder.*** Bar codes would inevitably interfere with experiments involving short DNA sequences.[109] Experience in the explosive "taggants" debate suggests that this will likely be the most durable objection.[*]

[*] The 25 year-old debate over the use of "taggants" in explosives is instructive. On the one hand, there is widespread consensus that such measures can be defeated by sophisticated terrorists, are subject to countermeasures, and are limited to commercially-produced materials. On the other hand, there is near-unanimity that taggants remain useful in "facilitating the investigation of almost all significant criminal bombings in which commercial explosives were used." In essence, the deciding factor is how much taggants interfere with the normal functioning of explosive, fertilizer, and ammunition. *See e.g.* Office of Technology Assessment, *Taggants in Explosives* (1980) at p. 9; US Treasury, Interim Progress Report on Marking, Rendering Inert, and Licensing of Explosive Materials" (1997); and US National Academy of Sciences, "Marking, Rendering Inert, and Licensing of Explosive Materials: Interim Report" (1997).

Three-quarters of the members we interviewed believe that genetic bar codes were either immediately useful or deserved further study.

A.5 Other Proposals: Licensing Biologists

Some members argued that screening should be limited to confirming that orders were being placed by responsible biologists.[110] In fact, commercial companies already do this.[111] Even if it were desirable, it is unclear whether the community could persuade companies to stop current screening practices in favor of examining purchasers' credentials.

B. Developing Norms and Practices for An Emerging Community.

The accompanying paper by Laurie Zoloth argues that individuals have an ethical obligation to (a) obtain advice from independent experts before conducting experiments of concern, or (b) to report dangerous behavior by others.[112] Without such obligations, the pace of experiments is always dictated by the community's most adventurous members so that community opinion become meaningless.[113] This section describes various institutional options for increasing consultation and communication within the community.

B.1 Make Advice About Experiments of Concern Freely Available to Both Members and Non-Members.

Most members who were asked agreed that individuals should seek independent expert advice before conducting "experiments of concern" that could potentially push synthetic biology in directions that made it more useful to terrorists.[114] Many members already have both formal resources (*e.g.* safety committees) and personal contacts who fulfill these functions. However, the Fink[115] and Wellcome Trust[116] Reports both express doubt that these bodies have sufficient biosecurity expertise to screen experiments of concern. For this reason, eight of the fourteen members asked agreed that it would be useful to have a body they could consult.[*] Such a body would also provide essential guidance to non-members (including, potentially, future biohackers) who lack access to normal university and NIH resources.

[*] Most of the other members thought that such a body would be duplicative of formal institutions, although not necessarily harmful.

Design Issues. Despite widespread support, most members emphasized that an ethics advisory committee would have to be very carefully designed. Design issues include:

> ***Defining "Experiments of Concern."*** While there is still no "official" or "consensus" definition embracing all possible "experiments of concern," members agreed that the Fink Report definitions were well known and would provide a useful starting point.[117] Our flexibility principle suggests that the community should to adopt those definitions even if it later needs to amend them.
>
> ***Academic Competition.*** Several members interviewed for this project expressed concern that independent experts could take advantage their position to steal ideas for proposed experiments.[118] These concerns can be mitigated by (a) directing inquiries to identified individuals and (b) documenting all inquiries.
>
> ***Mitigating Bureaucracy.*** Several members noted that informal consultation would be counterproductive if they led to an additional layer of bureaucracy. This danger would, however, be minimized if the consultative body made clear decisions and documented its reasoning.[119]

Building a Model Institution. In the US, many state and county bar associations operate Ethics Hotlines for attorneys who need advice. Callers' identities are invariably kept confidential; additionally, many lines accept anonymous inquiries.[120] Some hotlines also produce and publish short opinions explaining their decisions.[121] This practice reduces arbitrariness, sharpens existing ethics principles, and provides guidance to the broader community. UC Berkeley is in the process of extending this model to synthetic biology. Its Bioethics Advisory Committee ("BEAC") will provide biosafety/biosecurity advice to synthetic biologists at UC Berkeley and Lawrence Berkeley National Laboratory.

The BEAC will respond to inquiries from any experimenter, whether or not s/he is part of the UC system. Institutions at other universities are urged to follow suit.

Resolved: Experimenters considering an "experiment of concern" within the meaning of the Fink Report should obtain expert independent advice before proceeding. The community has an ethical obligation to make such advice freely available, particularly to non-members who lack access to university- or company-funded safety committees.

B.2 Endorse Members' Ethical Obligation to Investigate and Report Dangerous Behavior.

All members interviewed for this project agreed that scientists have an ethical obligation to report dangerous or inappropriate behavior. In some cases, members believe that safety committees and other appropriate official channels already exist to do this.[122]

Resolved: Members have an ethical obligation to investigate and, if necessary, report behavior that they believe poses a significant danger to human life, the environment, and property. Members may satisfy this obligation through existing channels, by calling authorities, or by contacting community bodies established for this purpose.

B.3 Other Proposals for Developing Norms and Practices.

Our interviews disclosed various novel proposals that have not yet been widely discussed within the community. These include:

Buddy System. Laurie Zoloth has suggested a provocative extension of normal academic mentoring in which PhD. advisors hold periodic reunions to track former students and identify troubling behavior. Members were divided over the suggestion.[123]

Include Ethical Statements in Grant Proposals. One member pointed out that a resolution calling on members to include ethical analysis in each grant application would encourage applicants to compete along ethical as well as scientific dimensions.[124]

Education. Two members argued that early education of young scientists and undergraduates about ethics and responsible design practices would improve biosafety/biosecurity.[125] However, education would have only limited value against malicious individuals.[126]

Promote International Cooperation. Members believe that scientists in different countries should pursue closer cooperation on biosecurity matters.[127]

C. Maintain an Ongoing Institutional Commitment to Biosecurity and Biosafety.

This document provides a snapshot of a rapidly changing technology. The picture five years from now could well be different. For this reason, the community needs to monitor and potentially respond to future biosecurity/biosafety threats as they emerge. This will require new institutions to ensure, in the words of a recent National Research Council report, that "regular and deliberate reassessments of advances in science and technology and identification of those advances with the greatest potential for changing the nature of the threat spectrum."[128]

.

C.1 Create A Community-Wide Clearinghouse for Identifying and Tracking Emerging Biosafety and Biosecurity Issues.

Communities in potentially dangerous industries frequently share information about risks to accelerate community learning and reduce the chance of accidents. The practice is particularly well developed in aviation, where the US Federal Aviation Authority's "Aviation Safety Reporting System"[129] receives and compiles data from 30,000 voluntary reports each year. The FAA uses this information to identify emerging safety hazards and issue advisories.

Most members interviewed for this project agreed that an on-line clearinghouse or working group should be established to share information about potential accidents[130] and biosecurity threats.[131] Although members expressed skepticism about how much information such a site would yield, they nevertheless concluded that such a site was a sensible investment given (a) its low expected cost and (b) the chance that it might yield substantially more information than anticipated.

Design Issues. Several members mentioned design issues that should be considered in designing a site:

> ***Breadth.*** One member cautioned that a site could create the false impression that synthetic biology is inherently more dangerous than other forms of genetic engineering or microbiology. This impression could, however, be negated if the site was deliberately broadened to include potential accidents involving genetic engineering or even microbiology as a whole.[132]
>
> ***Attribution, Confidentiality or Anonymity?*** Several members remarked that an anonymous site could become a focal point for naïve, irresponsible or hoax statements that the public would then attribute to the community. This argues

against classic **anonymous** sites like the US Navy's "Anymouse" [133] program.[*] On the other hand, an **attribution** site that publicly disclosed contributors' identities would likely deter participation. **Confidential sites** like the FAA's ASRS System (*supra*) provide a potentially appealing compromise.

Partial Overlap. Some members noted that information reported to the site would potentially duplicate that sent to university safety committees,[134] although a community-wide would presumably help to span institutional barriers. Other members worried that the most useful information was already published in academic journals.[135] The extent to which the site would develop additional but still useful information is an empirical question.

Web Page and Working Group. The Synthetic Biology conference series provides a natural focal point for reporting, analyzing, and sharing new biosafety/biosecurity developments. Members operating the site could deliver talks updating the community at each successive Synthetic Biology conference.

Resolved: Members have an ethical obligation to share facts, experiences, and conjectures that increase community awareness of, and ability to manage, biosafety and biosecurity risks. Community members are encouraged to establish confidential clearinghouses to collect, analyze, and disseminate this information.

C.2 Other Ideas for Developing Community Norms and Practices.

Various other proposals made during the course of our interviews remain preliminary. These include:

Conferences. Members approve of recent biosecurity workshops and want to see them continue.[136] Greater information sharing between universities is also desirable.[137]

Developing Formal Standards for Synthetic Biology. Some members think that existing recombinant DNA rules adequately cover synthetic biology.[138] However, a few members wondered whether it would be better to develop standards specific to synthetic biology.[139]

Codes of Ethics. Two recent National Research Council reports have recommended that biological research communities develop and adopt codes of ethics.[140]

[*] Community opinion appears evenly divided. Five of the nine members asked about anonymity thought that an anonymous site was appropriate.

NIH Advisory Body. One member suggested that the community could form an advisory group to help NIH review proposals to create novel organisms and recommend additional safeguards as necessary.[141]

Professional Society. In the near term, institutions like the proposed biosafety/biosecurity reporting site can be housed within the Synthetic Biology conference series. In the longer term, the community may want to start a professional society. A professional society would, *inter alia*, help members exercise greater self-governance on a variety of issues relating from security to intellectual property rights and communicating with the public.

International Law. The community could affirm that organisms created using synthetic biology methods are subject to the 1972 Biological and Toxin Weapons Convention.[142]

D. Invest in New, Safety- and Security-Enhancing Technologies.

Technology promises to make biosecurity/biosafety more effective. Members are uniquely qualified to prioritize technologies and should consider recommending promising ideas to funding agencies.

D.1 Endorse Biosecurity/Biosafety R&D Priorities.

Members interviewed for this project responded favorably to two possibilities, bar codes and inherently safe organisms. Other promising categories may emerge during Town Halls and Synthetic Biology 2.0.

Bar Codes. As previously explained, bar codes provide a promising technology for detecting organisms and deterring improper use of commercial gene sequences.

Resolved: Funding agencies should invest in research to explore the use of "bar code" technology to detect and trace the origins of genetically modified organisms.

Inherently Safe Organisms. In principle, current biosafety risks can be reduced still further by performing experiments in host organisms that have been deliberately engineered to minimize the chances for survival and propagation outside the laboratory. However, current examples of such technologies – *e.g.* using organisms that are oxotrophic or depend on materials like tetracycline not found in the wild – are not robust and offer only limited protection.[143]

Members generally agreed that research into these techniques should be actively funded and pursued.[144] Promising research directions include, but should not be limited to, organisms that can readily be killed using simple chemicals (*e.g.* salt or common antibiotics), organisms that depend on specialized nutrients or environments not found in Nature, co-dependent organisms that would likely become separated from one another in the wild, and organisms that cannot reproduce more than a pre-set number of times. Properly designed organisms should also be stable against evolutionary pressures that might otherwise delete these engineered safety features.[145] Some members expressed doubt that the community well ever produce a completely safe organism. That said, even an imperfect technology could be worthwhile if it improved safety.[146]

Inherently safe organisms are not a panacea. For example, inherently safe organisms cannot be used for projects in which organisms are released into the wild or for research agendas that do not follow a parts-and-chassis view of synthetic biology.[147] Nevertheless, it might make sense for a future conference to call on members to adopt such technologies whenever feasible.

Resolved: Funding agencies should invest in research to engineer host organisms for synthetic biology experiments that have little or no chance of surviving, propagating, or interacting with organisms outside the laboratory.

D.3 Other Ideas.

Various other proposals made during the course of our interviews remain preliminary. These include:

> ***Prize Incentives.*** Instead of endorsing existing technology ideas, the community could potentially call on agencies to offer prizes for new applications.[148] Since the grant system is already designed to elicit new ideas, the gains from such a strategy would likely be limited.[149] A prize system might nevertheless offer potential advantages to the extent that (a) it offered larger reward than researchers could normally expect from grants, or (b) it extended beyond the relatively small group of researchers who normally compete for synthethic biology support. Everything else being equal, the utility of prizes would depend on how many ideas are likely to be generated from students and other groups outside the normal grant system.
>
> ***US Biodefense Policy Review.*** Synthetic biologists could potentially organize a blue ribbon committee to review current US biodefense priorities.[150]

IV. Conclusion

Six years of almost continuous discussion have given synthetic biologists a solid understanding of biosafety/biosecurity risks and the available possible policy instruments for reducing them. The challenge now is implementation. This document has presented a menu of choices that could potentially improve security at modest cost. Synthetic Biology 2.0 offers a chance to turn this understanding into action.

---

[1] For a brief history of self-regulation following Asilomar, *see* National Research Council, *Biotechnology Research in an Age of Terrorism: Confronting the Dual Use Dilemma*," (2004), hereinafter "Fink Report" at viii.
[2] *Id.* at viii.
[3] See, e.g., Royal Society and Wellcome Trust: "Do No Harm: Reducing the Potential for the Misuse of Life Science Research (2004) at p. 1 ("Self governance by the scientific community was favoured, rather than new legislation"). The report is available at http://www.royalsoc.ac.uk/displaypagedoc.asp?id=10360.
[4] Anon., "First Annual Meeting on Synthetic Biology," (2004), available at http://openwetware.org/images/7/79/SB1.0_overview.pdf.
[5] Robert Carlson, "Synthetic Biology 1.0," *Future Brief* (2005), available at http://www.futurebrief.com/robertcarlsonbio001.asp. Discussions covered various proposals including licensing scientists, strict controls on the distribution of technology and reagents, and using artists' signatures to trace the source of altered DNA. Much of this debate was, however, controversial and the discussion inconclusive. *Id.*
[6] MIT News Office, "Study to Explore Risks, Benefits of Synthetic Genomics" (2005) (announcing 15 month, $570,000 grant).
[7] Conversation with Mike Stebbins (March 10, 2006).
[8] National Research Council, Globalization, Biosecurity, and the Future of the Life Sciences (2006) at p. viii.
[9] George Church, "Let Us Go Forth and Safely Multiply," *Nature* 438:423 (2005): "A code of ethics and standards should emerge for biological engineering as it has done for other engineering disciplines. The community recognizes this need, but discussions are fragmentary. The next international meeting on synthetic biology (in May 2006 at the University of California, Berkeley) should make significant progress in that direction."
[10]For updated agenda information, *see* Synthetic Biology 2.0," available at http://pbd.lbl.gov/sbconf/.
[11] Conversations with Drew Endy (March 21) and George Church (March 9, 2006).
[12] George Church, "Let Us Go Forth and Safely Multiply," *Nature* 438:423 (2005): "A code of ethics and standards should emerge for biological engineering as it has done for other engineering disciplines. The community recognizes this need, but discussions are fragmentary. The next international meeting on synthetic biology (in May 2006 at the University of California, Berkeley) should make significant progress in that direction."
[13]The ability to order ready-made genes and oligos drastically cuts the cost of traditional genetic engineering projects by freeing skilled laboratory workers for other tasks. This means that more experiments can be done within existing budgets, allowing companies to bring more – and more ambitious – products to market. Adam Arkin (personal communication). The resulting efficiency gains are presumably proportional to the current value of biotechnology products, which is believed to exceed $50 billion. Biotechnology Industry Association, *Bio 2005-2006 Guide to Biotechnology*, available at http://www.bio.org/speeches/pubs/er/BiotechGuide.pdf#search='bio%202005%20%202006%20guide'
[14] For detailed list of possible benefits, *see* Ray Gesteland, Synthetic Genomes: Policies and Impacts (2004) and NRC, "Globalization, Biosecurity, and the Future of the Life Sciences,"(2006) at pp. 121-123.
[15] *See, e.g.*, Bernadette Tansey, "Science Tweaks Nature's Toolbox," *San Francisco Chronicle* (August 20 2005)available at http://www.sfgate.com/cgi-bin/article.cgi?f=/c/a/2005/08/20/BUG4LEAGRT1.DTL&sn=001&sc=1000.
[16] D. Endy, "Foundations for Engineering Biology," *Nature* **438:**449 (2005)

[17] Conversation with John Mulligan (Feb. 24, 2006).
[18] Today's biosafety debate has changed relatively little since the Asilomar conference of 1975.*See generally,* Philip McClean, Historical Events in the rDNA Debate, http://www.ndsu.nodak.edu/instruct/mcclean/plsc431/debate/debate3.htm Similarly, modern biosecurity policy reflects a range of technologies developed between World War II *See, e.g,* Jeanne Guillemin, *Biological Weapons: From the Invetion of State-Sponsored Programs to Contemporary Bioterrorism* (Columbia Univ. Press: 2005) and Robert Harris and Jeremy Paxman, *A Higher Form of Killing: The Secret History of Chemical and Biological Warfare* (Random House: 2002). Genetically engineered bioweapons were massively explored by Soviet workers during the 1980s. *See e.g.*, Guillemin, *supra*, and Ken Alibeck,and Stephen Handelman *Biohazard* (Random House: 1999).
[19] *See generally,* S. Maurer, "What's So Hard About Terrorism?" available at http://www.cs.washington.edu/education/courses/csep590/05au/lectures/slides/Maurer_Sept7.ppt#56; *Accord,* National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences*, (2006) at pp. 190, 196 (greater biosecurity awareness among scientists would "change the risk calculus of potential offenders").
[20] *Accord,* National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences* (2006) at p. viii (scientists "need to survey the threat horizon continually").
[21] M. Dando, *The New Biological Weapons: Threat, Proliferation and Control* London: 2001 at p. 1. The psychological effect of bioweapons is probably overstated in any case. Social psychology research shows that human beings place relatively little weight on how (as opposed to when) they die. Furthermore, people tend to fear rare, unfamiliar risks more than familiar ones. *See generally,* Paul Slovic, *The Perception of Risk* (EarthScan: 2000). Small scale bioweapons may be more frightening *before* they are used.
[22] Phil Hirschkorn, "New York reduces 9/11 death toll by 40," CNN October 29, 2003, available at http://www.cnn.com/2003/US/Northeast/10/29/wtc.deaths/
[23] Government cost-benefit calculations typically value lives saved at $1-6 million each. Cass Sunstein, "Valuing Life, A Plea for Disaggregation," 54 Duke L.J. 385 (2005). Casualty rates would have to be in the thousands to erase the benefits that conservatively run into the tens of billions.
[24] For a survey, *see* W. Seth Carus, "Unlawful Acquisition and Use of Biological Agents," in Joshua Lederberg (ed.), *Biological Weapons: Limiting the Threat* (MIT Press: 2000); *see also*, Jeffrey Bale and Gary Ackerman, "How Real is the 'WMD Terrorism' Threat?" available at http://www.cs.washington.edu/education/courses/csep590/05au/lectures/.
[25] The Japanese program employed roughly 6,000 workers. Harris and Paxman, *Higher Form of Killing* at p. 80. The much larger US program employed "several hundred" scientists and research staff and invested billions of dollars from World War II through the late 1960s. J. Guillemin, *Biological Weapons*, Columbia Univ. Press 2005 at p. 109 (spending in the early 1960s totaled $300 million per year).
[26] The Soviet program reportedly employed a staggering 32,000 scientists and staff working at forty separate facilities with a budget of "several hundred million" per year. Roughly half of the employees worked to develop diseases; the other half made cures. B. Preston, "The Bioweaponeers," *The New Yorker* (March 9, 1998) at p. 53 (half of the employees made diseases, the other half medicines); Tom Mangold and Jeff Goldberg, *Plague Wars: The Terrifying Reality of Biological Warfare* (1999) at p. 93.
[27] The South African program employed fewer than 10 scientists. Milton Leitenberg (personal communication). *See also*, C. Gould and P. Folb, *Project Coast: Apartheid's Chemical and Biological Warfare Programme* (UNIDIR & Center for Conflict Resolution: 2002).
[28]Camp Detrick's approximately 1800 scientists and staff principally worked on identifying suitable "weaponizable" disease agents from 1945 to 1950. Mangold and Goldberg, *Plague Wars, supra,* at p. 32, 34. Japanese program used used hundreds of animals and, eventually, more than 10,000 human subjects. Harris and Paxman, *A Higher Form of Killing* at pp. 80-81.
[29] M. Dando, *The New Biological Weapons, supra* at p. 11
[30] Isolating in wild would be difficult. There are more than seventy different strains of *bacillus anthracis*, but only a small minority are highly virulent. J. Tucker, *Biosecurity: Limiting Terrorist Access to Deadly Pathogens* (United States Institute of Peace, 2004) at p. 15, available at http://www.usip.org/pubs/peaceworks/pwks52.pdf.

[31] Individual laboratories house large collections leftover from research on outbreaks and epizootics. The FBI has estimated that there may be as many as 22,000 such laboratories in the US alone. .J. Guillemin, *Biological Weapons*, Columbia Univ. Press 2005.
[32] J. Tucker, *Biosecurity: Limiting Terrorist Access to Deadly Pathogens* (United States Institute of Peace, 2004) at p. 13, available at http://www.usip.org/pubs/peaceworks/pwks52.pdf; *Fink Report*, *supra* n. 1, at pp. 2 (US oversight "will ultimately afford little protection if it is not adopted internationally") and 86 ("Only an international set of standards will help to minimize the misuse of biotechnology."); National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences* (2006) at p. viii ("Science does not stop at our borders"); Falkenrath *et al.*, *America's Achilles' Heel*, *supra*, at 115-16 (describing controls implemented in the 1990s and noting how terrorists could evade them by using front companies or placing orders overseas).
[33] J. Cello, A. Paul, and E. Wimmer (2002) Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science* **297**: 1016–1018. Although important as a demonstration, the experiment was "a very laborious and difficult way to accomplish this synthesis." Fink Report, *supra*, n. 1 at p. 22.
[34] *Science* (T. M. Tumpey *et al*. **310,** 77–80; 2005).
[35]British Joint Staff estimated that that an anthrax attack would require 636 four-pound bomblets per square mile in theory and 8500 bomblets in practice. Mangold and Goldberg, *Plague Wars, supra,* at p. 33; Harris and Paxman, *A Higher Form of Killing, supra,* at pp. 102, 106. Assuming that each bomblet contained ¾ wet agent by weight, the required quantity of agent runs to 12.5 tons or about 4.8 tons per kilometer. Attacks using aerosols could be somewhat more efficient. Nevertheless, the fact that postwar exercises invariably used warships suggests that tons of material were used. Mangold and Goldberg, *Plague Wars, supra,* at p. 35, 37.
[36]Sprayers reportedly have a 26% efficiency compared to 15 percent for bomblets. M. Dando, *The New Biological Weapons: Threat, Proliferation, and Control*. Lynne Rienner: London (2001). Postwar estimates reportedly found that required bombloads could be reduced by an order of magnitude if more contagious diseases were substituted for anthrax. Harris and Paxman, *A Higher Form of Killing, supra* at p. 135.
[37] Population density for large US cities typically runs from 5,800 people per km^2 to a high of 26,400 in midtown Manhattan. S. Glasstone and P.J. Dolan, *The Effects of Nuclear Weapons* (US Government Printing Office 1977) at p. 544. The authors remark that densities for midtown Manhattan would be "much higher" during the workday.
[38] Mangold and Goldberg, *Plague Wars, supra,* at pp. 18-19, 2. The Unit could reportedly produce 660 pounds of plague, 1400 pounds of anthrax, 2000 pounds of typhoid, or 2200 pounds of cholera as required. Harris and Paxman, *A Higher Form of Killing, supra* at p. 78. The facility used 900 tanks, each of which could produce 40 grams of bacteria every few days. Russian investigators estimated that it could produce eight tons of bacteria per month. *Id.* A US neo-fascist group reportedly managed to manufacture 30-40 kg. of typhoid culture during the 1970s. Falkenrath *et al., America's Achilles' Heel, supra,* at 38.
[39] Falkenrath *et al., America's Achilles' Heel, supra,* at pp. 117 - 118. A modern 500 liter fermenter can reportedly produce about 100 kg. of biomass in 72 hours. Ray Zilinskas (personal communication).
[40] The Soviet accident occurred in Sverdlovsk when a 24-inch filter exploded releasing an estimated 1 to 3.5 kilograms of anthrax from a rooftop site. Casualty estimates range from 64 to 600 people. Vaccine was subsequently administered to tens of thousands of residents. Mangold and Goldberg, *Plague Wars, supra,* at pp. 69, 404, 406.
[41] In 1968 the US program reportedly conducted tests demonstrating that a single aircraft dispersing three grams of powder per meter could deliver an LD-50 dose over a 30 km depth. Mangold and Goldberg, *Plague Wars, supra,* at p. 39; J. Guillemin, *Biological Weapons*, Columbia Univ. Press 2005 at p. 111 (test delivered dose over nearly 1,000 square miles); B. Preston, "The Bioweaponeers," *supra* at p. 60; Department of Defense, "Fact Sheet: Project Shipboard Hazard and Defense (SHAD) DTC Test 68-50 (reporting use of staphylococcal enterotoxin over "a 40 to 50 kilometer downwind grid."), available at http://www1.va.gov/shad/docs/DTC_Test_68-50_SHAD_DoD_Fact_Sheet.pdf. Assuming Manhattan-type population densities, the estimate naively suggests that 100 grams of material is sufficient to deliver an LD-50 dose to 5,000 people. This simple calculation assumes an unreasonable geometry in which peak

population density occurred along the full length of a 30 kilometer corridor. It also neglects the fact that much of the population would be sheltered indoors.

[42] Paul DeArmond, "The Anthrax Letters: Five Deaths-Five Grams-Five Clues," *Albion Monitor* (Sebastapol CA.) August 16 2002 available at www.monitor.net/monitor/0208a/anthrax.html.

[43]A three year Iraqi program failed to achieve pilot scale production of *Bacillus thuringiensis* in the late 1990s, although the country later went on to produce 40 tons per year. Iraq Survey Group Final Report: Evolution of the Biological Warfare Program, available at http://www.globalsecurity.org/wmd/library/report/2004/isg-final-report/isg-final-report_vol3_bw-01.htm.

[44] M. Dando, *The New Biological Weapons, supra* at p. 36.

[45] *Id.*at p. 37.

[46] Mangold and Goldberg, *Plague Wars, supra,* at p. 77, 79 (reporting approximately 20 injuries per year from 3000 member workforce).

[47] The most recent example involves the disruption of Al Qaeda's so-called Bojinka conspiracy in 1995 after a fire broke out in a terrorist bomb factory. CNFF, Terrorism Trial Begins in New York – 3 Men Accused of Plotting to Bomb US Planes," (May 13, 1996), available at http://www.cnn.com/US/9605/12/terror.plot/. For earlier examples, *see* S. Maurer, "What's So Hard About Terrorism?" available at http://www.cs.washington.edu/education/courses/csep590/05au/lectures/slides/Maurer_Sept7.ppt#56.

[48] Richard Falkenrath, Robert Newman & Bradley Thayer, America's Achilles' Heel: Nuclear, Biological, and Chemical Terrorism and Covert Attack (MIT Press 1998) at p. 20.

[49] Steven M. Block, "Living Nightmares: Biological Threats Enabled by Molecular Biology" in S. Drell, A. Sofaer & G. Wilson (eds.), *The New Terror: Facing the Threat of Biological and Chemical Weapons* (Stanford: 1999) at p. 47 (citing 1997 JASONs study: "Clearly, some elements of this 'wish list' seem rather far away from current state of the art."). The fact that nation states have yet to develop such weapons suggest that terrorist groups would have a hard time producing them.

[50] Iraq Survey Group Final Report: Evolution of the Biological Warfare Program, available at http://www.globalsecurity.org/wmd/library/report/2004/isg-final-report/isg-final-report_vol3_bw-01.htm.; Mangold and Goldberg, *Plague Wars, supra,* at p. 79 (wet agents based on cholera and dysentery can be stored for weeks; anthrax lasts several months).

[51] B. Preston, "The Bioweaponeers," *supra,* at p. 60 (tularemia lasts "only a few minutes" in sunlight); M. Dando, *The New Biological Weapons, supra* at p. (botulinin toxin degrades in sunlight at a rate of 7.8% per minute).

[52] J. Bale and G. Ackerman, "Recommendations on the Development and Methodologies and Attributes for Assessing Terrorist Threats of WMD Terrorism" (CNS 2005).

[53] *Id.*

[54] Mangold and Goldberg, *Plague Wars, supra,* at p. 33, 37.

[55] J. Bale and G. Ackerman, Recommendations on the Development and Methodologies and Attributes for Assessing Terrorist Threats of WMD Terrorism (CNS 2005). The effects of humidity are presumably related to how fast aerosol droplets evaporate.

[56] Mangold and Goldberg, *Plague Wars, supra,* at p. 34, 38.

[57] M. Dando, *The New Biological Weapons, supra* at p. 11, 41 (genetic engineering to effect enhanced aerosol and environmental stability)..

[58] *Id.* at p. 35. This conclusion is reinforced by experience in bioremediation, in which companies have almost always tried to locate suitable naturally occurring organisms rather than genetically engineer new ones. Conversation with T. Hazen (Feb. 21, 2006).

[59] M. Dando, *The New Biological Weapons, supra* at p. 41.

[60] Antibiotic resistance is largely irrelevant for very large WMD attacks, which would require the overnight delivery of impossible quantities of drugs. B. Preston, "The Bioweaponeers," *supra* at p. 60.

[61] M. Dando, *The New Biological Weapons, supra* at p. 41.

[62] Steven M. Block, "Living Nightmares: Biological Threats Enabled by Molecular Biology" in S. Drell, A. Sofaer & G. Wilson (eds.), *The New Terror: Facing the Threat of Biological and Chemical Weapons* (Stanford: 1999) at p. 47 (describing 1997 JASONs study: "Clearly, some elements of this 'wish list' seem rather far away from current state of the art").

[63] Mangold and Goldberg, *Plague Wars*, *supra*, at p. 180 (Soviet program used genetic engineering to create antibiotic resistance in the 1980s); B. Preston, "The Bioweaponeers," *supra* at p. [60-63] (describing how Oblolensk group published a paper on genetically engineered vaccine-resistant anthrax strains in the British journal *Vaccine*.)
[64] B. Preston, "The Bioweaponeers," *supra* at p. 52.
[65] Falkenrath *et al.*, *America's Achilles' Heel*, *supra*, at pp. 99, 113.
[66] Falkenrath *et al.*, *America's Achilles' Heel*, *supra*, at p. 122.
[67] Steven M. Block, "Living Nightmares: Bioilogical Threats Enabled by Molecular Biology" in S. Drell, A. Sofaer & G. Wilson (eds.), *The New Terror: Facing the Threat of Biological and Chemical Weapons* (Stanford: 1999) at p. 47 ("Clearly, some elements of this 'wish list' seem rather far away from current state of the art").
[68] B. Preston, "The Bioweaponeers," *supra* at p. 52 ("[H]e told me the formula for the Alibekov anthrax. He uttered just one sentence. The Alibekov formula is simple and the formula is somewhat surprising, not quite what you'd expect. Two unrelated materials are mixed with pure powdered anthrax spores. It took a lot of research and testing to get the trick right and Alibek must have driven his research group hard and skillfully to arrive at it. 'There are many countries that would like to know how to do this, he said."
[69] For the ideal size of bioweapons particles, *see* B. Preston, *supra* at p. 59. Viruses are less likely to be damaged during biopowder production because of their small size. Bacteria are not much smaller (and are usually larger) than the than the 1 to 5 micron diameter needed to optimize inhalation. *See* http://www.nanomedicine.com/NMI/10.4.2.5.htm.
[70]B. Preston, "The Bioweaponeers," *supra*, at p. 52.
[71] *See e.g.*, Centers for Disease Control, "Summary of Notifiable Diseases, United States, 1995" *Morbidity and Mortality Weekly Report*, available at H:\5. Teaching\Intro to Homeland Security\3. WMD\Notifiable Disease Stats, United States, 1995.htm; Centers for Disease Control, "Human Plague – United States 1993 – 94," *Morbidity and Mortality Weekly Report*, available at http://www.cdc.gov/mmwr/preview/mmwrhtml/00026077.htm; Anon., Plague Cases in the USA 1944 – 1993, available at http://www.imsa.edu/programs/pbln/problems/bernie/bubonicplagueusa.html.
[72] "In 1970, when a man infected with smallpox appeared in an emergency room in Germany, seventeen cases of smallpox appeared in the hospital on the floors above. Ultimately the German government vaccinated a hundred thousand people to stop the outbreak. Two years later in Yugoslavia, a man with a severe case of smallpox visited several hospitals before dying in an intensive care unit. To stop the resulting outbreak, which forced twenty thousand people into isolation, Yugoslav health authorities had to vaccinate virtually the entire population of the country within three weeks." B. Preston, "The Bioweaponeers," *The New Yorker* (March 9, 1998) at p.63.
[73] John M. Barry, *The Great Influenza: The Epic Story of the Greatest Plague in History* (Penguin 2005) at pp. 371-73 (mutations made 1918 influenza steadily less lethal as it traveled from the American East to West Coasts and then on to the Midwest.)
[74] M. Dando, The New Biological Weapons: Threat, Proliferation, and Control. Lynne Rienner: London (2001) at p. 41.
[75] Conversations with Terry Hazen (Feb. 21, 2006) and Adam Arkin (March 14 2006).
[76] Conversation with Terry Hazen (Feb. 21, 2006).
[77] J.O. Lloyd-Smith, S.J. Schreiber, P.E. Kopp & W.M. Getz, "Superspreading and the effect of individual variation non disease emergence. *Nature* 438:355 (17 Nov. 2005) (noting that skewed infectivity among human hosts makes outbreaks rarer but also more explosive and noting that "other population processes dependent on small numbers of individuals may yield similar insights."); *see also*, Alison P. Galvani and Robert M. May, "Dimensions of superspreading," *Nature* 438:293 (17 Nov. 2005) (explaining why distribution of "superspreaders" within population affects "both the probability that an epidemic will take off and the subsequent course of the epidemic").
[78] *See also, Fink Report*, *supra* n. 1 at p. 23 ("There have been no reported cases of disease caused by recombinant microorganisms despite the widespread use of gene splicing techniques in academic laboratories and in the production of pharmaceuticals.")
[79] Conversation with Amy Shutkin (Jan. 31 2006).

[80] Ronald Jackson, "Expression of Mouse Interleukin-4 by a Recombinant Ectromelia Virus suppresses Cytolytic Lymphocyte Responses and Overcomes Genetic Resistance to Mousepox, *Journal of Virology* 75:1205-10 (2001).
[81] Conversation with George Church (Mar. 9 2006).
[82] *Id.*
[83] Conversation with Marcus Graf (March 14, 2006).
[84] *Id.*
[85] Conversations with John Mulligan (Feb. 24, 2006), Claes Gustaffson (March 6, 2006), Marcus Graf (March 14, 2006), and Marcus Fischer (March 8, 2006).
[86] *See* Craic Computing LLC, "Products," available at http://www.craic.com/products.html
[87] Interviews with Claes Gustafsson (March 6, 2006) and John Mulligan (Feb. 24, 2006).
[88] Screening practices can be enforced by using a "testing group" to submit sample orders for controlled oligos and reagents. George M. Church, "A Synthetic Biohazard Non-Proliferation Proposal," (2004). Industry routinely uses similar methods to gather commercial intelligence. Conversation with Marcus Graf (March 14, 2006).
[89] Conversation with John Mulligan (Feb. 24, 2006).
[90] Conversation with Rob Carlson (Feb. 11, 2006).
[91] National Research Council, *Globalization, Biosecurity, and the Future of the Life Scienes*, (2005) at pp. 6 (Recommendation 2: "The Committee recommends adopting a broader perspective on the 'threat spectrum.'"); 175 (recommending that scientists "[r]ecognize the limitations inherent in any agent-specific threat list and consider instead the intrinsic properties of pathogens and toxins that render them a threat and how such properties have been or could be manipulated by evolving technologies") and 177 (scientists should "[a]dopt a broadened awareness of threats beyond the classical 'select agents' and other pathogenic organisms").
[92] Conversations with George Church (March 9, 2006), Mike Stebbins (March 10, 2006), John Mulligan (Feb. 24, 2006), Claes Gustaffson (March 6, 2006), Marcus Graf (March 14, 2006), and Marcus Fischer (March 8, 2006).
[93] *See e.g.*, National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences*, at p. 177 (select agent lists should be extended to include "biologically active molecules, synthetic molecules or life forms").
[94] Conversation with Marcus Graf (March 14, 2006).
[95] Conversations with George Church (March 9, 2006), Mike Stebbins (March 10, 2006), John Mulligan (Feb. 24, 2006), Claes Gustaffson (March 6, 2006), Marcus Graf (March 14, 2006), and Marcus Fischer (March 8, 2006).
[96] Conversations with George Church (March 9, 2006), Mike Stebbins (March 10, 2006), Marcus Graf (March 14, 2006), John Mulligan (Feb. 24, 2006), and Claes Gustafsson (March 6, 2006).
[97] Conversations with. Holman (Feb. 23, 2006), Ron Weiss (Feb. 14, 2006), and Claes Gustafsson (March 6, 2006).
[98] Conversation with Marcus Fischer (March 8, 2006).
[99] Conversation with Chris Voight (March 6, 2006); George M. Church, "A Synthetic Biohazard Non-Proliferation Proposal" ((2004) at p. 1.
[100] Conversation with John Mulligan (Feb. 24, 2006).
[101] Conversation with Drew Endy (March 21 2006) (Sloan group ideas).
[102] Conversations with Tom Knight (March 2, 2006) and Sven Panke (Feb. 6, 2006).
[103] Conversation with Victor De Lorenzo (Feb. 27, 2006).
[104] Conversations with Adam Arkin (Feb. 23), Andrew Ellington (Feb. 14), and Victor de Lorenzo (Feb. 27).
[105] Conversation with David Schaffer (Feb. 9, 2006); Andrew D. Ellington, "Intelligence Countermeasures for Biological Threats (n.d.) at p. 10 (describing use of natural genetic variablility to trace the origin of agents.)
[106] According to Andrew Ellington, "The potential utility of organismal sequence taggants was recently demonstrated by the apparent difficulties in determining the provenance of the B anthracis strains used in the bioterrorism attacks on Senators and others. While the strain was eventually traced back to the dead

Texas cow from which it had originally been obtained, the problem was that this particular strain had apparently been transferred from USAMRIID to a number of military and other contractors and collaborators. While it is possible that some genetic variance may eventually be found to be associated with each of these transfers, attempting to delineate the different strains based upon random mutations that may (or may not) have arisen will be extremely difficult. This situation can be contrasted with a scenario in which each organism, upon transfer, was embedded with some manner of sequence taggant. IN this instance, the 'bar code' that identified the sender and receiver would accompany the organism throughout its history, and would make attribution of the organism trivial." Andrew D. Ellington, "Intelligence Countermeasures for Biological Threats (n.d.) at p. 13; *See also*, Paul DeArmond, "The Anthrax Letters: Five Deaths-Five Grams-Five Clues," *Albion Monitor* (Sebastapol CA.) August 16 2002 available at www.monitor.net/monitor/0208a/anthrax.html.

[107] Conversations with Adam Arkin (Feb. 23, 2006) and Dan Fletcher (Feb. 3 1006).

[108] *See, e.g.*, Robert Carlson, "Synthetic Biology 1.0," *Future Brief* (2005), available at http://www.futurebrief.com/robertcarlsonbio001.asp. and conversations with Adam Arkin (Bef. 23, 2006), Tom Knight (March 2 2006) and Jörg Stelling (Feb. 21, 2006).

[109] Conversation with Andrew Ellington (Feb. 14, 2006).

[110] George Church, "A Synthetic Biohazard Non-Proliferation Proposal (2004); *see also*, conversations with Adam Arkin (Feb. 23, 2006) (screening for approved grantees) and Sven Panke (Feb. 6 2006) (screening for accredited purchasers)

[111] Conversations with John Mulligan (Feb. 24, 2006), Marcus Fischer (March 8, 2006) and Marcus Graf (March 14, 2006).

[112] *See also*, National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences* (2006), p. 9 (suggesting that scientists "report[] … activity to national authorities when it appears potentially malevolent in intent").

[113] The argument is reminiscent of Kurt Vonnegut's Tralfalmadorians, who knew that the universe would end when one of their test pilots experimented with the wrong fuel. Kurt Vonnegut, *Slaughterhouse Five* (Dell: 1969).

[114] The "experiments of concern" framework was originally developed by the Fink Report. For a full discussion of the categories *see*, *Fink Report*, *supra* n. 1 at pp. 88 – 90. *See also*, National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences* (2006), p. 54 (describing possible experiments of concern).

[115] According to the Fink Report, "[M]embers of the [Institutional Biosafety Committees] will require substantial education in the potential risks associated with advanced biotechnology research in order to [evaluate experiments of concern] competently. Many IBCs may need to add expertise in immunology, virology, pathology, and epidemiology to undertake this new responsibility." Fink Report, *supra.* at 90. The report also noted that Institutional Biosafety Committees do not currently have formal jurisdiction over "experiments of concern." *Id.* at 91. The Report also recommends that the entire "Institutional Biological Safety committee/Recombinant DNA Advisory Committee process [be] augmented to include the assessment of the potential for misuse as a criterion for approval or denial of proposed experiments." *Id.* pp. 86-87.

[116] "It was agreed that existing regulatory processes did not assess whether experiments should be undertaken. Many of the current systems, such as those for dangerous pathogens, genetically modified organisms and animal experiments, address whether an experiment can be conducted safely, rather than whether the experiment should be conducted at all based on a consideration of the potential misuse of the research. For example, an experiment to reconstruct the 1918 influenza virus would be permitted providing it was conducted in a category 4 laboratory to ensure the required level of containment. However, there would not be any discussion of whether it would be wise to undertake the experiment." Royal Society and Wellcome Trust: "Do No Harm," *supra* n. 3 at p. 4.

[117] Conversation with Roger Brent (Nov. 16, 2005).

[118] Conversations with Luis Serrano (Feb. 16, 2006) and Ron Weiss (Feb. 14, 2006).

[119] Conversation with George Church (March 9, 2006).

[120] *See, e.g.*, The California Bar Association Ethics Hotline homepage, available at http://www.calbar.ca.gov/state/calbar/calbar_generic.jsp?cid=10131&id=1118.

[121] *See, e.g.*, The Los Angeles Country Bar hotline opinions posted at http://www.lacba.org/showpage.cfm?pageid=427.
[122] Conversations with Rob Carlson (Feb. 11, 2006), Andrew Ellington (Feb. 14, 2006), John Mulligan (Feb. 24, 2006), Sven Panke (Feb. 5, 2006), Kris Prather (Feb. 14, 2006), David Schaffer (Feb. 9, 2006), Pamela Silver (Jan. 28 2006) and Chris Voigt (March 6, 2006).
[123] Conversations with George Church (March 9, 2006) and Drew Endy (March 21 2006).
[124] Conversation with Tim Han (Jan. 31, 2006)
[125] Conversation with Christina Smolke (Feb. 2, 2006); *see also* Fink Report, *supra* n. 1 at p. 87 (recommending that professional societies update ethical codes to include biosecurity); National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences* (2006) at pp. 8 (recommending ethical codes as mechanism to "facilitate the recognition of potentially malevolent behavior (i.e., experiments aimed at purposefully developing potential weapons of biological origin) or potentially inappropriate experiments that might unwittingly promote the creation of a more dangerous infectious agent."), 188 (recommending explicit national and international codes of conduct and ethics for life scientists"), 190 ("Nor will codes of ethics likely deter anyone who is firmly committed to applying biotechnology for malevolent purposes, such as … a dedicated member of a terrorist group.") and 198 ("In considering such codes, the Committee concluded that their primary effect would be to create an enabling environment that would facilitate the recognition of potentially malevolent behavior (i.e., experiments aimed at purposefully developing potential weapons of biological origin), or potentially inappropriate experiments that might unwittingly promote the creation of a more dangerous infectious agent.").
[126] Wellcome Trust, Do No Harm," *supra* n. 3, at p 1 ("Some skepticism was expressed about the value of codes of conduct").
[127] Conversation with Drew Endy (March 21 2006) (Sloan group ideas); *see also, Fink Report, supra* n.1 at p. 10 (proposing international forum on biosecurity).
[128] National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences*, at pp. 177-78.
[129]The ASRS maintains a confidential, voluntary incident reporting system and a database of reported incidents. The purpose is to identify system or latent errors, as well as overt hazards, and to alert the industry about these errors. The ASRS receives more than 30,000 reports annually and issues alerts to the industry on a regular and as needed basis. Most aviation experts agree that these efforts have resulted in an ever-increasing level of civilian airline safety." http://www.aorn.org/journal/2002/aprrc.htm and http://asrs.arc.nasa.gov/main_nf.htm.
[130] Conversations with Carlos Bustamonte (Feb. 3 2006), Rob Carlson (Feb. 11, 2006), George Church (March 9, 2006), Jim Collins (March 8, 2006), John Mulligan (Feb. 24, 2006), David Schaffer (Feb. 9, 2006), Luis Serrano (Feb. 16, 2006), Christina Smolke (Feb. 2, 2006), Ron Weiss (Feb. 14, 2006), Tom Knight (March 2, 2006), and Jörg Stelling (Feb. 21, 2006).
[131] Conversation with George Church (March 9, 2006). Most of the remaining interviewees expressed doubt that a clearinghouse was necessary given the existence of other institutions.
[132] Conversation with George Church (March 9, 2006).
[133]For a short description of the Anymouse program, *see* ALMAR 010/03, available at http://www.usmc.mil/almars/almar2000.nsf/45be3083aa37f0d48525685a004b4bcc/c8dd75f302b9f06f85256cbe005129d4?OpenDocument. Sample reports can be found in D. Nelson and D. Parsons, *Danger: Life and Death Story's from the US Navy's* Approach *Magazine*, Osceola WI, 1991.
[134] Conversations with Drew Endy (March 21 2006), Chris Voigt (March 6, 2006), and Kris Prather (Feb. 14, 2006).
[135] Conversations with Sven Panke (Feb. 5, 2006) and Adam Arkin (Feb. 23, 2006).
[136] Conversations with Jörg Stelling (Feb. 21 2006)(citing forthcoming EU-USA workshop as model) , Luis Serrano (Feb. 16, 2006) (same) , and Victor De Lorenzo (February 27, 2006) (same); *see also*, Fink Report, n.1 at p. 3 (calling for regular meetings and symposia). Several members endorsed a variant of the conference idea ("Asilomar") in which a small select group sets policy. *See, e.g.*, Conversations with Adam Arkin (Feb. 23 2006) and Kris Prather (Feb. 14, 2006).
[137] Conversation with David Schaffer (Feb. 9, 2006).

[138] Conversation with Chris Voigt (March 6, 2006), Jim Collins (March 8, 2006), David Schaffer (Feb. 9, 2006) and Pamela Silver (Jan. 28, 2006).
[139] Conversations with Dan Fletcher (Feb. 3, 2006), Ron Weiss (Feb. 14, 2006), Kris Prather (Feb. 14, 2006), Jörg Stelling (Feb. 21, 2006), and Adam Arkin (Feb. 23, 2006).
[140] Fink Report, *supra* n. 1 at p. 88; National Research Council, *Globalization, Biosecurity, and the Future of the Life Sciences* (2006) at p. 188.
[141] Conversation with Dan Fletcher (Feb. 3, 2006).
[142] Jonathan B. Tucker and Raymond A. Zilinskas**,** "Microbes by Design: The Security Implications of Synthetic Biology" *New Atlantis* (forthcoming), available at http://www.thenewatlantis.com/. Tucker & Zilinskas argue that a "general understanding" already exists, but argue that it would be "worthwhile" to make this "crystal clear." *Id.*
[143] Conversation with Chris Voigt (March 6, 2006),Victor de Lorenzo (Feb. 27, 2006), George Church (March 9, 2006), and Luis Serrano (Feb. 16, 2006).
[144] Conversation with George Church (March 15, 2006), Adam Arkin (Feb. 23, 2006), and Andrew Elllington (Feb. 14, 2006),
[145] Conversation with Adam Arkin (Feb. 23, 2006)., Tom Knight (March 2, 2006), and George Church (March 9, 2006).
[146] Conversations with Ron Weiss (Feb. 14, 2006), Jörg Stelling (Feb. 21, 2006), and Victor de Lorenzo (Feb. 27, 2006).
[147] Conversation with Andrew Ellington (Feb. 14, 2006).
[148] Conversation with Drew Endy (March 21, 2006) (Sloan group ideas).
[149] For a modern description of grants, *see* S. Scotchmer, *Innovation and Incentives* (MIT Press: 2005).
[150] Conversation with Drew Endy (March 21, 2006) (Sloan group ideas).

Appendices

Contents

Appendix A

Appendix A: Proposed Resolutions

The following list provides a partial menu of resolutions that community members may want to debate and vote on when they meet for Synthetic Biology 2.0 on May 23, 2006:

A. Supporting and Extending Responsible Industry Screening Practices.

A. 1 Resolved: Gene synthesis companies have an ethical responsibility to screen orders consistent with "best practices" within the industry, including but not limited to the routine use of automated searches (equivalent to current Blackwatch release or higher) and hand examination of all suspect sequences by qualified scientists. Companies that practice such screening should publicly certify the fact by January 1, 2007. Thereafter, community members pledge not to place orders with any company that fails to comply with this resolution.

A.2 Resolved: Better screening software and machine-readable, detailed sequence watch-lists are urgently needed to improve screening. A community-wide initiative is currently underway to create these tools on or before December, 2006. Members will have an opportunity to review and endorse these products when they meet for Synthetic Biology 3.0.

B. Developing Norms and Practices for An Emerging Community.

B.1 Resolved: Experimenters considering an "experiment of concern" within the meaning of the Fink Report should obtain expert independent advice before proceeding. The community has an ethical obligation to make such advice freely available, particularly to non-members who lack access to university- or company-funded safety committees.

B.2 Resolved: Members have an ethical obligation to investigate and, if necessary, report behavior that they believe poses a significant danger to human life and property. Members may satisfy this obligation through existing channels, by calling authorities, or by contacting community bodies established for this purpose.

C. Maintain an Ongoing Institutional Commitment to Biosecurity and Biosafety.

C.1 Resolved: Members have an ethical obligation to share facts, experiences, and conjectures that increase community awareness of, and ability to manage, biosafety and biosecurity risks. Community members are encouraged to establish confidential clearinghouses to collect, analyze, and disseminate this information.

D. Invest in New, Safety- and Security-Enhancing Technologies.

D.1a Resolved: Funding agencies should invest in research to explore the use of "bar code" technology to detect and trace the origins of genetically modified organisms.

D.1.b Resolved: Funding agencies should invest in research to engineer host organisms for synthetic biology experiments that have little or no chance of surviving, propagating, or interacting with organisms outside the laboratory.

We anticipate that additional proposed resolutions may be added to this list as a result of community input between now and May 23.

Appendix B

Appendix B: “Experiments of Concern”

The Fink Report[1] defines seven classes of experiments that it believed would require the “review and discussion by informed members of the scientific and medical community before they are undertaken or, if carried out, before they are published in full detail.” These consist of experiments that:

1. Would demonstrate how to render a vaccine ineffective.

This would apply to both human and animal vaccines. Creation of a vaccine resistant smallpox virus would fall into this class of experiments.

2. Would confer resistance to therapeutically useful antibiotics or antiviral agents.

This would apply to therapeutic agents that are used to control disease agents in humans, animals, or crops. Introduction of ciprofloxacin resistance in *Bacillus anthracis* would fall in this class.

3. Would enhance the virulence of a pathogen or render a nonpathogen virulent.

This would apply to plant, animal, and human pathogens. Introduction of cereolysin toxin gene into *Bacillus anthracis* would fall into this class.

4. Would increase transmissibility of a pathogen.

This would include enhancing transmission within or between species. Altering vector competence to enhance disease transmission would also fall into this class.

5. Would alter the host range of a pathogen.

This would include making nonzoonotics into zoonotic agents. Altering the tropism of viruses would fit into this class.

6. Would enable the evasion of diagnostic/detection modalities.

This could include microencapsulation to avoid antibody-based detection and/or the alteration of gene sequences to avoid detection by established molecular methods.

7. Would enable the weaponization of a biological agent or toxin.

This would include the environmental stabilization of pathogens. Synthesis of smallpox virus would fall into this class of experiments.

The Committee described the foregoing list as “illustrative” and noted that it would probably need to be revised or expanded over time.

---

[1] National Research Council, *Biotechnology Research in an Age of Terrorism: Confronting the Dual Use Dilemma*,” (2004) at pp. 88-90.

Appendix C

Technical Barriers to Successful Biological Attacks with Synthetic Organisms

by
Raymond A. Zilinskas, Ph.D.
April 10, 2006

1. Introduction

Pathogens and their products, particularly toxins, may be deliberately released into the environment for the express purpose of causing disease and death among human, animal, and plant populations. If the release occurs in a military context, to gain strategic or tactical advantage over an enemy, it is called biological warfare (BW); if it is carried out by non-military persons or groups in pursuit of political, religious, or social objectives, its is called bioterrorism. In either case, pathogens and toxins are used for weapons purposes.

Modern biotechnology techniques, including genetic engineering, have in the recent past been applied by Soviet scientists to enhance the pathogenic characteristics of bacterial and viral species and render them more effective for BW then their wild counterparts.[1] The possibility that scientists working for terrorist groups would do the same has been discussed by several security experts.[2] That probability that synthetic biology, the latest manifestation of modern biotechnology, might be applied for illicit and military purposes has been recently analyzed by Tucker and Zilinskas.[3] Following up on their discourse, the assumption underlying this article is that malicious persons or groups will at some point in the future develop a synthetic organism for hostile purposes. This article discusses the formidable challenges facing anyone who would attempt to synthesize an organism for biological weapon's use.

To begin, a common misconception is that a pathogen or toxin is a weapon.[4] In fact, the process of "weaponizing" an agent commences only after the pathogen or toxin is in hand in the laboratory. From the history of past BW programs, we know that a biological weapon is a system consisting of four components that all must work in unison for it to be able to inflict mass casualties. In addition, natural factors and stresses may render a pathogen useless after it has been released during a biological attack. Since synthetic biology practitioners may be unaware of the substantial technical barriers facing the development and use of biological weapons, I will discuss two major problem areas that face anyone who might, sometime in the future, attempt to deliberately cause damage with a synthetic microorganism.[5] These problems relate to (1) biological weapon requirements and agents, and (2) meteorological and environmental factors and stresses. A concluding section discusses whether synthetic organisms are likely to be weaponized and, if so, the vital element that probably will be missed by weapons developers and thus will prevent successful weaponization.

2. Biological Weapon Requirements and Agents

A biological weapon is more than a quantity of pathogenic agents; instead, it is a system consisting of (1) a suitable pathogen; (2) an appropriate "formulation"; i.e., a combination of the pathogen and chemical additives; (3) an appropriate container to

safely store and transport the formulation; and (4) an efficient mechanism to disperse the formulation. In addition, if the formulation is to be delivered by aerosol, a fifth factor is essential, namely favorable atmospheric and meteorological conditions. Each of these factors is next considered in detail, as are their implications for a malevolent synthetic biologist intent on weaponizing a newly synthesized organism.

A. Suitable pathogens

In the past, state BW programs have tended to focus their efforts on a relatively small group of pathogens, including the bacterial species *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis* (the causative agents of anthrax, plague, and tularemia, respectively) the viruses that cause smallpox, Venezuelan equine encephalitis, and Marburg hemorrhagic fever, and a few proteinaceous toxins such as botulinum toxin and *Staphylococcal* enterotoxin B. However, security analysts generally agree that the menu of pathogens from which terrorist groups could choose is far larger than for national military programs because the latter usually do not find foodborne and waterborne pathogens militarily useful. State programs also tend to avoid contagious agents, although the Soviets developed two of them (smallpox virus and plague bacterium) for long-range strategic attacks. The U.S. Centers for Disease Control and Prevention (CDC) has developed three groupings of biological threat agents, of which Group A agents are the most threatening and Group C the least.[6]

For the purpose of this article, I will assume that the malevolent synthetic biologist probably would not synthesize one of the CDC's threat agents in the laboratory but would create a new and unique life form. In that case, the problem of synthesizing an organism for weapons use would be two-fold. First, the perpetrator would have to create an organism that is pathogenic to the target population, be it human, animal, or plant. There are many aspects to pathogenicity, but I will limit my discussion here to two – infectivity and virulence – and will address only biological weapons directed against humans. Second, a would-be bioterrorist would have to take account of several additional factors to end up with a useful weapons agent, of which I will discuss four – hardiness, resistance to defenses, specificity, and detection avoidance.

i. Infectivity

Infection is the process whereby microorganisms attach to certain tissue cells of the host (such as the cells lining the intestines or the air sacs of the lungs), enter the cells (thereby penetrating the host's first line defense), and colonize the host tissues. Whether or not a microorganism is able to infect human beings depends on the outcome of a series of complex interactions between the invader and the host. Thus, one objective of the bioweapons scientist would be to enhance the pathogen's ability to infect the members of the target population. He might attempt to do so by, for example, increasing the invasive abilities of microorganisms being developed for BW. Since body surfaces usually are the host's first and primary defense against invading pathogens, increasing a pathogen's ability to penetrate these barriers would facilitate infection. For example, a scientist could try to develop a microorganism that secretes two kinds of powerful enzymes: proteinases that break up peptide bonds, and phospholipases that hydrolyze phospholipids. Scientists may also imbue a pathogen with the ability to secrete enzymes that break down

antibodies secreted by skin cells, such as immunoglobulin A (IgA). One can imagine a multitude of other approaches that affect the balance between the host and pathogen so as to facilitate infection.

ii. Virulence.

Pathogenicity or virulence refers to the ability of the pathogen, once it has successfully colonized the host, to traverse the bloodstream or the lymphatic system, evade the intrinsic defenses of the host, enter the target tissues, and obtain nutrients for itself.

Like infectivity, virulence (meaning the ability to produce morbidity and mortality in a host) is a complex process requiring the coordinated activity of many gene products. For example, it has been estimated that over 200 genes in *Salmonella typhimurium* (a common cause of bacterial food poisoning), or approximately 4% of the organism's entire genome, have functions related to virulence.[7] The most important determinants of virulence are called "virulence factors," several of which often act in unison to destroy the host's defenses and bring about the symptoms of disease.[8] It is reasonable to assume that each bacterial and fungal pathogen possesses its own array of virulence factors. Thus, if a classical microbiologist was able to add virulence factors to a microorganism being developed for BW, or could enhance a pathogen's intrinsic virulence factors so they would work more efficiently, the modified microorganism or pathogen probably would make a better BW agent.

It is probable that a bioweapon scientist wishing to add or modify virulence factors could select from a large menu of candidates. Virulence factors can be divided into three groups—those that produce local effects, those that produce distant effects, and those that allow the pathogen to evade host defenses.

Virulence factors that produce local effects: After taking up residence in a host's tissue, some pathogens secrete enzymes and other substances, such as coagulases, kinases, lecithinases, and proteases, which break down the host's cells and intracellular matrices located near the foci of infection. For example, the so-called "flesh-eating" bacteria are strains of Group A *Streptococcus* whose virulence factors facilitate a rapidly progressing subcutaneous infection. These bacteria also produce superantigen exotoxins, such as *Streptococcus pyogenes* exotoxin A, which are associated with streptococcal toxic shock that has 30-80% mortality. Type III secretion systems, which are found only in gram- negative pathogens [i.e., bacteria that do not absorb the Gram stain], assist in delivering toxins into host cells.[9]

Virulence factors that produce distant effects: Some virulence factors are released by the colonized pathogens and are carried by the host's circulatory or lymphatic system to distantly located organs. Among these types of virulence factors are toxins. Many bacterial pathogens are able to secrete toxins, of which hundreds have been identified to date. There are two general types of bacterial toxins—endotoxins and exotoxins. Gram-negative bacteria, such as *Vibrio cholerae* and certain strains of *Escherichia coli*, usually produce endotoxins. Endotoxins are lipopolysaccharides that are part of the cell wall of

gram-negative bacteria. They either are secreted in small quantities during cell growth or are released when a cell is lyzed. However released into the host's circulatory system, endotoxins are responsible for many of the pathogenic effects of gram-negative bacteria.

Exotoxins are proteins produced by either gram-positive or gram-negative bacteria. They may be secreted continuously by bacteria or, more commonly, in bursts at the end of growth, during sporulation, or during the lysis of a bacterial cell. Exotoxins stimulate the host's immune system to produce interleukin-1 from macrophages and tumor necrosis factor from phagocytes; these immunoregulators in turn produce fever, shock, and death. There are three types of exotoxins: cytotoxins, which destroy host cells; neurotoxins, which interfere with neural transmission; and enterotoxins, which damage the cell lining of the gastrointestinal tract by overstimulating the cellular immune response.

Virulence factors that allow the pathogen to evade host defenses: Pathogens have evolved numerous strategies to evade host defenses and to utilize substances produced by the host for their own purposes. Thus, many pathogenic bacteria are able to secrete special proteins, called siderophores, that remove iron from the host's carrier proteins and make it available to the bacterial cell. Some pathogens, such as *Streptococcus pneumoniae* and *Cryptococcus neoformans*, produce a capsule that protects the bacterium from phagocytosis by the white blood cells of the host. There also are species of *Staphylococcus* and *Streptococcus* that secrete leukocidins, which are capable of destroying the host's white blood cells, and hemolysins that lyze red blood cells. Some bacteria (such as rickettsiae) and viruses (such as HIV and herpes virus) hide within the host's cells, thus evading the host's immune response. Some strains of *E. coli* O157:H7 are able to secrete heat shock proteins, which protect the organism against fever.

It can be seen from the foregoing discussion that most virulence factors are proteins secreted by the invading pathogen that interfere with the normal immune responses of the host, thereby allowing the pathogen to propagate relatively unhindered in the host's tissues. It would appear that the genes controlling the production of some of these proteins would not be difficult to identify and transfer to microorganisms being developed for BW purposes. For example, a gene coding for the production of the SEG superantigen by *Staphylococcus aureus* has been identified and cloned.[10] The transfer of this gene from one cell to another would not be technically difficult. In addition, researchers have developed methodologies for the transfer and efficient expression of the genes encoding botulinum and tetanus toxins in *E. coli*, the ubiquitous and generally harmless bacterium that resides in the human intestinal tract.[11]

Holding even more promise to the bioweaponeer is the report that research in Australia aiming to develop a better method for controlling rodents unexpectedly produced a genetically engineered virus that killed rodents. The Australian researchers had set out to develop a modified form of mousepox virus (also called ectromelia virus) that would trigger a specific antibody response in the host female mouse and destroy her eggs.[12] To boost the antibody response, the investigators inserted into the virus a gene coding for the production of a natural hormone called interleukin-4 (IL-4), which plays

an important role in the immunological defense systems of mammals.. In earlier research involving a different virus, the vaccinia virus, this procedure increased the antibody response in mice while decreasing the efficiency of virus-clearing killer T cells. However, after the IL-4 gene was inserted into the mousepox virus, the genetically modified virus did not sterilize the mice but instead caused a fulminating mousepox infection that killed most of the recipients. Even more remarkable, the genetically engineered virus also killed or severely harmed mice who had been vaccinated against mousepox. As the authors wrote, "These data therefore suggest that virus-encoded IL-4 not only suppresses primary antiviral cell-mediated immune responses but also can inhibit the expression of immune memory responses."[13]

The Australian findings raised an alarm among the public and scientific community.[14] One worry is that this technique could be used to produce more virulent forms of human viruses for application as weapons. An Australian scientist was quoted as stating, "This shows that something we had thought was hard—increasing the pathogenicity of a virus—is easy."[15] Ron Atlas, a prominent environmental microbiologist, observed, "If there is a lesson in this, it's that you can create a more virulent pathogen. In 99 percent of the cases you would not, but in others you can, and here's an example."[16]

As the research results noted above demonstrate, it is probable that classical microbiologists attempting to weaponize bacteria or viruses would have a plethora of choices as to which virulence factors they could use when weaponizing bacterial pathogens. Because the genomes of many pathogens have been mapped and sequenced, the scientists would be working with a familiar pathogen whose characteristics are mostly known. In contrast, a synthetic biologist probably would be limited to trying to weaponize one novel organism and, possibly, variants thereof. The mere fact that this organism might have included some genes coding for virulence factors would not necessarily make it into a pathogen. Rather, the malevolent synthetic biologist would need to recreate genetic complexes of genes that, when working in unison, allow the new pathogen to infect a host and then cause it to become sick and die. The new pathogen would also have to possess defenses that allow it to fight off the host's immunological responses elicited by the invader, something that natural pathogens have evolved over the eons to do. In view of these very substantial technical difficulties, I do not believe that any synthetic biologist will be able to transform a newly created organism to a pathogen for the foreseeable future.

iii. Hardiness.

Hardiness refers to the ability of an organism to survive being enclosed in a storage container or munition and, after release as a fine-particle aerosol, to endure the physical and chemical stresses encountered in the open environment. A classical microbiologist therefore might attempt to enhance the hardiness of bacteria, fungi, and viruses in two ways. First, he could try to enhance the organism's ability to resist desiccation, withstand UV radiation, and survive decontamination procedures. Second, he could attempt to stabilize genetically determined traits, such as virulence, in the weaponized agent. If he is successful in increasing hardiness, the BW agent would

survive longer after release, thereby increasing its potential for causing casualties. If he is successful in maintaining virulence, the payload of biological agent would have a longer shelf life, thus lessening the need to reload them frequently with fresh batches of agent.

From experience gained over many years of research and testing, the ability of many pathogens to survive in the open environment or under many other conditions are well known. From this experience it is known that some pathogens, such as *Yersinia pestis* bacteria, are fragile, meaning that they will survive for only minutes after being released into the open air. Other pathogens, such as *Bacillus anthracis* spores, are hardy and able to survive in the open air for hours and in the soil for years. We also known that the degree of hardiness an organism possesses is dependent on several factors, including cell wall construction and the effectiveness of a microorganism's DNA repair mechanism, both of which are controlled by a multitude of genes. No similar data would be available to a synthetic biologist who is constructing a new organism. For example, it is highly doubtful that anyone is in a position to design and produce a DNA repair mechanism for any new organism. It probably will take synthetic biology many years before any of its practitioners can factor in hardiness during the process of designing and constructing a synthetic organism. If this is so, probably all synthetic organisms created in the foreseeable future will be fragile and thus unable to withstand the stresses of containment, dispersal, and/or open environment.

iv. Resistance.

Resistance refers to the ability of a microorganism to defeat the actions of therapeutic drugs such as antibiotics and preventives such as vaccines. The means by which a classical microbiologist might attempt to enhance the capabilities of microorganisms to resist drugs and preventives would probably vary considerably from type to type. With respect to bacteria, a scientist might attempt to develop a strain that is resistant to antibiotics used by the target population or alter a bacterium's antigenic characteristics to defeat vaccines. The advantage to the bioterrorist of using highly resistant strains in an attack would be greater casualty generation and higher lethality among those attacked.

Today, a classical microbiologist would not find it difficult to develop antibiotic-resistant bacterial strains through the application of genetic engineering techniques. However, this approach would not guarantee that the altered strains would be better suited for weapons use than their antibiotic-susceptible relatives. The reason is that no one can know at the outset of research whether or not the newly developed antibiotic-resistant strains will manifest additional but negative characteristics that make them unsuitable for weapons purposes.[17] For example, an antibiotic-resistant strain might also be less virulent or hardy than the parent strain (or both). Multiple effects from a single genetic modification, or "pleiotropy," have been a common problem with genetically engineered organisms that have been developed in the past for specific civilian purposes. Accordingly, there is good reason to believe that similar difficulties would beset scientists developing synthetic organisms for either licit or illicit purposes.

What this means for a synthetic biologist seeking to weaponize a newly created organism is that he is entering into a risky endeavor with no guarantee of success. If we assume for the purpose of discussion that this biologist has been able to develop an organism that is pathogenic (i.e., infective, virulent, and hardy), he still would not know if his creation is resistant to any or all antibiotics. Such knowledge could only be gained from testing antibiotics against the organism, first *in vitro* and then *in vivo*. If testing revealed the synthetic microbe to be extremely sensitive to antibiotics, the creator would have to return to the laboratory and endeavor to insert antibiotic-resistance genes into his creation. This action might generate pleiotropic effects, necessitating a new round of research to remove the undesired characteristics while retaining the desirable ones. Several such cycles of research, testing, new research, new testing, and so forth, might have to be carried out before the weaponization of the novel agent had been completed. Alternatively, efforts at weaponization might in the end fail.

v. Specificity.

Specificity refers to a pathogen's propensity to prefer a specific host. A scientist working for bioterrorists might find it useful to either to increase a pathogen's preference for a specified target population or to decrease its ability to attack populations other than the target population. By doing so, the probability of a biological weapon causing collateral damage would be decreased, thus increasing the biological aggressor's ability to control the weapon. When considering biological weapons against humans, the ultimate manifestation of specificity would be an "ethnic weapon"; i.e., a weapon that would selectively harm a particular population group that is characterized by specific genes.

Host preferences among pathogens vary widely. At the one end of the scale, some species of viruses (for example, poxviruses) and bacteria (for example, *Mycobacterium lepri*) tend to be species-specific. At the other end of the scale, many bacterial and fungal species attack more than one animal or plant species. For example, there are subspecies of *Pseudomonas aeruginosa*, a ubiquitous bacterium, that can cause disease in every kind of animal, be it vertebrate or invertebrate, warm blooded or cold-blooded, and in virtually all tissues.[18]

The biological interactions between hosts and pathogens, be they bacteria, fungi, or viruses, are exceedingly complex, some of them having evolved over millions of years. While research on the genetic basis of host-pathogen relationships is beginning to produce results, knowledge about these relationships is still rudimentary. Thus, for the foreseeable future, it is most unlikely that even the most qualified synthetic biologist would be able to synthesize an organism that is pathogenic to a select target population.

vi. Detection avoidance.

There are two types of detection avoidance. The first involves the deliberate altering of properties possessed by well-characterized BW agents, such as engineering a bacterium or virus to express surface antigens it normally does not express. If this goal were accomplished, the target population would have difficulty detecting and identifying the modified form of pathogen with existing methods. Second, an organism could be

deliberately altered to defeat immunological defense systems present in a target population. A BW agent that is able avoid detection would greatly complicate the situation facing an attacked population because it would delay a correct diagnosis and the provision of appropriate treatment.

While detection avoidance would be an important goal for a weapons scientist developing existing pathogenic bacteria and viruses for BW purposes, it probably would not be so for a synthetic biologist who is developing a new organism. Since the microbial agent would be new and unique, there probably would be no way to detect and identify it by existing clinical microbiology methods and nor vaccines to protect against it.

B. Formulation

It is known from experience that natural pathogens can survive only briefly in the open air and that therefore they need to be protected by some type of formulation technology. This situation probably would face the creator of the new pathogen, who would need to formulate it. In national BW programs, weaponized pathogens were packaged in either wet or dry formulations. Producing a wet formulation of a bacterial pathogen begins by inoculating a small amount of the selected bacterial strain (seed culture) into a culture medium and placing the mixture into a fermenter. The fermentation process then is allowed to proceed for a set number of hours at an optimal temperature until a maximum number of pathogens has been propagated. At that time, the fermentation process is terminated. After cooling, the mass of pathogens (biomass) is separated from the nutrient broth, usually by centrifugation, and then is stored under refrigeration. At this stage of the process, the biomass resembles a mud-like slurry in consistency and appearance. After the pathogen cells or spores have been propagated and harvested, and prior to use, they must be suspended in a special solution containing preservatives, adjuvants, and other chemicals that protect the pathogens and remove or negate electric charges that otherwise would tend to clump the particles. The final emulsion or mixture is commonly called “formulation,” and resembles cloudy water. Each weaponized pathogen requires a specific formulation. The wet formulation can either be disseminated directly or dried into a fine powder, which entails an additional two steps (see below).

A major technical problem facing anyone who intends to disseminate a wet formulation is how to force it through a nozzle to create an aerosol consisting of particles less than 10 microns in diameter. If the formulation has not been constituted correctly, the wet slurry will clog the nozzle, and/or the particles contained in the aerosol will clump because of electrostatic attraction, thus generating particles of a size much larger than 10 microns.

The development of a formulation suitable for protecting a specific agent and facilitating effective aerosolization demands considerable expertise in several disciplines. Thus, at a minimum, the development of agent formulations would require an interdisciplinary R&D team constituted by scientists having expertise in bacteriology or virology, biochemistry, fermentation processes, and downstream processing. Efforts to

develop and produce formulations for use as aerosols would also require the service of an aerobiologist or an appropriately trained physicist.

The issue of what it takes to develop a dry agent formulation has become acute since the so-called "anthrax letters" were delivered to public figures in September-October 2001. Without doubt, it is more difficult to develop and produce a dry formulation than a wet slurry. First, it is necessary to dry the wet biomass in special equipment (such as a spray-dryer or freeze-dryer) and then mill the dried material into a fine powder whose particles are no larger than 10 microns in diameter. These operations are technically difficult to accomplish and present an extreme biohazard to persons in the vicinity. As with preparation of a wet formulation, it probably would take several cycles of experimentation and testing before an effective dry formulation could be developed.

Formulation technology is not a science; instead, designing formulations is an art form and the final product requires much field testing. A synthetic biologist would probably be unprepared and unequipped to undertake serious formulation development and testing. It is therefore possible that even a highly lethal synthetic organism would ultimately fail for weapons use because its creator could not protect it from rapid decay while in storage and after aerosol dissemination.

C. Container for storage and transport (munition)

In order to carry out an attack, a bioterrorist must be able to deliver a requisite quantity of the formulation to a staging area from whence the attack will be mounted. To do this, the requisite quantity of formulation will have to be transported in some kind of container from the site of production or storage to the staging area. The container would have to be designed in such a way that the pathogens it contains do not lose virulence over time. Additionally, the stored formulation needs to remain safely contained (so it does not produce a leak that could harm the operator and non-targeted persons), but must be capable of being disseminated at the appropriate time. Under some circumstances, the container also could be a munition; i.e., the container could be designed to both carry the formulation securely and be equipped with a mechanism for dispersing it on command.

A synthetic biologist probably would not find it difficult to design a container in which he could safely store and transfer his creations, but dissemination would be more challenging.

D. Methods for dispersing BW agents

As noted above, terrorists mounting a biological attack could use contagious or non-contagious pathogens. There are preferred methods of dispersal for each type of pathogen.

i. Dispersing contagious agents.

If the agent of choice was a contagious pathogen, such as a hemorrhagic fever virus or the plague bacterium, initiating an epidemic within the target population would not be difficult, especially if the population is susceptible to the selected pathogen.[19] The easiest method probably would be for the attacker to use the biological equivalent of a

suicide bomber; i.e., a person who has been deliberately infected with a contagious agent and dispatched to the target population before disease symptoms appear (this is called the prodromal phase of disease). In many viral diseases, including influenza, the infected individual is more contagious in the prodromal state than after disease symptoms have appeared. With other viral diseases, the person must be showing signs of the disease before he is infective. For example, a person afflicted with smallpox is not contagious until a rash appears. Moreover, an individual in the prodromal phase of smallpox would be severely ill and probably bedridden, or at least too weak to walk around.

For the purpose of this article, contagious agents will not be considered further because it is difficult to imagine that even a highly qualified synthetic biologist could design a synthetic organism that not only is pathogenic but also contagious.

ii. Dispersing non-contagious agents.

Carrying out an effective attack with a non-contagious pathogen, be it a bacterium or a virus, is a technically difficult process. In particular, the dissemination must ensure that the agent being dispersed remains viable for a sufficiently long time to reach the airways of the targeted population and that each and every individual in that population is exposed to an infectious or toxic dose of the agent released. While there potentially are many ways of dispersing biological agents, the discussion here is limited to four methods of potential utility to terrorists, namely dispersal by injection, explosion, food or beverages, and aerosol.

Dispersal by injection

Injection is most likely to be employed when a biological attack is carried out for the purpose of assassination.

A malevolent synthetic biologist might be intent on murder, perhaps to eliminate a competitor, or he might wish to test his creation on an unsuspecting victim. If so, all the considerations discussed above related to infectivity, virulence, hardiness, etc. would come into play in the design and application of an injectable pathogen.

Dispersal by explosion

Explosive dispersion is generally used to disseminate agents contained within bombs and artillery shells, or rocket and missile warheads. Typically, these munitions contain a tube filled with an explosive charge (burster), such as TNT, which is placed in the center of a chamber containing the biological agent. At the moment of impact, the burster explodes, rupturing the outer wall of the munition and expelling the payload over a limited area. Whatever explosive is used in the burster, further dispersal of the expelled agents depends on meteorological forces, in particular the wind. However, explosive dispersal generates heat and shearing force that kills 95 to 99 % of the payload. Further, a proportion of the surviving cells are driven harmlessly into the ground and another proportion is transformed into very small particles that are unlikely to settle in the lungs and thus will not infect and cause disease. For all these reasons, anyone who wishes to disperse an agent by explosive means must use a very hardy pathogen in order to maximize the survival rate of the dispersed agent.

It is doubtful that synthetic biology is anywhere near the stage of development that would allow one of its practitioners to design and produce an organism that would be able to survive explosive dispersal.

Dispersal by food or beverages

Foodborne or waterborne attacks would involve the deliberate introduction of a pathogen or toxin into food, beverages, or fomites during their manufacturing or packaging process. Alternatively, the agent could be introduced when edibles are at the point of being distributed or are being used as ingredients for other preparations. Carrying out this type of attack might simply involve carrying a flask containing the agent formulation and pouring a small amount of it into the food, beverage, or fomite while it is being prepared or, in the case of foods and beverages, before they are served. It bears noting in this regard that most pathogens are destroyed by boiling and other cooking procedures, so their introduction would have to take place towards the end of the food preparation process, when it is about to be packaged or just before serving.

The problem facing a synthetic biologist who might wish to attack a population through the use of a food- or beverage-borne agent would be to design an organism that would be able to live or remain potent in four types of environments: (1) a container that would store and transport the agent; (2) the food or beverage into which the agent will be introduced; (3) the host's digestive system; and (4) the host's tissues, where the agent will be beset by specific and non-specific immune defenses. This kind of development would most likely be so technically difficult that it most likely could not be accomplished for the foreseeable future.

Dispersal by aerosol

The aerosol method of delivery is of greatest concern since its application is the one that is most likely to generate mass casualties. However, for this method to be maximally effective, three factors must to be taken into account by the attacker. The first relates to the altitude at which microorganisms are released – the release must be done at a low altitude. Field experiments carried out at the Dugway Proving Ground have demonstrated that when particles of between one and five micron size are sprayed by an aircraft that flies at an altitude of over a few hundred meters, atmospheric turbulence will dissipate the particles quickly, so that they cannot be detected by detectors placed as close as a few hundred meters downwind from the line source. Billions of microorganisms that had been dispersed in the course of a field trial have thus been rapidly diluted below the infectious dose. Second, many environmental factors will act to stress the released agent; these factors are discussed in the next section.

Were a terrorist group to attempt to mount an airborne attack, it probably would use a spray or nebulizer system that can be purchased at hardware stores or farm suppliers. The main components of the usual type of sprayers and nebulizers used in agriculture and by painters are a hopper tank (which holds the bulk material to be sprayed), a source of compressed air, a feeding line through which bulk material is conveyed by compressed air, and a nozzle through which the bulk material is ejected. Sprayers break up and spread the formulation contained in the hopper tank by squirting it

under pressure through the nozzle. In general, nozzles break up formulations unevenly, resulting in a very wide range of aerosol particle sizes. Although some of the aerosol particles produced this way will be in the required size range (approximately 1 to 5 microns), most will not. Very large and very small aerosol particles are both wasteful. The very large spray droplets end up settling rapidly onto the ground, while the very small spray droplets disperse widely and hence are diluted beyond effectiveness. For these reasons, off-the-shelf sprayers and nebulizers are not optimal for the dispersal of BW agents but need to be adapted for that purpose.

A synthetic biologist who sought to disperse his creation by aerosolization would face the same technical problems as any other criminal or terrorist who chose this method of attack. In addition, he would need to perform experiments to determine if his creation would be able to survive the shear forces that would beset it as it was forced through a tiny nozzle.

3. Environmental Factors

Anyone releasing an aerosol to attack a community will face formidable technical and practical challenges. One set of difficulties concerns controlling the aerosolized agents after release. Briefly, the atmosphere must be stable where the attack is to take place because the less turbulence, the higher the likelihood of producing a high concentration of the agent at or near ground level. Two meteorological forces, wind and inversion layer, commonly affect atmospheric stability. Thus, wind cannot be too forceful (or be completely absent); a favorable wind for an aerosol attack is one that propels the released agents from the line dispersal zone over the area occupied by the target population. A suitable inversion layer is one that keeps the aerosol cloud close to the ground, which usually is in the early morning.

The second set of difficulties pertains to natural forces that stress the agents constituting an aerosol. Microorganisms released into the open environment as a component of an aerosol face many threats to their survival or integrity. In particular, physical atmospheric factors, such as relative humidity and temperature, will directly affect the survival of aerosolized agents. Thus, pathogens constituting an aerosol cloud will begin to desiccate after being released into the open environment, leading to their rapid death. As a rule of thumb, the higher the temperature and the lower the relative humidity, the faster the aerosolized microorganism will desiccate.[20]

In addition to the foregoing what might be called “known” factors, there are poorly understood factors that influence biological decay rates. In particular, in the late 1960s, British scientists identified a phenomenon called the “open air factor” that appeared to have a significant impact on the survival of bacterial cells. They found that when *E. coli* cells were exposed to air from outdoors, the decay rate increased from 0.2 % per minute to between 1.5 and 20 % per minute (averaging 3 – 10 % per minute). This effect was noted with many organisms, including some with BW potential, such as the organisms responsible for tularemia and brucellosis. Significantly, spores from B. *anthracis* were unaffected.[21] Some researchers believe that the “open air factor” is related to the presence of ozone and certain types of hydrocarbons in the atmosphere.[22]

Because of these limitations, a successful biological attack using vegetative (non-spore) *B. anthracis* cells would be difficult to accomplish unless they have been weaponized and are appropriately formulated. Similar limitations apply to vegetative cells of other bacterial species such as *Y. pestis* and *F. tularensis*. Conversely, as is discussed above, spores, such as those formed by *B. anthracis*, remain stable for several hours in the atmosphere in the absence of sunlight (ultra-violet radiation kills spores, but it takes considerably longer to do so). Therefore, spores dispersed under the favorable meteorological conditions explained above would have a high probability of surviving long enough to cause mass casualties with high mortality among a target population.

A malevolent synthetic biologist, like anyone aiming to utilize a biological weapon that depends on aerosol dispersal, would have to contend with all of the difficulties enumerated above. Whether a synthetic organism would be better, equal, or worse at surviving natural forces than classical BW agents could not be determined in advance, and only field testing would provide definitive answers.

3. Concluding Thoughts

Often during discussions about the applicability of advanced biotechnology techniques for weaponization of pathogens, someone will state that nature already has produced plenty of virulent pathogens, so why would anyone bother about trying to improve on nature? A corollary to this opinion is that there is no need for a nation or subnational entity to take on risky research to weaponize pathogens because they only need to access natural sources to secure pathogens suitable for waging war or bringing about terror. In view of advancing biotechnologies, including synthetic biology, this seems like a disingenuous argument. Certain nations, especially the Soviet Union, operated a huge BW program that tried very hard to improve on nature through genetic engineering and other advanced biotechnologies, including genetic engineering, to develop pathogens uniquely suited for warfare. In general, I believe that powerful technologies, including the biotechnologies, will at some time in the future be applied for military, criminal, and terrorist purposes. On the basis of this assumption, it is not too early to bring this possibility to the attention of the synthetic biology community and urge it to start thinking about how its creations might be misused and what can be done to prevent that from happening. This article suggests, however, that even if a synthetic microorganism were to exhibit pathogenic properties, it would not necessary be suitable for weapons use. To reiterate the important point, a biological weapon is more than merely a collection of pathogens, it is a intricate system that must be developed by experts in several disciplines before it is likely to be efficient at generating mass casualties. A lone synthetic biologist who created an organism with putative pathogenic properties in the laboratory would face an arduous developmental and testing process before he had a usable biological weapon.

Based on the foregoing discussion, it is clear that a synthetic microorganism would have to be tested for infectivity and virulence, probably on animal models or, covertly, on humans, before its developer would be certain of its pathogenicity. If a pleiotropic effect is noted that decreases the new creation’s value for weapons use, further research and experimentation would be needed to remove the unwanted

characteristics while retaining the desirable properties. The implication of these uncertainties is that synthetic biology research undertaken for the purpose of creating a new pathogen for weapons use is risky for two reasons. First, it might fail. It is possible that a synthetic organism cannot be made into a pathogen. Second, even if an organism with apparently enhanced pathogenic properties were developed, there is a substantial possibility that pleiotropic effects would become manifest in the modified organism, necessitating further research, development, and testing to remove them. It could take a long time and considerable effort before an organism exhibiting superior qualities for weaponization was in fact created; and conversely, the entire effort might ultimately fail.

The need for field-testing a newly synthesized organism has been noted several times above. Field-testing is important for three reasons. First, the behavior of a synthetic microorganism in the open environment must be observed and measured. This step is necessary for such reasons as making certain that newly developed pathogens will survive long enough to cause damage and that their level of virulence remains stable despite the stresses that environmental forces exert on them. Second, the ability of munitions to disseminate the agent effectively must be assured. Third, it is only through field testing that the developers can ascertain that a biological weapons system, comprised of the munition and the agents it carries, will operate dependably and predictably.

It is true that not all past national BW programs carried out extensive testing of their biological weapons. The Iraqis, for example, had only a rudimentary field-testing program for their BW devices. Therefore, Iraqi scientists probably never properly assessed the military value of their weapons.[23] The reason the Iraqis chose to forego thorough testing is not known, but it could be that the two agents they concentrated on, *B. anthracis* and botulinum toxin, were fairly well understood. Therefore, even if Iraq's biological weapons had proven technically inefficient, they still would have been sufficiently effective for purposes of terrorizing Iraq's adversaries, most of whom were unprotected by vaccination and personal protection gear. However, a synthetic biologist having just created a new organism could not be so assured, and it would only be through field testing that he would gain the knowledge that not only was his new creation pathogenic, but that it could survive in the environment long enough to infect and sicken a large number of the target population.

Although the foregoing discussion has dealt with entirely synthetic organisms and the difficulties involved in converting them to pathogens, it bears mentioning that there might be an alternative approach for a synthetic biologist to create a pathogen, which would involve starting out with a "minimum genome" pathogen. A research group at the Venter Institute has been working for some time on the bacterium *Mycoplasma genitalium*, which is notable because it has the smallest known bacterial genome (482 protein-coding genes, 43 RNA genes), yet possesses all of the biochemical machinery needed to metabolize, grow, and reproduce. The group recently reported that it developed a form of *M. genitalium* that has been stripped down to the absolute minimum number of genes required to support independent life.[24] The goal of this "minimum genome project" is to build a microbial platform to which new genes can be added, creating a synthetic

organism with known characteristics and functionality. It might be possible for a synthetic biologist to use a similar approach, but to use a frank pathogen as the starting microorganism. The idea then would be to strip this pathogen of as many genes as possible but leaving those that imbue it with pathogenic properties. New genes useful for weaponization purposes would then be added to this specialized microbial platform, resulting in a new life form that could be applied by militaries or terrorists. Were this approach to be attempted, however, I believe that all the difficulties of effective delivery discussed above would apply equally to this new life form.

Endnotes and References

[1] Popov, Serguei. "Interview – Serguei Popov," *Journal of Homeland Defense*, November 1; http://www.homelandsecurity.org/journal/Interviews/PopovInterview_001107.htm (2000).
[2] Poste, George. "Biotechnology and Terrorism," *Prospect*, April 25, 2002; Zilinskas, Raymond A. and W. Seth Carus, 2002. *Possible Terrorist Use of Modern Biotechnology Techniques*, (Washington, D.C.: Chemical and Biological Defense Information Analysis Center). (For Official Use Only)
[3] Tucker, Jonathan B. and Raymond A. Zilinskas, "Microbes by Design: Assessing the Risks of Synthetic Biology," *New Atlantis* (in press).
[4] Some security analysts believe that certain inert biological materials, such as toxins, are potential BW agents, but for the purpose of this article, such materials are chemicals and therefore will not be further considered.
[5] For the purposes of this article I assume that the synthetic organism under consideration is bacteria-like, rather than virus-like or fungus-like. However, considerations being presented here would be approximately the same for other life forms.
[6] CDC Strategic Planning Workgroup. "Biological and chemical terrorism: Strategic plan for preparedness and response. Recommendations of the CDC Strategic Planning Workgroup," *Morbidity and Mortality Weekly Report* 49(RR04):1-14 (2000).
[7] Marcus, Sandra L., John H. Brumell, Cheryl G. Pfeifer, and B. Brett Finlay. "*Salmonella* pathogenicity islands: big virulence in small packages," *Microbes and Infection* 2:145-156 (2000).
[8] Weinrauch, Yvette and Arturo Zychlinsky. "The induction of apoptosis by bacterial pathogens," *Annual Review of Microbiology* 53:155-187 (1999).
[9] Gauthier, Annick and B. Brett Finlay. "Type III secretion system inhibitors are potential antimicrobials," *ASM News* 68(8):383-387 (2002).
[10] Abe, Jun, Yasuhiko Ito, Michiko Onimaru, Takao Kohsaka, and Tae Takeda. "Characterization and distribution of a new enterotoxin-related superantigen produced by *Staphylococcus aureus*," *Microbiology and Immunology* 44(2):79-88 (2000).
[11] Zdanovsky, Alexey G. and Marina V. Zdanovskaia. "Simple and efficient method for heterologous expression of clostridial proteins," *Applied and Environmental Microbiology* 66(8):3166-3173 (2000).
[12] Jackson, Ronald J., Alistair J. Ramsay, Carina D. Christensen, et al. "Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte response and overcomes genetic resistance to mousepox," *Journal of Virology* 75(3):1205-1210 (2001).
[13] Jackson et al. 2001.
[14] Broad, William J. "Pandora's box of germ warfare?" *International Herald Tribune*, January 25, p. 1 (2001); Finkel, Elizabeth. "Engineered mouse virus spurs bioweapon fears," *Science* 291:585 (2001).
[15] Finkel, 2001.
[16] Broad, 2001.
[17] The effects of pleiotrophy are random; they could either enhance or reduce an engineered organism's ability to survive, infect, and reproduce, although in practice negative effects predominate.
[18] Lyczak, J.B., C.L. Cannon, and G.B. Pier. "Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist," *Microbes and Infection* 2(9):1051-1060 (2000).

[19] For the purpose of bioterrorism, there is only one contagious bacterial pathogen, *Y. pestis*, which can cause pneumonic plague. However, as the rarity of this disease indicates, *Y. pestis* does not appear to be very contagious via the aerosol route of transmission.
[20] Walter, M.V., B. Marthi, V.P. Fieland, and L.M. Ganio. "Effect of aerosolization on subsequent bacterial survival," *Applied and Environmental Microbiology* 56(11):3468-3472 (1990).
[21] May, K.R., H.A. Druett, and L.P. Packman. "Toxicity of open air to a variety of microorganisms," *Nature* 221(5186):1146-1147 (1969).
[22] de Mik, G. and Ida de Groot. "The germicidal effect of the open air in different parts of the Netherlands," *Journal of Hygiene* 78:175-187 (1977).
[23] Zilinskas, Raymond A. "Iraq's biological weapons: the past as future?," *Journal of the American Medical Association* 278(5):418-424 (1997).
[24] Marvin Frazier and J. Craig Venter, "Harnessing the power of genomics for energy and the environment," *Industrial Biotechnology*, vol. 1, no. 1 (March 2005), pp. 6-8.

Ethical Issues in Synthetic Biology: Security and Regulation of Experiments of Concern

A White Paper on the Ethics of Self-Governance in New Scientific Community

Town Hall Meeting Series, Spring, 2006

Laurie Zoloth Northwestern University
Center for Bioethics, Science and Society, Northwestern University

Introduction: a brief summary of the science

This paper will consider one of the many ethical issues that are engaged an emerging field and an emerging community of basic research scientists. The field is "synthetic biology" which is an intellectual project that involves interdisciplinary efforts from molecular genetics, genomics, cell biologists, material engineers, metabolic biological engineers, artificial life researchers, computational and structural biologist, chemists, nanotechnology experts and systems biologists. The field uses the following self-definition:

> "Synthetic biology is a) the design and construction of new biological parts, devices and systems, and b) the redesign of existing natural biological systems for useful purposes."

On the joint website that defines the project, the members of the community add:

> "We are a group of individuals from various institutions who are committed to engineering biology in an open and ethical manner. We are currently working to help specify and populate a set of standard parts that have well-defined performance characteristics and can be used (and re-used) to build biological systems; to develop and incorporate design methods and tools into an integrated engineering environment; reverse engineer and re-design pre-existing biological parts and devices in order to expand the set of functions that we can access and program; reverse engineer and re-design a 'simple' natural bacterium" [1]

Such language is rich in its assumptions, and moral claims and that such linguistic claims imply a particular framing of the issue, a particular stance toward "naturalness" and indeed to "usefulness," has not escaped the attention of this author, nor that such framing endows the field with a set of a priori set of ethical principles. Indeed, research on these topics is warranted and is the ongoing subject of other work[2]. The task of this paper is more limited in scope: it is to consider how such a self-defined field in biology can undertake the considerable challenge of the establishment of normative rules to govern and manage the uses of such a powerful science and technology, how the challenges for the emergence of any powerful new field in science that comes of age in a new climate of bio-weaponry, internet access and globalization can be met.

Synthetic biology is far more than a new nomenclature for Genetic Engineering. The promise of synthetic biology extends and expands the earlier ideas of recombinant DNA (rDNA) technology. Let us begin by consider the following cases as examples of the technology:

Case Examples: theory and basic research

Jack Szostak has long been interested in RNA and the progression of life. Starting with a few key elements: membrane, "cell" globules, clay, and simple RNA enzymes, he is studying basic properties of cells.

> "(T)hese ersatz sacs may passably mimic the wrappings of primitive life: cell membranes. But infusing in them the real "stuff" of life requires more work. Lately, Szostak, a professor of genetics, has been putting simple RNA enzymes inside, showing that they can conduct their characteristic activities. Thus some of life's chemistry is compatible with artificial membranes, he says, something that required a careful tweaking of the membrane chemistry. He has also made the sacs grow spontaneously, and even divide - with help.[1] "It's a simplified model of the situation we'd really like to have," says Szostak: a growing, dividing, living organism of totally synthetic origins. But even at present, he says, "These simple membrane systems do pretty fascinating things."[3]

Szostak and others are interested in what the core elements of life may be. One way to understand this problem is by "top-down" deconstruction of existing organism, seeking a minimal genome.[4] The approach of many investigative synthetic biologists is to work from the "bottom up," constructing new entities using systems or genetic pathways that exist in nature.

> "David W. Deamer, professor emeritus of chemistry and biochemistry at the University of California, Santa Cruz, and a cadre of pioneers expanded the quest three decades ago, launching an attempt to build a "protocell." According to Deamer, such an entity must meet 12 requirements for life including having membrane enclosures (1) that can capture energy (2), maintain ion gradients (3), encapsulate macromolecules (4), and divide (5). Macromolecules must be able to grow by polymerization (6), evolve in a way that speeds growth (7), and store information (8). Add to that information store the ability to mutate (9) and to direct growth of catalytic polymers, and you have 10.[2]"

> "Albert Libchaber of Rockefeller University engineered a DNA plasmid to express proteins and put them into membranous sacs. They could produce proteins for a few hours but would eventually peter out when the raw materials ran low inside the compartment. They needed to keep the supply coming. So, he and Vincent Noireaux, now an assistant professor at the University of Minnesota, designed them to produce a channel-forming protein, alpha hemolysin.[3] Suddenly, finished proteins tagged with Green Fluorescent Protein inserted themselves into the artificial membrane allowing nucleotides and other molecules to enter. These

> "cells" survive for up to four days, but it's only a small victory. In the quest to build life, defining success is hard, Libchaber says. Is it success simply to create a cell that functions? Or must it also reproduce? "I think in our case at least, the first step has been achieved." Next, he wants to make them divide, something that's only been done thus far through physical manipulation."

The idea of developing a synthetic biological organism that can divide into copies of itself is more than a goal of purely investigative research: one clear plan for the work is in its application and use. For engineers, such restructuring is a part of how their field is intended: using biological parts is merely a difference in kind, and not intent. In this manifestation, naturally occurring solutions to problems may be discarded in favor of one with a better (more intelligent) design.

> "Indeed, in attempts to create artificial life Chen along with Steen Rasmussen of Los Alamos National Laboratory have thrown out much of the conventions found in nature. They've turned the protocell model inside out, designing a micelle with information coding and metabolic machinery on its exterior.[4] Extant thus far mostly on paper, these micelles use peptide nucleic acids (PNAs), DNA-mimics with a pseudopeptide backbone conjugated to a light-sensitive molecule. When exposed to light, the photosensitive chemical discharges an electron triggering chemical reactions to convert nearby nutrients into new fatty acids and PNA based on the PNA template. These incorporate into the micelle, which grows until it spontaneously pinches in half and divides."

The Case of Practical Use: The Keasling lab, artemesin and malaria

Jay Keasling at the University of California at Berkeley has turned his attention to the problem of malaria, disease with three important challenges: it affects a huge number of the world's population, killing more than 1 million annually, but nearly all of its sufferers (300 million) are concentrated in the poorest regions of the globe; it relays on a host-carrier mechanism with a high rate of mutation and the multi-drug resistant form is becoming dominate; the best treatment found in nature is rare, hard to refine and thus too expensive for most patients. Keasling has developed a

> "technique for transplanting yeast and plant genes to construct an entirely new metabolic pathway inside bacteria can be used generally to produce a broad family of so-called isoprenoids - chemical precursors to many plant-derived drugs and chemicals of interest to industry, including the anticancer drug taxol and various food additives. Isoprenoids, found widely in microbes, plants and marine organisms, currently are very expensive for the chemical industry to synthesize from scratch and nearly as expensive to extract from plant material.(Unlike standard pharmaceutical rDNA techniques,) where protein drugs are produced primarily through fermentation by recombinant yeast that seldom have more than one gene inserted in them, Keasling assembled 10 genes, including control elements, from three different organisms - bacteria, yeast and wormwood- and got them to work together successfully.

Wormwood genes produce artemesin in yeast, which can replicate the plant process, and the "cassette" is then placed into e-coli, which can divide rapidly enough to allow for commercial production. Further, such a system could allow this new "product" to evolve in response to any resistance that is developed, for it is a biological system[5,6] It is important to note that the rights to the project was donated to Amyris Biotechnology, with will sell the drug at no cost (25 cents for the needed 3 day dose) instead of the $2.40 needed with the traditional drug. The testing is overseen by the Gates Foundation via the Non Profit, OneWorldHealth Organization.

These case examples do not describe the breadth of the entire field, yet suggest the general intentions of the investigators. Such research has long excited concern and interest in bioethics-in fact, prior even to the naming of the field as "bioethics" genetic manipulation was a core concern for the philosophers and theologians who reflected on modern science.

The history of Genetic Engineering

John Fletcher and Albert Jonson cite the period of the late 1960s as the first mention of modern concerns. Fletcher notes that Marshall Nirenberg wrote of programming cells with synthetic messages and of the implications of such an advance in 1967[7]. In 1973, Singer and Soll noted such concerns in a letter to Science, and in 1974, the community of scientists capable in this technology, lead by the NAS, gathered to create guideline for their work at Asilomar. Asilomar guidelines stressed too aspects of regulation. First, that the concerns about safety should be taken serious enough to create Level 4 biocontainment for the work, next, that the e-coli used in the work to replicate the DNA needed to be artificially altered for greater control, and finally, that each and every rDNA experiment face not only a standard IRB review, but a specialized review by a specialized national committee (the Recombinant DNA advisory Committee, or The RAC). The scientist declared a moratorium on all work until such mechanisms could be established. In 1974, the NIH established the RAC. The RAC held its first public meeting in 1975. [8]

The RAC was guided in the work by a new Presidential Bioethics Commission (itself a version of an earlier National Commission on the Protection of Human Subjects (which issued the Belmont Report of 1978) In 1982, the President's Commission for the Study of Ethical Problems in Medical and Behavioral Research, issued its report "Splicing Life," which emphases, as the NAS/ Asilomar process, the need for safety and the need for RAC oversight. This is widely considered a foundational text. It drew on far wider ethical, theological and social implications for its framework, yet like the RAC it ultimate normative focus was on safety, the IRB process and RAC Review for "broader issues" and relied on the consent and withdrawal rules for human subjects for enforcement. It delineated extra care when treating vulnerable populations (loosely defined); it suggested decorum in regard to publication practices, set in place concerns and guidelines for tissue use, largely supporting the need for privacy of the subject in genetic research. It set in place a process for response to emerging technology by waiting until actual cases could be proposed—a process called casuistry in ethical reasoning.

In 1984, the RAC created a new group with the sole purpose of looking closely at human gene therapy, called the Human Gene Therapy Working Group, the first task of which was to create a mechanism for scientist approaching the RAC. ("Points to Consider" The RAC first approved all basic research projects involving rDNA in US labs, the all gene marking research, and finally all gene therapy[9] protocols in conjunction with the FDA. The FDA's role was primarily safety based, as it is with standard drug protocols, focusing on safety and efficacy in the clinical trial process and on all genetically altered products. By 1991, the FDA had published its own Points to Consider (Appendix M "Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into One or More Human Research Participants) and began review of all individual gene therapy protocols. The RAC now exists within the NIH to review new technologies and recommend new regulatory changes should these be understand as necessary for clinical gene transfer research (CGTR)[10] . The RAC now is a part of complex regulatory system which includes the Office of Biotechnological Activities Office, the FDA, biosafety (IBC) committees (producing guidelines for accidents in the lab) at federally funded institutions, IRBs, and voluntary compliance oversight by non-federally funded companies. Like Synthetic biology, noted Leroy Walters, chair of the first RAC GTR committee

> "Gene therapy research was clearly a hybrid field. On the one hand, it was highly technical and required the expertise of molecular biologists and human geneticists. On the other hand, gene therapy research was human subject research which was governed by its own set of rules and which was quite comprehensible to laypeople." [11]

The Federal Rules were complex and reached across many agencies: the EPA created computer modeling for the accidental release of synthetic microbes and studying TSCA implications; the Dept of Agriculture does inspection and certification of rDNA (foot to mouth virus, nematodes , plant germ plasm); the FTC may regulate deceptive practices; the CDC has a 24 hour hotline for reports of leakages in shipping of agents if interstate travel is involved; NIOSH and OSHA do research on worker safety—with no specific regulatory plans; the DOT may regulate rDNA as hazardous material; the Dept of Commerce and National Board of Standards may regulate by products in feedstocks, and regulate patents and trade secrets standards; the State Department works with the UN when it considers rDNA, the DOE has a research protocol on Chinese hamsters; several NSF committees suggest regulations (Law and Social Sciences committee and the and Ethics and Values in Science and Technology or EVIST); NAS grants and committees also regulate and oversee certain rDNA projects and the OTC also conducted its own studies. Currently, all clinical GTR protocols connected with institutions that receive federal funding are submitted to Office of Biotechnological Activities for RAC review, (simultaneously with local IRBs so that the RAC review is first) and are passed via email review or go to full public hearing, and also have IRB and IBS committees at each university. New rules involve efforts at "harmonization" of oversight, including attention to data-monitoring, research ethics education, and conflict of interest issues. RAC protocols that are not selected for full review are published, as are all the email correspondence about each protocol. Adverse incidents are reported to both the FDA and

the OBA with the same form when they occur.(All of these changes reflect issues that emerged after Jesse Gelsinger, a patient in a Phase 1 Gene Transfer trial died at the University of Pennsylvania after a number of errors in such regulation and oversight.)[12]

Of critical interest to the Synthetic Biology community is the plan to create two entities. The first is the Gene Transfer Safety Advisory Board (GTSAB) a "nontraditional super-data safety monitoring board, composed of some RAC and some FDA members, ad hoc consultants and others as needed to comprehensive review all data that emerges from all CGTR, in order to see larger trends, error patterns or paradigms and present these at public RAC meetings. [13] In addition, there are plans for a massive data base called the Genetic Modification Clinical Research Information System (GeMCRIS) which will contain information on protocols, available online, with some protected and some public access.[14]

Others have noted other characteristic in common: the mediagenic, mediapathic aspects, the uneasiness with theological or eschatological claims, the need for public education, and the need for cross-field study and analysis of data.[15] However, the RAC did not seriously reflect on two critical issues at stake in the new field of Synthetic Biology. RAC rules are largely about how to protect human individuals who would be used as the "test bed" for gene transfer, asking questions like: what are potential harms and benefits to research subjects? How will potential harms and benefits be communicated so they can consent? How will selection among research subjects be made? (Justice and access issues) and how will privacy and confidential be preserved?[16]

The New Problem of SynBio I: The Social Scale of the Very Small Perturbation

New technologies, not anticipated by the first decades of the RAC changed some of the classic protective paradigms in rDNA and other synthetic research. First, animal models may be utterly misleading (e.g. British phase 1 clinical trials) and not offer any guidance to the use of technology in humans or with humans, or in human environments. Second, "Future tense consent" may be impossible for very long term research (VLTR). Third, new research may use unexpected routes and disciplines (nanotechnology, materials engineering, artificial life, computer science, chemistry) and finally, and most importantly, Syn Bio projects usually do not involve the standard idea of the manipulation of human genes via vectors, patient by patient but image large uses—screening of huge populations, environmental alterations in large landscapes, nanotechnological solutions to social scale issues. Such a "social scale" may have population implications. The idea, well displayed in the Keasling research, that genetic alteration could implicate millions of people allows for serious and just responses to some of humankind's most vexing diseases. Yet it also suggests a degree of applicability and replicability of some powerful bioagents.

New Problems of SynBio II: BioSecurity in a Changing World

The RAC was created in a more sanguine era. Since 9/11, even the complex network of oversight noted above may seem incomplete when confronted with the actual

use of bioterrorism for political ends and the increasing power, scalability and universalizability of Synthetic Biology. For the past two years, synthetic biologist at the key American labs, still limited in number and scope, have met to consider this issue. The problem is well characterized and numerous solutions have been offered. In 2005, a year long intensification of this discussion was held, and research was done on how the scientist within the still self described and self contained community understood the problem of regulation, and the new issues of biosafety and security that lay outside previous RAC discourse. Following the National SynBio 1.0 meeting, and an associated NAS meeting, a team of researchers, led by Stephan Maurer of the Goldman School of Public Policy, extensively interviewed the PIs and post-doctoral students of the labs at UC Berkeley, MIT, (need precise numbers) and the University of Texas most centrally involved in the work, and created a draft consensus document aimed at stimulation of the genre of robust discussion that could lead to an Asilomar type discourse. Like that earlier model, the community is still small enough to create adherent rules. This will change. As the techniques and tools are more widely appreciated and the student base grows, more people will need to be accountable to some process of regulation.

The RAC process was also limited in another way: like much of bioethics, it had no theory of evil. In fact much of bioethics thinks about reasons to reflect about the good act, and hence theorizes nearly exclusively about the good. Many critiques of gene transfer are concerns that attend to "enhancement" or of the increased privileges therein, or of concerns that the wealthy misuse the science. Few theories focus on such aspect of ideas such as malaria—here the sin is either foolishness, or triviality or the in-authenticity of the rich who might alter the genetic provenance of their children for competitive advantage, or narcissism[17].However, such critiques so not develop the scenario of genetic transfer done for deliberate harm. (The parents made be misguided, but not murderous.) Having no robust theory of evil in bioethics or science allows a certain silence to develop around several core issues in Synthetic biology, for all the existing regulations are based on premises of innocent error prevention or overly enthusiastic persuasion by scientists to an unwitting or foolish public, as the core problem.

Further, there is a strong liberty based, rights theory approach within the field, stressing individual autonomy, freedom and creativity of research as a free speech act. Hence, many of the existing regulations are concerned with privacy issues, or protection of the patient—but allow for the free expression of any scientific notion that to be expressed. Yet it must be noted that all new science is inherently subversive, in that it exists outside existing structures of knowledge and beneath the text of the known, well characterized or proven. In this sense, the best, most creative and risky science creates a "knowledge frontier." Like all frontiers or borderland areas, this creates the conditions for a powerful trade zones, in which the risk and benefit constraints within existing knowledge terrain are altered. In such frontier, free exchange (of ideas, goods, services, knowledge) flourishes under the least restrictive interventions by the state.

The Critical Issue of BioSecurity and Dual Use

In the paper "From Understanding to Action: Community Bases Options for Improving Safety and Security in Synthetic Biology"[18] documents extensively, synthetic biologists understand the potential for the use and also for the abuse of new technology. In

particular, any technology that reshapes conditions understood as stable, or "natural" create the intriguing problem of the moral choice faced by the creators—would the new tool be used for agriculture or other human industry, or weaponry? This is as true for SynBio engineers as it was for the inventors of metallurgy, wheeled vehicles, gunpowder, forks, or nuclear fusion. In many cases (gunpowder) the technology was tightly controlled by a central authority to prevent dual use by enemies. In others use was determined by the marketplace from the beginning—however, in all cases, dual use proved to be simple inevitable.

In the most recent period, nuclear arms technology, learned and practiced under clear security guidelines, western educational systems and labs, was then sold by scientists for personal gain and patriotic passion. Place against the new sorts of economies, and the new sorts of national and religious fervor, the new technologies of SynBio present a serious cause for alarm. The scale of the experiment, the openness of the field and the each of portability present a particular sort of challenge. The same wide use applicability that makes the science so promising also makes it far more dangerous than conventional weaponry, for a re-engineered virus or bacteria could wreck for more havoc than bombs.

Oversight has been inconsistent. In December of 2005, Milton Leitenberg noted in a US Government report on the issue of threat assessment of biological weapons, that:

> "The entire area of oversight of problematical "dual use" research in molecular genetics and its applications in the United States appears to range from inadequate at the local levels to virtually nonexistent at the national level and in terms of BWC treaty compliance."[19]

Leitenberg reminds the reader of the pathway of IBC and NIH national oversight, and of local IBC rules, but notes that:

> "failure to adhere to NIH guidelines does not apply except on a voluntary basis to a very large population of institutions that are not recipients of NIH funding, such as US government biodefense laboratories, and US Government contractors as well as hundreds of commercial biotechnology enterprises."

He then goes on to note two cases. One involves making a vaccine resistant strain of anthrax presumably to find ways to combat it, ("Project Jefferson") and one in which a method of countering the action of botulinum toxin is sought, which involves making a biologically stable version of the botulism neurotoxin by using genetic alterations, so that it could be studied more easily, but such stabilization would also allow it to be weaponized. (Previously, BT was deemed too unstable to have such a dual use.) Neither project has been reviewed by the IBC, nor the RAC, whose guidelines do not currently include consideration of the security or proliferation implications of dual-use research.[20] In this report, he does note that the NAS has taken this problem on to some extent, recommending that seven categories of "experiments of concern" be added to the NIH oversight process:

> "In response to the recommendations of the NAS committee report, the administration announced the establishment of a National Science Advisory

> Board for BioSecurity (NSABB) on March 4, 2004. Its mandate was to last for 2 years. The NSABB was established by the DHHS and housed within the NIH The staff of the NSABB was not appointed until 11 months later…no membership of the Board was made until its first meeting June 30, 2005. …the NSABB will not itself review individual project protocols; it will only respond to requests for guidance. …But most importantly, the NSABB is to have *no* (emphasis in original, ed.) oversight over classified BW-relevant research, which is the location in which the most problematical dual-use research is likely to take place." [21]

It is the contention of the still small community of self-identified synbiologists that the potential for harm, even for the developed of weapons capable of "mass destruction," is not trivial. It is also the opinion of many who were interviewed for this report, that it is perhaps inevitable, yet it could clearly be made more difficult.

It is outside the area of expertise of even an expansive field such as bioethics to assess these concerns, however, it is the contention of this report that a clear moral obligation exists in all field of inquiry to protect the society in which it is given the privilege and freedom and funding to exist. This duty is correlative to the right of free inquiry.

"To live outside the law you must be honest": the creation of ethical limits in a mutable world.

Open frontiers need some way of ordering and limiting the power and the exchange of precious commodities. Moreover, the marketplace alone also needs a way to protect people, goods, services and relationships from exploitation or the danger created by evil activities, or by the misuse of the freedom implied by the frontier social economy. There are several traditional ways of regulation of human activities. All of these ways are consistent with the self-governing model, for each could be freely chosen by a mutually consenting community, yet each one suggests a different set of assumption about the issues at stake.

1.) The first such way would be to invest one person, or to accept the State's authority to delegate one person, as a "sheriff" with police power to regulate and punish. This could be accomplish in states with a strong central authority structure, widespread voluntary (or coerced) agreement with the police power of the state, and the ability to enforce rules with a large enough force to enable compliance. Such include the FBI, or other mechanism, but classic examples include the American Western frontier, the Chinese dynastic era, or the British colonial system.

2.) The second such way to establish compliance is strong religious code to which all can agree. Here, the issue of doing good and avoiding evil is set by the missionary with a strong text. Classic examples include the use of missionaries in the empires of the 16th-19th centuries, and theocracies in today's era.

3.) The third such way is to allow the growth of an outlaw gang with a strong peer to peer code that regulates behavior of the advantaged and

self-selected elite. There are strict codes that do two types of regulation—the inner life world and moral order of the group is regulated by a strong moral code of conduct and the "civilians" are protected from the activities of the group waged on their behalf. In fact, the citizens of the frontier can count on the group for protection from other external sources of harm or danger. Classic examples include the American mafia, the James Brothers of the American West, and certain immigrant landsmenschaften or ethnic groups in the urban centers of the early 20th century.

4.) The final regulatory idea is the one of the creation of the ethical expert, with an individual commitment to personal, completely individual moral agency, which may or may not comply with the law of the state. Classic examples include Gandhi, Martin Luther King, or Harriet Tubman.

5.) Public funding means calls for public oversight. Science as frontier has another corollary: it is supported and sustained in large part by public support—and public support increasingly means that the public wants transparency and participation in the science it funds. Moreover, as claims of rapid, even remarkable advances fuel science, and the science itself grows increasingly complex, the media drives a heightened sense of conflict around this issue. SynBio is both mediagenic and mediaphobic, and give reference to an increased sense of both power for transformative and salvatic advances and fear of error in its use. In a world understood as increasely "flat" both in access and in popular entitlement to science, the idea that the technology is dangerous, or that scientists cannot adequately handle it, or that scientists are likely to be seduced into misuse by such power, monetary greed or a sense of amoral arrogance is a common feature of contemporary discourse—a cultural trope that can be observed in any number of film treatments of advanced science. Such fears can lead to Other regulatory measures (the crowd.) In this regulatory measure, the polis may decide to ban the exchange, ban the moral actors, or exclude all support for the exchange. Classic examples are the first years of human embryonic stem cell research.

One may use popular culture as a metaphor and template for these ideas, for they are clearly popularly understood in the literary genre of the American Western ("Shane") and in the "Star Wars" films based on such a shared mythic linguistics. Here we have the Jedis as the template for the idea of a grouping with a strong personal and group peer code, a long period of training and the nearly exclusive use of new and powerful technology with a deep "dual use" potential (in Westerns, it was the Colt revolver and newly developed sharp shooting skills only available to a few.) One may also turn to classic texts: Plato in The Republic in which philosophers had certain duties of citizenship) or many differing religious traditions in which specified and highly trained clergy had access to special tools of power, prophecy and technology—tools that could

be use for both blessing and weaponry. [22] Indeed, as Shattuck reminds us, the theme of forbidden knowledge has a long tradition in the philosophy of knowledge.[23]

Normative challenges

Which sort of regulation is best suited for our problem—in which the theory of evil is not as yet defined? All have drawbacks. Police power can be distrusted by academics, who can have a well developed sense of privacy, a keen unease with state intuitions and some history of anti-authority in a constitutive sense, and in the general sense of science as a genre of disruptive knowledge. Rules, and "training" classes, forms and standard IRB forms can easily become trivialized, mockable, and rote exercises in which regulations can become formulaic and easily outwitted. The crowd can be easily overzealous. Lack of oversight can result in the "loss" of colleagues—to the lure of other calls, some of which, as in the case of nuclear technology, can be both lucrative and frankly dangerous to the larger society that unwittingly trained them.

I will suggest that a new culture is needed in science—an ethical and moral culture which is of particular importance since the spectacle and the phenomenon of the Korea laboratory fraud scandal, in which an entire lab, from PI to student, was swept up in a manipulation of data. What is needed is a lab culture which is strong enough and decent enough to teach and model impeccably honest moral science citizenship in the as yet small academic field of synthetic biology. This will mean the careful construction of specific rules of conduct and behavior for each lab, voluntarily taken up. The elements of such a culture include:

- **PI as strong moral leader with responsibility for her or his students.**
  Each PI of the labs involved must understand his or her mentorship quite seriously and frankly, as a life long duty to follow the work of his or her students with serious attention. In a sense, the teacher bears the duty of continuing attention for she or he has trained a person in a sort of knowledge that demands moral attention.
- **Yearly reunions with papers.**
  Each lab should be funded to have a yearly reunion with the lab residents and trainees. This will mean that returning students will present their work for peer consideration and reflection, including the social and ethical implications of the work. If a person is suddenly absent from this pleasant event, it should raise concerns, but if a person remains engaged and responsive, it is far less likely that he or she is working on a sinister tangent.
- **Buddy system for all grad students**
  Each incoming graduate student should, early in her graduate career, be paired with a peer. Ideally, this should happen within the lab, but may happen across disciplines and labs. This is to help to underscore the idea that "no one is ever truly alone in the lab." This idea, of the public nature of science, is key—the moral error of science fraud is to believe that one can be "alone" in the sense that one could be unobserved, performing in secret. As a scientist, however, one is not alone in this sense—one is in the presence of the witnessing community, of history, and in the world of other moral agents.[24]
- **Need for education and reflection—not "training"-- in ethics and moral philosophy.**

Thoughtful education in ethics is not synonymous with "ethics training." As understood and enacted to fulfill guidelines, such training is useful, but ethics education cannot be done with websites or software—there is a need for individual moral agency, and for this task, serious questions about the complexities of good and evil, difficult moral choices, the nature of the love of country, the nature of obligation to the other and the limits of the search for knowledge, all must be read about and studied with the same seriousness that young scientists give to understanding siRNA or nanobiobarcodes. The source of this education is found in the history and traditions of philosophy, and theology, classic theories of political and economic history and the humanities.

☐**Each scholar must ask the question—**
Am I a good scientist and what does that mean? Such a question must be at the core of the scientific enterprise—it is not one that can come as a set of external moral orders or linguistic authorizations derived from outsiders, yet the position of the outsider can serve a useful function. The public belief in the goodness of science is grounded in a sense of scientific optimism and care, in integrity as much as it is in efficacy. Thus, one must recall here the admonition of Sydney Brenner: stand up for all humanity; always tell the truth.

This report, is understood as the ethical background for the proposals suggested in the larger report on self-governance in synthetic biology. It suggests an agenda larger than one concerning experiments of (known) concern, addressing the larger concerns of motive, goal and meaning that are necessary to consider why evil might be done, as well as how it might be thwarted in this new field.

The hope of the project, which I was privileged to have been invited, was to craft a process by which, as the report suggests, "specific ideas to be turned into reasonable rule." Thus, this paper is part of a call for a flurry of ideas, leading to policies, planning and culture of reason in an emerging field in which reason and imagination, wisdom and skill, all have invaluable roles.

---

[1] Website for project: http://syntheticbiology.org/Who_we_are.html
[2] These issues are obvious to any bioethicist, humanities scholar, historical or social theorist that reads that preceding definition, and include: a sense that nature is a commodity whose existence can be improved for human use; a sense that the biological world can be understood as a sort of machine, with parts that bear scant relationship to a particular whole; that the givenness of order as we find it is mutable and temporal; a sense that our intervention is sanguine, for such a world can be taken apart and reconstructed much like any machined element of society. Reflected as well is a positive and pragmatic idea that the power of basic research can be harness. This footnote is not intended as a critique of these ideas, but as a note that the authors and the reader understand that such are the ideas behind the declarative sentences. The ontological and epistemic phenomenon of this work is not the subject of this limited white paper.
[3] New Scientist, "Is this life?"
[4] This problem has been considered in the Ventner lab and reviewed by Caplan, et al for its bioethical implications.
[5] UC Berkeley news release, April, 2006, see also, Nature Biotechnology, forthcoming, July 2006.
[6] Abstract of article, Keasling, et al: "Malaria is a global health problem that threatens 300–500 million people and kills more than one million people annually[1]. Disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum*[2, 3]. Synthetic antimalarial drugs

and malarial vaccines are currently being developed, but their efficacy against malaria awaits rigorous clinical testing[4, 5]. Artemisinin, a sesquiterpene lactone endoperoxide extracted from *Artemisia annua* L (family Asteraceae; commonly known as sweet wormwood), is highly effective against multi-drug-resistant *Plasmodium* spp., but is in short supply and unaffordable to most malaria sufferers[6]. Although total synthesis of artemisinin is difficult and costly[7], the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of artemisinin[8, 9]. Here we report the engineering of *Saccharomyces cerevisiae* to produce high titres (up to 100 mg l^{-1}) of artemisinic acid using an engineered mevalonate pathway, amorphadiene synthase, and a novel cytochrome P450 monooxygenase (*CYP71AV1*) from *A. annua* that performs a three-step oxidation of amorpha-4,11-diene to artemisinic acid. The synthesized artemisinic acid is transported out and retained on the outside of the engineered yeast, meaning that a simple and inexpensive purification process can be used to obtain the desired product. Although the engineered yeast is already capable of producing artemisinic acid at a significantly higher specific productivity than *A. annua*, yield optimization and industrial scale-up will be required to raise artemisinic acid production to a level high enough to reduce artemisinin combination therapies to significantly below their current price"

[7] Fletcher, John, Human Gene Therapy Georgetown, 1990, p, 57
[8] King, Nancy, "RAC Oversight of Gene Transfer Research: A Model worth Extending?" Journal of Law, Medicine and Ethics, 2002, pp 381-389.
[9] the term is itself contested—some contend, such as Mildred Cho, that "no therapy has ever resulted"
[10] Gene Therapy, op cit, and King, op cite. For the most complete account of both the complex history and the resulting complex scaffold of committees, see Leroy Walters and Julie Gage Palmer, The Ethics of Human Gene Therapy, Oxford, New York, 1997
[11] Walter, Leroy, Senate testimony, Feb 2, 2000. As cited in King, op cite, page 382.
[12] King, op cit, p. 385
[13] Ibid, p. 385
[14] Ibid, p 386.
[15] King, op cit, pp 396, also Zoloth, "Ethics of the Mutable World" NAS Conference, San Francisco, August, 2005.
[16] Walter, Leroy, Ethics op cite
[17] There is an extensive literature on this topic that makes this argument: Eric Parens (beginning with a Hastings Center project, extending to a report in the Hastings Center Report, and followed by a book on the issue, makes the claim cogently, and Leon Kass and Francis Fukayama, in both individually authored books and in the President's Council Report make the claim as well.
[18] Maurer, Stephen, Lucas, Keith, Terrell, Starr, Goldman School of Public Policy, University of California at Berkeley, funded by the MacArthur foundation, 2006, for presentation at SynBio 2.0 Berkeley, California, May 2006.
[19] Leitenberg, Milton, "Assessing the Biological Weapons and Bioterrorism Threat," December, 2005, US Government. Pps 82-115.
[20] Ibid, p. 82.
[21] Ibid, p, 83.
[22] Zoloth, Laurie, "Reasonable Magic: Forbidden Knowledge and the Human Embryonic Stem Cell Debate," Kennedy Institute of Ethics Journal, Spring, 2000.
[23] Shattuck, Roger, Forbidden Knowledge
[24] A theological note is also in order: for theologians and religious ethicists would also note: one is in the presence of a witnessing Other, of God. The point is made more clearly in this sense.

PHYSICS MODELING OF AIRBORNE WEAPONS

Estimates of Outdoor Attacks using a Gaussian Plume Model

Keith V. Lucas, Ph.D.

October 2006

Information Technology & Homeland Security Project
The Richard & Rhoda Goldman School of Public Policy
University of California, Berkeley

ABSTRACT

This paper explores the use of simple analytic models to estimate the amount of biological agent required for a weapon of mass destruction. In particular, we estimate the amount of anthrax required for an outdoor attack using a Gaussian Plume Model under two different boundary conditions. For one set of parameters and general modeling assumptions, we estimate the amount of anthrax required to deliver an LD_{20} lethal dose 2,050 meters downstream from release to be 4 grams to 464 grams; the upper bound is more appropriate to areas densely populated with building, trees, and other objects that can absorb anthrax as it nears the ground. For identical conditions, we estimate the amount of anthrax required to kill 1,000 of 5,000 people distributed over a football-field sized area starting 2,000 meters downstream from release to be 4 grams to 452 grams. For the conditions explored, the amount of anthrax required to guarantee an average attack rate over the target area is similar to the amount required to deliver the same attack rate to a single point within the area. This is a consequence of the problem parameters. A larger target area and smaller distance between release and target would produce a larger difference between the two estimates.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The author is grateful to the following scientists for their assistance: Sanjiva K. Lele, Department of Aeronautics & Astronautics, Stanford University; Lynn Hildemann & Mark Jacobson, Department of Civil & Environmental Engineering, Stanford University; William S. Andrews, Department of Chemistry & Chemical Engineering, Royal Military College of Canada; and Lisa V. Lucas, Water Resources of the United States, U.S. Geological Survey.

INTRODUCTION

This work is motivated by a larger public policy question: how hard is it to create a biological "weapon of mass destruction" (WMD)? More specifically, is the amount of biological agent required to kill at least 1,000 people (our definition) prohibitively large to relegate its use to state-level actors, or is the amount required sufficiently small to enable its use by ad hoc groups? Would you need a bomb or an envelope? Large amounts of a biological agent typically require large-scale production, a daunting effort typically reserved to state-level actors. Smaller amounts, however, could be acquired through non-production means such as theft or black-market purchase, reducing barriers to entry. The answers to these questions require a multidisciplinary approach, including biology, atmospheric physics, and the security of anthrax repositories, to name a few.

This paper concentrates on the physics portion of the problem, defined from release to human exposure. We explore the use of simple analytic models to estimate the amount of a biological agent required for a WMD attack. In particular, we estimate the amount of anthrax required for an outdoor attack using a Gaussian Plume Model (GPM). GPMs generally assume statistically stationary, homogeneous turbulence[1] and "simple flows, such as unidirectional steady-state flow over relatively flat terrain."[2]

The GPM assumptions are not appropriate for all use cases. In some emergency planning and response analyses, for example, the questions of interest, the nature of the physical environment, or both may warrant higher-fidelity models that incorporate observational data, weather forecast predictions, and detailed flow physics calculated using computational fluid dynamic techniques.[2] For the public policy question at hand, however, we are interested in order-of-magnitude statistically averaged estimates – does a biological WMD attack require, on average, grams or kilograms? The GPM is sufficient for this purpose and provides a simple mathematical framework for analysis.

THE GAUSSIAN PLUME MODEL

Consider the release of anthrax powder from a building roof approximately 20 meters high. The wind blows steadily at 5 meters/second towards a crowd of 5,000 at an outdoor concert. The temperature, humidity, and other weather conditions prevent the anthrax from being driven rapidly to the ground or from being swept upward into the atmosphere. Consequently, the anthrax travels with the wind towards the crowd, subject primarily to the effects of gravity and atmospheric turbulence. The crowd is two kilometers downstream sitting in an area the size of a football field. The neighborhoods between the release point and the concert are residential, containing trees and low-lying houses. How much anthrax is needed to kill at least 1,000 people at the concert?

These conditions have sufficient physical predictability to permit the use of analytic modeling techniques. For example, we can estimate the amount of anthrax delivered to a downstream position using a Gaussian Plume Model (GPM), a fluid dynamics formulation that specifies downstream concentration as a function of wind speed, release height, and empirical models of turbulent transport. While the outdoor concert scenario may seem overly simplistic, it is not unrealistic. Similar conditions occurred in Sverdlovsk, U.S.S.R in April 1979, when a military microbiology laboratory released anthrax and killed approximately 68 people (see *Appendix: Sverdlovsk 1979*).

Scenarios that are more complex require more sophisticated and potentially less tractable models. These scenarios include winds that vary significantly in speed and direction, non-stationary target

victims, changing atmospheric stability conditions, long distances between release point and target, and intricate surface characteristics (*e.g.* buildings, trees, hills, and valleys). Techniques for modeling these scenarios range from three-dimensional unsteady fluid dynamic simulations (at the high-fidelity end) to multi-directional GPMs.[2,3] The choice depends on the conditions and questions being explored.

To answer public policy questions, we are interested in the order-of-magnitude statistically averaged estimates provided by a unidirectional GPM. We describe this approach and various modeling alternatives in the remainder of this section.

THE LAGRANGIAN FORM OF THE GAUSSIAN PLUME MODEL

The Gaussian Plume Model (GPM) specifies the concentration of a trace species* downstream of a release point as a function of source quantity, average wind velocity, and turbulent diffusion.† Two approaches exist for deriving the GPM: an Eulerian and a Lagrangian. In the Eulerian approach, the continuum equations of fluid dynamics are applied to a "control volume" to derive a solution. The Lagrangian approach follows the path a single trace particle, employing probability distributions to determine a statistical solution for the initial and boundary conditions. The two approaches yield similar and related GPM results. The following summary of the Lagrangian formulation follows from Seinfeld and Pandis;[1] we have adapted their discussion for a more general audience, but one with specific interest in airborne weapons.

THE TRANSITIONAL PROBABILITY DISTRIBUTION FUNCTION

Let $c(\vec{x},t)$ be the instantaneous concentration (moles per unit volume) of a trace species at position $\vec{x}$ and time t. From a statistical perspective, we are interested in the ensemble-averaged concentration, $\langle c(\vec{x},t)\rangle$, which is the concentration of the trace species at a given position and time averaged over many experimental trials. Physically, the concentration is a function of fluid dynamic conditions; we can also represent it, however, as a function of a positional probability distribution.

Let $\psi(\vec{x},t)$ be the probability that a *single* trace particle is inside the fluid volume $\vec{x}+d\vec{x}$ at time t, having been previously located at at $\vec{x}'$ at time $t'<t$. The ensemble mean concentration $\langle c(\vec{x},t)\rangle$ is then the sum of the probability density functions for *all* trace particles in the fluid:‡

$$\langle c(\vec{x},t)\rangle = \sum_{i=1}^{m} \psi(\vec{x},t) \tag{1}$$

where m is the number of particles in the fluid. In other words, $\langle c(\vec{x},t)\rangle$ is the aggregate probability that all trace particles in the fluid can be found in the volume $\vec{x}+d\vec{x}$ at time t. This probability density function, $\psi(\vec{x},t)$, is itself composed of two other probability density functions multiplied

* A trace species is transported passively by its fluid host and has a negligible effect on flow dynamics.

† Turbulent diffusion is not the same mechanism as molecular diffusion, though it shares a common term. Molecular diffusion is a process by which fluid dynamic properties, such as momentum and energy, are transferred molecule to molecule. Turbulent diffusion is the transport of macroscopic flow constituents, such as a trace species, by turbulent eddies in the flow – it is the fluid dynamic transport of material by statistically random flow features.

‡ Reference 1, page 883.

together and integrated over three-dimensional space. The resulting formulation (see Seinfeld and Pandis) accounts for all particles initially in the fluid and those introduced between times t' and t:*

$$\langle c(\vec{x},t)\rangle = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} Q(\vec{x},t\,|\,\vec{x}_o,t_o)\cdot\langle c(\vec{x}_o,t_o)\rangle\cdot d\vec{x}_o + \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\int_{to}^{t} Q(\vec{x},t\,|\,\vec{x}',t')\cdot S(\vec{x}',t')\cdot d\vec{x}'dt' \qquad (2)$$

Assumptions

- No chemical reactions
- Trace species has negligible impact on flow dynamics

In Equation (2), $Q(\vec{x},t\,|\cdots)$ is the *transition probability density function*,† $S(\vec{x}',t')$ is a source of trace species located at $\vec{x}'$ at time t' (a sink is a negative source), $\vec{x}_o$ is a point in space at the initial time t_o, and $\vec{x}'$ is a point in space at an arbitrary time t', where $t_o < t' < t$.

Equation (2) is the general result for any trace species not undergoing chemical reaction.‡ At this point, no assumptions have been made about *how* the trace particles are transported by the fluid. Particles are transported by an unspecified velocity field whose effect is captured in the transition probability density function $Q(\vec{x},t\,|\cdots)$, which is also unspecified. Indeed, knowing the probability density function for a specific set of conditions is analogous to knowing the fluid dynamic solution for the flow field. This is the approach taken in Seinfeld and Pandis to derive the GPM.§

The first term in Equation (2) is the contribution to the ensemble-averaged concentration by the trace species initially in the fluid. Under general conditions, the fluid has an initial concentration of trace species distributed throughout space in some way; the species may be concentrated in a small volume or distributed along a line, across a plane, or in any other manner. The integrand of the first term expresses the likelihood that an infinitesimal element of trace species initially at $\vec{x}_o$ resides in $\vec{x}+d\vec{x}$ at time t; the volume integral aggregates the effects of all infinitesimal elements initially in the fluid over all space.

The second term in Equation (2) is the contribution to the ensemble-averaged concentration by sources of trace species distributed in both time and space. Under general conditions, sources of trace species may turn on or off, may move relative to the mean fluid flow, may be positive (source) or negative (sink), and may be located anywhere in the fluid. The integrand of the second term expresses the likelihood that an infinitesimal element of trace species released at $(\vec{x}',t')$ resides in $\vec{x}+d\vec{x}$ at time t; the volume integral aggregates the effects of all infinitesimal elements released in the fluid over all space and time.

While Equation (2) is not used directly to derive the GPM,[1] an important analogy can be made between the probability density function and the flow's velocity field, as discussed below. As such, Equation (2) provides an important interpretation of Gaussian Plume models.

---

* Reference 1, page 884.

† Reference 1, page 883.

‡ Reference 1, page 886.

§ Reference 1, pages 890 – 892.

CONSERVATION OF TRACE SPECIES MASS

In fluid dynamic terms, a trace species in a fluid is a passive scalar: it is transported by the velocity field and has a negligible effect on flow dynamics. Formally, the evolution of the trace species through the flow field is governed by the advection equation:[*]

$$\frac{\partial c}{\partial t}+\frac{\partial}{\partial x}(uc)+\frac{\partial}{\partial y}(vc)+\frac{\partial}{\partial z}(wc)=S(x,y,z,t) \tag{3}$$

where x, y, z are the spatial coordinates in the stream-wise, cross-stream, and vertical directions, respectively; u, v, w are the flow velocities in the x, y, z directions, respectively; and $S(x,y,z,t)$ represents sources and sinks of trace species. Equation (3) states that the mass of a trace species is conserved as it is transported by the fluid, with changes in mass due solely to sources and sinks.

The Gaussian Plume Model follows from Equation (3) given three assumptions:[†] (a) the velocity field is a stationary random process;[‡] (b) the probability density function for the flow's random velocity field is a Gaussian distribution; and (c) the "maximum correlation between the velocities at two times occurs when those time are equal."[§] The second assumption is valid in the case of stationary, homogenous turbulence.[**]

For the case of an initial unit-strength pulse of trace species released at height h in a flow with non-zero x-directed mean velocity and zero y- and z-directed mean velocities, Equation (3) yields the following form of the Gaussian Plume Model:[††]

$$\langle c(x,y,z,t)\rangle=\frac{1}{(2\pi)^{3/2}\sigma_x(t)\cdot\sigma_y(t)\cdot\sigma_z(t)}\times\exp\left(-\frac{(x-\bar{u}t)^2}{2\cdot\sigma_x^2(t)}-\frac{y^2}{2\cdot\sigma_y^2(t)}-\frac{(z-h)^2}{2\cdot\sigma_z^2(t)}\right) \tag{4}$$

The Gaussian Plume Assumptions

- Velocity field is a stationary, random process (*e.g.* stationary, homogeneous turbulence).
- Velocity field has a Gaussian probability distribution function.
- Maximum correlation between two velocities occurs at the same time.
- Trace species is a passive scalar (it has a negligible effect on the flow).

Other Assumptions

- Mean flow has non-zero x-directed velocity and zero y- and z-directed velocities.
- Trace species is released as a pulse of unit strength at time $t=0$.

---

[*] Reference 1, pages 890 – 891.

[†] Reference 1, page 890.

[‡] A stationary random process is one in which the statistical properties of a variable at two different times depend only on the time difference and not on the individual values of time. For more details, see Reference 1, page 890.

[§] Reference 1, page 890.

[**] Reference 1, page 892. Stationary, homogeneous turbulence is sometimes referred to as "box turbulence;" it is a stationary random process that is statistically identical at every point in space (i.e. homogeneous).

[††] Reference 1, page 892.

- No boundaries present in the flow ($z = 0$ is not a surface).

In Equation (4), $\bar{u}$ is the mean velocity in the stream-wise direction, and σ_x, σ_y, and σ_z are the dispersion coefficients in the stream-wise, cross-stream, and vertical directions, respectively; the source is located at $(0,0,h)$ in (x,y,z) coordinates.

The dispersion coefficients σ_x, σ_y, and σ_z model the spreading of the plume in the x, y, and z directions, respectively. For the Gaussian velocity distribution assumed, the dispersion coefficients are explicit functions of time and the velocity distribution variance. In practice, however, the dispersion coefficients have empirical relations that change with atmospheric stability conditions (see *Dispersion Coefficients* for more details).

Equation (4) states the important result that "the mean concentration of a tracer released in a flow where the velocity is a stationary, Gaussian process has a distribution that is, itself, Gaussian."* This establishes a link between Equation (4), the solution to a fluid dynamic equation, and Equation (2), an expression of probability.

RELATING THE TWO EQUATIONS

The physics of Equation (4) are encapsulated in the probability density function $Q(\vec{x},t|\cdots)$ of Equation (2). $Q(\vec{x},t|\cdots)$ is not an arbitrary probability density function, but a specific function that articulates a solution to the appropriate governing equation of fluid dynamics, namely Equation (3). For the conditions leading to Equation (4), the trace species terms in Equation (2) are:

$$\langle c(\vec{x}_o,t_o)\rangle = 0 \text{ (no initial concentration), and} \tag{5}$$

$$S(x',y',z',t') = \delta(x-0)\cdot\delta(y-0)\cdot\delta(z-h)\cdot\delta(t-0) \tag{6}$$

Where $\delta(x-0)$ is the Dirac Delta Function, defined as 1 at $x = 0$ and 0 otherwise. Equation (6) is an instantaneous point source, or *pulse*, at $z = h$. Using these simplifications and comparing Equations (2) and (4) yields:

$$Q(x,y,z,t|x',y',z',t') = \frac{1}{(2\pi)^{3/2}\sigma_x\cdot\sigma_y\cdot\sigma_z}\times\exp\left(-\frac{((x-x')-\bar{u}(t-t'))^2}{2\cdot\sigma_x^2}-\frac{(y-y')^2}{2\cdot\sigma_y^2}-\frac{((z-z')-h)^2}{2\cdot\sigma_z^2}\right) \tag{7}$$

Equation (7) is the probability density function for the specific conditions and assumptions of Equation (4). It is a Gaussian distribution and it represents the fluid dynamic advection of a passive scalar. While it could be used in Equation (2) to calculate the trace species concentration at a given position and time, Equation (4) is a simpler equation for achieving the same results.

* Reference 1, page 891.

SUPERPOSITION OF GPM SOLUTIONS

Equation (3) is linear in trace species concentration. Consequently, its solutions can be superposed to form compound solutions. Equation (4) is one specific solution to Equation (3) for the case of an initial unit-strength pulse released at height h in a flow with non-zero x-directed mean velocity and zero y- and z-directed mean velocities. Other solutions follow from other source conditions.

For example, the concentration for an initial release height of $-h$ for the same flow field yields:

$$\langle c(x,y,z,t)\rangle = \frac{1}{(2\pi)^{3/2}\sigma_x(t)\cdot\sigma_y(t)\cdot\sigma_z(t)}\times\exp\left(-\frac{(x-\bar{u}t)^2}{2\cdot\sigma_x^2(t)}-\frac{y^2}{2\cdot\sigma_y^2(t)}-\frac{(z+h)^2}{2\cdot\sigma_z^2(t)}\right) \tag{8}$$

The solution for two initial sources, one at height h and another at $-h$ is simply the sum of Equations (4) and (8):

$$\langle c(x,y,z,t)\rangle = \frac{1}{(2\pi)^{3/2}\sigma_x(t)\cdot\sigma_y(t)\cdot\sigma_z(t)}\times\exp\left(-\frac{(x-\bar{u}t)^2}{2\cdot\sigma_x^2(t)}-\frac{y^2}{2\cdot\sigma_y^2(t)}\right)\times\left[\exp\left(-\frac{(z-h)^2}{2\cdot\sigma_z^2(t)}\right)+\exp\left(-\frac{(z+h)^2}{2\cdot\sigma_z^2(t)}\right)\right] \tag{9}$$

Superposition is a powerful tool in GPM analysis, enabling us to solve a single complicated problem by solving multiple smaller, less-complicated problems. Equation (9), for example, is the model for a single unit-strength pulse of trace species released at height h above a totally-reflecting ground plane located at $z=0$; a totally-reflecting ground plane means that all trace elements striking the ground bounces back into the plume.

SLENDER PLUME FORMULATION

The GPM solution to Equation (3) takes on a special form in the limit $\sigma_x \to 0$ for a continuous point source of strength q. For a continuous source, the source term, S, in Equation (3) is $q\cdot\delta(x-0)\cdot\delta(y-0)\cdot\delta(z-h)$. The units of q are mass per unit time – q is the rate at which trace species is introduced into the flow.

At this limit, Equation (3) yields the slender plume approximation:*

$$\langle c(x,y,z,t)\rangle = \frac{q}{2\pi\cdot\bar{u}\cdot\sigma_y\cdot\sigma_z}\times\exp\left(\frac{-y^2}{2\cdot\sigma_y^2}\right)\times\exp\left(\frac{-(z-h)^2}{2\cdot\sigma_z^2}\right) \tag{10}$$

Assumptions

- The Gaussian Plume Assumptions
- Mean flow has non-zero x-directed velocity and zero y- and z-directed velocities.
- Trace species is released as a continuous point source of strength q
- $\sigma_x \to 0$ (zero dispersion in the stream-wise direction).

* Reference 1, pages 917 – 918.

GROUND REFLECTION & ABSORPTION

Equations (4) and (10) are appropriate in the absence of surfaces, such as a ground plane at $z = 0$. Surfaces can be modeled using the principle of superposition: add multiple point, line, and surface sources together to mimic the effects of surfaces within the flow field. This modeling approach is analogous to elementary aerodynamic analyses of potential flow around airfoils and other shapes.

TOTALLY REFLECTING GROUND PLANE

A *totally reflecting* surface at $z = 0$ can be modeled by mirroring the point sources above the ground plane with sources of equal strength and position below the ground plane. Hence, a trace element crossing the ground plane from above is compensated by another trace element crossing the plane from below at the same point in the (x, y) plane and at the same time; this gives the appearance of total reflection from either above or below the ground plane.

The mathematical expression for a *totally reflecting plane* at $z = 0$ for the *slender plume* approximation and a point source at $z = h$ is:

$$\langle c(x,y,z,t)\rangle = \frac{q}{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z} \times \exp\left(\frac{-y^2}{2 \cdot \sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2 \cdot \sigma_z^2}\right) + \exp\left(\frac{-(z+h)^2}{2 \cdot \sigma_z^2}\right)\right] \tag{11}$$

Assumptions

- Gaussian Plume Assumptions
- Mean flow has non-zero x-directed velocity and zero y- and z-directed velocities.
- Trace species is released as a continuous point source of strength q
- $\sigma_x \to 0$ (zero dispersion in the stream-wise direction).
- Totally reflecting boundary at $z = 0$.

TOTALLY ABSORBING GROUND PLANE

The mathematical expression for a *totally absorbing plane* at $z = 0$ for the *slender plume* approximation and a point source at $z = h$ is:

$$\langle c(x,y,z,t)\rangle = \frac{q}{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z} \times \exp\left(\frac{-y^2}{2 \cdot \sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2 \cdot \sigma_z^2}\right) - \exp\left(\frac{-(z+h)^2}{2 \cdot \sigma_z^2}\right)\right] \tag{12}$$

Assumption

- Gaussian Plume Assumptions
- Mean flow has non-zero x-directed velocity and zero y- and z-directed mean velocities.
- Trace species is released as a continuous point source of strength q
- $\sigma_x \to 0$ (zero dispersion in the stream-wise direction).
- Totally absorbing boundary at $z = 0$.

where a point source of negative strength is placed below the ground plane to "remove" trace elements permanently from the flow field once they cross the ground plane. The negative source is needed because a single source solution (one at $z = h$) allows for the statistical possibility that trace elements moving below the $z = 0$ plane can re-enter the flow above the $z = 0$ plane downstream; a two-source solution such as Equation (12) does not allow for this possibility.

A NOTE ON "GROUND PLANE" BOUNDARY CONDITIONS

The boundary conditions expressed in Equations (11) and (12) apply solely at $z = 0$, defining a precise location of the ground reflection/absorption plane. This precision is idealistic. In reality, the boundary "plane" is a boundary "layer" – the physical elements responsible for absorbing trace species, such as building, trees, and pools, act over a vertical distance from 0 to 10 or more meters. For the *totally reflecting* boundary condition, this has little consequence; that condition simply ensures that any trace species falling below the $z = 0$ plane is mathematically returned to the aboveground plume. The *totally absorbing* boundary condition is another matter. This condition removes the statistical possibility that an element passing through the $z = 0$ plane will appear downstream. In reality, however, this removal occurs throughout the boundary layer, not simply at the ground plane. Hence, the *totally absorbing* boundary condition likely *over estimates the amount of trace species migrating downstream*, statistically removing only those trace elements that reach $z = 0$, when in reality some elements should be removed before striking the ground. This means that *the totally absorbing boundary condition likely under estimate the source strength* required for a given downstream concentration.

DISPERSION COEFFICIENTS

The dispersion coefficients in Equation (4) and similar equations model the spreading of a Gaussian plume in the x, y, and z directions, respectively. Physically, the atmospheric dispersion coefficients are functions of turbulent transport and atmospheric stability. Mathematically, the dispersion coefficients are functions of time, which for the case of x-directed mean flow translates into functions of x. For the Gaussian velocity distribution assumed in Equation (4), the dispersion coefficients are also functions of the velocity distribution variance. In practice, however, the dispersion coefficients have empirical relations that change with atmospheric stability conditions.

The empirical nature of the dispersion coefficients may be responsible for the usage of GPMs in applications that may not otherwise be appropriate. According to Seinfeld and Pandis, "the justification for these applications is that the dispersion parameters … have been derived from concentrations measured in actual atmospheric diffusion experiments approximating those of the application."* The most commonly used coefficients are the Pasquill-Gifford curves for stability conditions ranging from extremely unstable to moderately stable.† Typically, atmospheric conditions such as temperature, humidity, time of day, and vertical wind shear determine the stability conditions; the appropriate dispersion coefficients are then used in a GPM to calculate trace species concentration as a function of downstream position.

The dispersion coefficients have units of length and generally vary with downstream conditions as powers of x. For the Pasquill-Gifford curves, the values of the y and z coefficients range from the same order-of-magnitude as the downstream distance to one or more orders-of-magnitude less. The

* Reference 1, page 926.

† Reference 1, page 927. This reference contains power-law approximations of these parameters for analytic modeling.

coefficients for neutral stability, for example, are approximately 70 meters and 30 meters for the y and z coefficients, respectively, at about 1,000 meters downstream of the source. Unstable atmospheric conditions produce wider plumes, while stable conditions produce narrower plumes.

Because empirical dispersion coefficients are measured in actual atmospheric experiments, the coefficients include gravitational effects, albeit to a limited extent. The vertical dispersion coefficient models spreading caused by both gravity and turbulent diffusion. For short distances downstream of release, vertical diffusion is approximately symmetric about the release plane. As the plume travels further downstream, however, its centerline, which defines mean concentration, bends downward under the action of gravity (in the absence of atmospheric instabilities). Eventually, the deviation from the release plane is sufficient to render a GPM approach invalid. As such, GPMs are typically used over shorter distances. Longer distances require an explicit modeling of gravity.

EXAMPLES

ESTIMATING THE SOURCE STRENGTH FOR A DESIRED POINT LD-VALUE

Let us return to the outdoor concert problem initially posed; the parameters are listed in Table 1. To these, we add the following from Meselson:[5,7] human breathing rate is approximately 0.0005 meters^3/second, the LD_{20} dosage for humans is 14,487 spores,[*] and anthrax contains approximately 10^9 spores per milligram. Additionally, we use the Brigg's dispersion coefficients for neutral atmospheric stability, also from Meselson.

For the problem described in Table 1, how much anthrax is required at the source to deliver a single LD_{20} dose at a specific location?

We begin with the *slender plume* GPM. Equations (11) and (12) can be generalized as:

$$\langle c(x,y,z,t)\rangle = \frac{q}{2\pi\cdot\bar{u}\cdot\sigma_y\cdot\sigma_z}\times\exp\left(\frac{-y^2}{2\cdot\sigma_y^2}\right)\times\left[\exp\left(\frac{-(z-h)^2}{2\cdot\sigma_z^2}\right)\pm\exp\left(\frac{-(z+h)^2}{2\cdot\sigma_z^2}\right)\right] \tag{13}$$

Assumptions

- Gaussian Plume Assumptions
- Mean flow has non-zero x-directed velocity and zero y- and z-directed velocities
- Trace species is released as a continuous point source of strength q
- $\sigma_x \to 0$ (zero dispersion in the stream-wise direction)
- Totally reflecting ($\pm \to +$) / absorbing ($\pm \to -$) boundary at $z = 0$

[*] LD_{20} is a single dose that kills approximately 20% of the people receiving it. For example, the LD_{20} dose for anthrax is 14,487 spores (using the independent action dose-response model described in *Appendix: Sverdlovsk 1979*); giving each of 100 people 14,487 spores would kill 20 people on average.

PARAMETER	SYMBOL	UNITS	VALUE
Release Height	h	Meters	20
Wind Speed	$\bar{u}$	Meters / second	5
Wind Direction	-	-	x direction (downstream)
Atmospheric Stability	-	-	Neutrally Stable
Dispersion Coefficients	σ_y, σ_z	Meters	Brigg's coefficients for neutral atmospheric stability (references 5 and 7)
Slender Plume Assumption	-	-	$\sigma_x \to 0$
Crowd Size	N_p	Number of people	5,000
Target Area	-	Square Meters	100 x 50 (little larger than football field)
Target Position	(x_c, y_c, z_c)	Meters	(2,000, 0, 0) (beginning of target area)
Surface Characteristics	-	-	Residential (trees and low-lying houses)
Human Height	h_{human}	Meters	1.75
Human Breath Volume	V_{breath}	Meters^3	0.0005 (references 5 and 7)
LD_{20} Dose	LD_{20}	Number of spores	14,487 (references 5 and 7)
Lethal Dose / Breath	-	-	1
Anthrax Mass	M	Grams / spore	10^{-12} (references 5 and 7)

Table 1: Parameters for sample problem.

We can rearrange Equation (13) to solve for the source strength:

$$q = \frac{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z \cdot \langle c(x,y,z,t) \rangle}{\exp\left(\frac{-y^2}{2 \cdot \sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2 \cdot \sigma_z^2}\right) \pm \exp\left(\frac{-(z+h)^2}{2 \cdot \sigma_z^2}\right)\right]} \tag{14}$$

This gives the source strength in terms of a specified target concentration. To relate this concentration to an LD_{20} value, we must make an additional assumption about how the target concentration is absorbed. Specifically, is a lethal dose of anthrax inhaled in a single breath or in multiple breaths? We assume a single lethal breath, which results in a *minimal source-strength prediction* – the least amount of source anthrax needed to deliver the desired dose to the desired location. For this assumption:

$$\langle c(x,y,z,t) \rangle = M \cdot LD_{20} / V_{breath} \tag{15}$$

Hence, Equation (14) becomes:

$$q = \frac{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z \cdot \left(M \cdot LD_{20}/V_{breath}\right)}{\exp\left(\frac{-y^2}{2\cdot\sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2\cdot\sigma_z^2}\right) \pm \exp\left(\frac{-(z+h)^2}{2\cdot\sigma_z^2}\right)\right]} \tag{16}$$

Assumptions

- Gaussian Plume Assumptions
- Mean flow has non-zero x-directed velocity and zero y- and z-directed velocities
- Trace species is released as a continuous point source of strength q
- $\sigma_x \to 0$ (zero dispersion in the stream-wise direction)
- Totally reflecting ($\pm \to +$) / absorbing ($\pm \to -$) boundary at $z = 0$
- Minimal source-strength prediction – single lethal dose

Where the Brigg's dispersion coefficients for atmospheric neutral stability are: [5,7]

$$\sigma_y = \frac{0.08 \cdot \mathrm{x}}{\sqrt{1 + 0.0001 \cdot \mathrm{x}}} \quad , \quad \sigma_z = \frac{0.06 \cdot \mathrm{x}}{\sqrt{1 + 0.0015 \cdot \mathrm{x}}} \tag{17}$$

Let us consider the solution at the middle of the concert field at the level of human height: (x, y, z) = (2,050, 0, 1.75). Using these and the other values in Table 1,

$$\sigma_y = \frac{0.08 \cdot 2050}{\sqrt{1 + 0.0001 \cdot 2050}} = 149.000$$

$$\sigma_z = \frac{0.06 \cdot 2050}{\sqrt{1 + 0.0015 \cdot 2050}} = 60.931$$

$$q = \frac{2\pi \cdot 5 \cdot 149 \cdot 60.931 \cdot \left(10^{-12} \cdot 14{,}487/0.005\right)}{\exp\left(\frac{-0^2}{2\cdot 149^2}\right) \times \left[\exp\left(\frac{-(1.75-20)^2}{2\cdot 60.931^2}\right) \pm \exp\left(\frac{-(1.75+20)^2}{2\cdot 60.931^2}\right)\right]}$$

Or,

$$q_{reflecting} = 4 \cdot grams^{*}$$

$$q_{absorbing} = 464 \cdot grams^{*}$$

[*] For reproducibility purpose, the values calculated were 4.374 grams for the reflecting boundary conditions and 463.984 grams for the absorbing boundary condition.

A change in boundary condition from *totally reflecting* to *totally absorbing* changes our estimate of the minimal source-strength by two orders of magnitude. Our estimate of the source-strength using a slender plume GPM is thus very sensitive to the ground plane conditions. We interpret the reflecting and absorbing results as lower- and upper-bound estimates, respectively, as defined within the context of our model. Specifically, we would interpret the results as follows: for the parameters and assumptions listed Table 1 and the general assumptions of Gaussian Plume Models, we estimate the amount of anthrax required to deliver an LD_{20} lethal dose 2,050 meters downstream to be approximately 4 grams to 464 grams.

This range can be narrowed with a further consideration: a preference for the *totally absorbing* boundary condition. A *totally reflecting* boundary condition assumes that none of the anthrax striking the ground between the source and the target remains on the ground – not in puddles, on damp leaves, or inside buildings. Conversely, the *totally absorbing* condition assumes that all of the anthrax sticks to the ground on contact, significantly increasing the amount of anthrax at the source required for a specified target dose. While both of these conditions represent ideals extremes, the *totally absorbing* condition seems more appropriate for the more densely populated areas (*e.g.* buildings and trees) that invite attack. The results are also consistent with those of U.S. Military Intelligence analysts for similar scenarios (see *Appendix: Sverdlovsk 1979*).

ESTIMATING THE SOURCE STRENGTH FOR A DESIRED AREA LD-VALUE

For the problem defined in Table 1, how much anthrax is required to kill 1,000 people at the concert? We have supposed that 5,000 people are distributed within an area roughly the size of a football field. Continuing with our approach of making a *minimal source-strength prediction*, killing 1,000 of 5,000 people translates into an average dose of LD_{20} for the entire field. According the Meselson *et al*, this corresponds to an average dose of 14,487 spores of anthrax for each person (using the independent action dose-response model described in *Appendix: Sverdlovsk 1979*),[5,7] or a total of 72,435,000 spores for the entire target population.*

It is not possible to proceed further without assuming a target population distribution – because the delivered dose varies in all three spatial dimensions, the location of each person on the field must be known. We assume a uniform distribution of people on a rectangular field, an assumption we will revisit at the end of this analysis. Under these conditions, the 5,000 people are arranged in a grid, 100 evenly spaced in the x direction by 50 evenly spaced in the y direction.

By definition, the Gaussian Plume Model has a Gaussian distribution in it spatial dimensions. As such, it is not possible to define a single LD value for all points in space – the LD values, or doses, vary exponentially in space. Thus, we seek a source strength that delivers an *average* LD value over the target area. We start by rewriting Equation (16) as an equation for dose:

$$dose = \frac{q \cdot \exp\left(\frac{-y^2}{2 \cdot \sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2 \cdot \sigma_z^2}\right) \pm \exp\left(\frac{-(z+h)^2}{2 \cdot \sigma_z^2}\right)\right]}{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z \cdot \left(M/V_{breath}\right)} \tag{18}$$

* An LD_{20} dose kills, on average, 20% of the people who receive it. To kill 20% of 5,000 people, all 5,000 people need to receive an LD_{20} dose. For the LD_{20} value of 14,487, the total dose is 5,000 x 14,487 or 72,435,000 spores.

where we have replaced LD_{20} with *dose* because the dose is an unknown in Equation (18). The average dose is simply 1/5,000 multiplied by the sum of Equation (18) applied at each of the 5,000 points in our 100x50-person grid:

$$\bar{d} = \frac{1}{N_p}\sum dose = \frac{1}{N_p}\sum \frac{q \cdot \exp\left(\frac{-y^2}{2\cdot\sigma_y^2}\right)\times\left[\exp\left(\frac{-(z-h)^2}{2\cdot\sigma_z^2}\right)\pm\exp\left(\frac{-(z+h)^2}{2\cdot\sigma_z^2}\right)\right]}{2\pi\cdot\bar{u}\cdot\sigma_y\cdot\sigma_z\cdot\left(M/V_{breath}\right)} \tag{19}$$

Where N_p is the number of people in the target area. Moving the constants outside of the summation and solving for q, we have an equation for the source strength required to deliver an average dose over the target area.

$$q = \frac{2\pi\cdot\bar{u}\cdot\left(M\cdot LD_{20}/V_{breath}\right)\cdot N_p}{\sum \frac{\exp\left(\frac{-y^2}{2\cdot\sigma_y^2}\right)\times\left[\exp\left(\frac{-(z-h)^2}{2\cdot\sigma_z^2}\right)\pm\exp\left(\frac{-(z+h)^2}{2\cdot\sigma_z^2}\right)\right]}{\sigma_y\cdot\sigma_z}} \tag{19}$$

Performing the calculation, we have:

$$q_{reflecting} = 4\cdot grams^{*}$$

$$q_{absorbing} = 452\cdot grams^{*}$$

The amount of anthrax required to guarantee an average attack rate over the target area is similar to the amount required to deliver the same attack rate to a single point within the area. This is a consequence of the problem parameters. The distance between release and target is sufficiently large to necessitate a significant number of spores at the source (approximately 4×10^{12} to 5×10^{15}). As a result, there are more than enough spores delivered to the target area to achieve an LD_{20} value at a single point and as an average over the entire area. A larger target area and smaller distance between release and target would produce a more significant difference between the two estimates.

As a numerical check, we used Equation (18) to calculate the total dose delivered by each estimated source strength. As expected, the total dose, summed over all points in the 5,000-person grid, sums to the 72,435,000 spores required to kill 20% of the population (for both boundary conditions).

Finally, let us revisit the logic of an evenly distributed crowd. In reality, the crowd is not evenly distributed. Fortunatley, this calculation is not very sensitive to the crowd's distribution, something that is not necessarily true under general conditions. In this case, the per-person source-strength varies from 4.218 grams to 4.593 grams over the evenly distributed crowd for the *totally reflecting* boundary condition; this is a range of 9% of the 4-gram average. For the *totally absorbing* boundary condition, the per-person source strength varies from 433.859 grams to 502.004 grams, a range of

* For reproducibility purposes, the values calculated were 4.265 grams for the reflecting boundary condition and 452.099 grams for the absorbing boundary condition.

15% of the 452-gram average. This sensitivity (9% to 15% in predicted source strength) is a function of target area; we expect greater variation for larger target areas and less variation for smaller target areas.

"THE BIOWEAPONEERS" & AEROSOL TERMINAL VELOCITY

In a 1998 New Yorker article about biological weapons entitled "The Bioweaponeers",[4] Bill Patrick, an American bioweapons expert, and Ken Alibek, a former U.S.S.R. bioweapons expert, claimed that a 10 to 12 mile-per-hour wind could transport powdered toxin 18 to 20 miles downstream. How is this estimate made?

A simple estimate can be made by calculating the distance trace particles travel at a mean wind speed before striking the ground under the action of gravity. Particles, like all objects falling to Earth, reach a *terminal velocity* at which the force of gravity is countered exactly by drag forces. Using this velocity:

$$x = \bar{u} \cdot t \approx \bar{u} \cdot \left(\frac{h}{V_T} \right) \tag{20}$$

where x is the distance traveled, $\bar{u}$ is the mean wind speed, h is the release height of the particle, and V_T is the particle's terminal velocity.

The terminal velocity for particles traveling at Reynolds numbers* below 0.1 is:†

$$V_T = \frac{1}{18} \cdot \frac{D_p^2 \cdot \rho_D \cdot g \cdot C_c}{\mu} \tag{21}$$

where D_p is the particle's diameter, ρ_D is the *spherical particle density*, g is gravity, C_c is the *slip convection effect*, **Error! Bookmark not defined.** and μ is the fluid viscosity.‡ Equation (21) is the ratio of gravitational forces to drag forces.

Assuming particles 5 microns in diameter (typical of airborne toxins) traveling through air at 1 atmosphere pressure and 298 degrees Kelvin (standard conditions), the Reynolds number is approximately 1.11 x 10^{-3}, $C_c = 1.012$, $\rho_D = 1 \cdot g/cm^3$, and $\mu = 1.72 \times 10^{-4}\ g/cm/s$.§ Gravity is 980 cm/s^2. Using these values in Equation (21) yields a terminal velocity of 7.21 mm/s.

Assuming a mean velocity of 5 meters/second (11.2 miles/hour) and a release height of 45 meters, Equation (20) yields a travel distance of 31 kilometers or 19 miles. This calculation is a function of atmospheric conditions, particle size, release height, and mean wind velocity. The "Bioweaponeers" articles supplied only the wind velocity and the estimated travel distance. The other parameters have been chosen for illustrative purposes, but are reasonable values.

* The Reynolds number is the ratio of inertial forces to viscous forces in a flow, determined using characteristic dimensional scales. A low Reynolds number implies a high ratio of viscous effects to inertial effects.

† Reference 1, Chapter 8.

‡ Reference 1, page 464.

§ Using various tables in Reference 1, Chapter 8.

APPENDIX: SVERDLOVSK 1979

In "1979, an unusual anthrax epidemic occurred in Sverdlovsk, Union of Soviet Socialist Republics. Soviet officials attributed it to consumption of contaminated meat. U.S. agencies attributed it to inhalation of spores accidentally released at a military microbiology facility in the city. Epidemiological data show that most victims worked or lived in a narrow zone extending from the military facility to the southern city limit. Farther south, livestock died of anthrax along the zone's extended axis. The zone paralleled the northerly wind that prevailed shortly before the outbreak. It is concluded that the escape of an aerosol of anthrax pathogen at the military facility caused the outbreak."[5]

THE MESELSON INVESTIGATION

In 1992, less than a year after the collapse of the Soviet Union, President Boris Yeltsin committed Russia to the 1972 biological weapons convention. In June of that year, Russia permitted Matthew Meselson, a Harvard biologist, to lead a research team to investigate the Sverdlovsk incident that had occurred 13 years earlier; Meselson and his team returned in 1993 for a follow up visit.[6]

According to Meselson *et al*,[5] "Compound 19", a microbiology facility, released an aerosol of anthrax pathogen on April 2, 1979.[*] The prevailing winds carried the anthrax downstream, killing 68 people in a "high-risk zone" of approximately 0.7 square miles. The first cases of illness appeared within 2 to 3 days; people and livestock were affected up to 4 km and 5 km, respectively.[5]

At the time, Sverdlovsk was a city of approximately 1.2 million, and the high-risk zone downstream of Compound 19 had a population density of approximately 10,000 people per square mile.[5,7] While these numbers suggest an attack rate of approximately 1%,[†] Meselson estimated the rate slightly higher at approximately 2% at a ceramics factory facility located approximately 2.8 kilometers downstream of Compound 19.[‡] This higher rate is reasonable because the incident occurred during the day, when many people were likely away from home. Meselson also concluded that "most or all infections resulted from the escape of anthrax pathogen on [April 2]. Owing to the inefficiency of aerosol disposition and resuspension, few if any inhalatory infections are likely to have resulted from secondary aerosols on subsequent days. A single date of inhalatory infection is also consistent with the steady decline of onsets of fatal cases in successive weeks."[5]

ANALYTIC PREDICTIONS

Meselson *et al* conducted a three-step calculation to estimate the amount of anthrax released at Compound 19:[5]

1. estimate the attack rate at a specific location (*e.g.* 2% at a ceramics factory facility located approximately 2.8 kilometers downstream of Compound 19);

---

[*] According to Wampler and Blanton (Reference 6), this was due to a "failure by maintenance personnel to replace a critical filter in a vent serving the anthrax production facility."

[†] Sixty-eight people died in a region of 0.7 square kilometers and a population density of 10,000 per square mile; using these numbers, an estimate of the overall attack rate is 68 / (0.7 * 10,000) or ~1%.

[‡] Meselson's primary estimate of a 2% attack rate was based on 10 deaths in a ceramics factory facility of 450 people, located approximately 2.8 kilometers downstream of Compound 19. In a follow up analysis (Reference 7), Meselson estimated rates for other locations, concluding that "the attack rate of 1-2% recorded at the ceramics factory is consistent with the rates estimated for locations upwind and downwind of it, providing some assurance that the ceramics factory rate used in the article for source strength estimation is not anomalous."

2. estimate the dosage at this location using a dose-response model and the attack rate;
3. estimate the source-strength using a Gaussian slender-plume model and the dosage.

DOSE-RESPONSE RELATION

According to Meselson, the dose-response model is the most uncertain part of the calculation. "Even if there were an agreed dose-response relation for non-human primates, which there is not, there would remain uncertainty regarding the relation applicable to the actual population at risk and the particular aerosol encountered at Sverdlovsk." [5]

Dose (spores)	Log-Normal (attack rate)	Independent Action (attack rate)
8000	0.500	0.115
4000	0.417	0.059
2000	0.337	0.030
1000	0.264	0.015
500	0.200	0.008
250	0.146	0.004
125	0.103	0.002
60	0.068	
30	0.045	
15	0.028	
8	0.018	
4	0.011	
2	0.006	
1	0.003	

Table 2: Dose-response relations for log-normal and independent action models. Doses are listed in number of anthrax spores; attack rates are listed as the proportion killed in the test population.

Meselson employed two models to estimate the anthrax dosage at the ceramics factory for the estimated attack rate, or *response*, of 2%. The first was a "log-normal" model for an LD_{50}* value of 8,000 spores and a slope of 0.7 probits† per log dose. The second was an "independent action" model for an LD_{50} value of 45,000 spores. These models are depicted in Table 2.

According to Meselson, the log-normal model "allows for heterogeneity in susceptibility among individuals", while the independent action model assumes homogeneous susceptibility and "that spores act independently, not cooperatively, in the initiation of inhalation anthrax".[7] The models are a combination of parameters from separate experiments with humans and two different types of monkeys; the human parameter (LD_{50} of 8,000 spores) is from the U.S. Department of Defense.[7]

Table 3 shows the dose response for various attack rates, including a prediction of a WMD magnitude incident and an LD_{50} incident.

Attack Rate (lethal dose)	Log-Normal (spore count)	Independent Action (spore count)	Comment
2%	9	13,12	Same attack rate as Meselson 1994
28%	1,219	21,327	Estimate for a WMD-magnitude incident ‡
50%	8,000	45,000	LD_{50} incident

Table 3: Doses (number of spores inhaled) for various attack rates (percentage of test population killed) for log-normal and independent action dose-response relations.

* LD_{50} is a single dose that kills approximately 50% of the people receiving it. For example, the LD_{50} dose for anthrax is 45,000 spores (the independent action model); giving each of 100 people 45,000 spores would kill 50 people on average.

† "In probability theory and statistics the probit function is the inverse cumulative distribution function, or quantile function of the normal distribution." Source: http://en.wikipedia.org/wiki/Probit.

‡ This calculation is based on preserving a single ratio: the overall attack rate to the attack rate at the ceramics factory. Considering that 68 of 7000 people in the high-risk zone died (~1%) and that 10 of 450 people in the ceramics factory died (~2%), we determine what the ceramics factory attack rate would be if this ratio was preserved and the high-risk zone attack rate were 1000 of 7000. We use 1000 as a "weapons of mass destruction" (WMD) threshold.

The log-normal values were calculated by linearly interpolating the log-normal table (Table 2) for the two points bracketing this value. For example, a nine-spore dose follows from linearly interpolating between (1.8% attack rate, eight spores) and (2.8% attack rate, 15 spores) for a 2% attack rate. The independent action values follow from Meselson:[7]

$$AR = 1 - \left(\frac{1}{2}\right)^{dose/LD_{50}} = 1 + \exp\left(\frac{0.69 \cdot dose}{LD_{50}}\right) \quad \text{(A-1)}$$

Using the first form of this equation, taking the natural logarithm of both sides, and solving for dosage, we have:

$$dose = LD_{50}\left(\frac{\ln(1 - AR)}{\ln(1/2)}\right) \quad \text{(A-2)}$$

The resulting number is rounded to an integer value of spores.

The values for LD_{28} and LD_{50} are somewhat artificial for the Sverdlovsk example because they result in mass casualties only if the entire population of 7,000 people were in the high-risk zone at the time of the accident. While this is an unlikely restriction for the Sverdlovsk scenario, these high LD values do yield mass casualties in cities of larger population densities and are thus included for illustrative purposes. In New York City, for example, the population density is 25,800 per square kilometer.* If only 10% of the people remain in the city on an average workday, a WMD-magnitude attack (1,000 or more people killed) requires an LD_{38} attack rate, which translates to 3,325 and 32,090 spores-per-dose for the log-normal and independent action models, respectively.

GAUSSIAN SLENDER-PLUME MODEL

Meselson *et al*[5] estimated the amount of anthrax released at Compound 19, or "source strength", using a Gaussian slender-plume model. Table 4 lists the parameters and assumptions used model.

Parameters & Assumptions		Value	Comments
A.1	Release Height	10 meters (m)	Approximate height of Compound 19 release point
A.2	Mean Wind speed	5 meters / second (m/sec)	Actual wind speeds varied from 4 to 6 m/sec
A.3	Dispersion Coefficients	Briggs values for neutral atmospheric stability	"During the period of northerly wind on 2 April, which followed the passage of a cold front, the wind speed was 4 to 6 m/s, the temperature –10° to –3°C, the relative humidity 50 to 66%, the sky cloudless, and the midday sun 39° above the horizon. These conditions of insulation and wind speed indicate that the atmosphere near the surface was of neutral stability."[5]
A.4	Vertical Mixing Limit	Infinite	Environmental conditions (see A.3) were such that atmospheric reflection was assumed negligible.
A.5	Aerosol Particle Diameter	Less than 5 microns (μm)	Typical of laboratory aerosol generator

* As of the United States 2000 Census, http://quickfacts.census.gov/qfd/states/36/3651003.html.

Parameters & Assumptions		Value	Comments
A.6	Infectivity Decay Rate	Negligible (less than 0.001/minute)	The anthrax does not lose its potency during transport
A.7	Deposition Velocity	< 0.005 m/sec	This "is insufficient to cause appreciable reduction of dosage at downwind distances less than 50 kilometers," the distance to the furthest animal infections.[5]
A.8	Human Breathing Rate	5 x 10^{-4} m^3/sec	Man engaged in light work
A.9	Indoor Protection	Negligible	"People indoors will be exposed to the same total dosage as those outside if filtration, deposition, and infectivity decay of the aerosol are negligible. The negligibility of these factors is supported by the absence of significant dosage reduction in field studies of protection afforded by tightly constructed buildings against an outside spore aerosol."[5]
A.10	Spores / milligram	1 x 10^9	Results of the dose-response model calculations

Table 4: Parameters and assumptions made by Meselson *et al.*[5] All values and justifications are from that paper.

These assumptions lead to a slender-plume formulation (A.2, A.3) of the Gaussian Plume Model for zero inversion effect (A.4) and total ground reflection (A.7). From Seinfeld & Pandis,[1] page 925:

$$\langle c(x,y,z,t)\rangle = \frac{q}{2\pi\cdot\bar{u}\cdot\sigma_y\cdot\sigma_z}\times\exp\left(\frac{-y^2}{2\cdot\sigma_y^2}\right)\times\left[\exp\left(\frac{-(z-h)^2}{2\cdot\sigma_z^2}\right)+\exp\left(\frac{-(z+h)^2}{2\cdot\sigma_z^2}\right)\right] \qquad \text{(A-3)}$$

where $\langle c(x,y,z,t)\rangle$ is the ensemble-averaged concentration (spores per unit volume), x is the downstream location (0 at the release point), y is the cross-stream location (0 along the centerline), z is the vertical location (0 on the ground), h is the release height, $\bar{u}$ is the mean downstream velocity (meters per second), q is the source strength (number of spores), and σ_y and σ_z are atmospheric dispersion coefficients in the y and z directions, respectively. In this model, time is solely a function of mean downstream velocity and spatial location ($t = x/\bar{u}$).

Physically, the atmospheric dispersion coefficients are functions of turbulent transport and not related to molecular diffusivity. Mathematically, the dispersion terms are functions of downstream location. For the assumptions employed (Table 4), the dispersion coefficients are approximated by the Briggs values for a neutrally stable atmosphere:[5]

$$\sigma_y = \frac{0.08\cdot x}{\sqrt{1+0.0001\cdot x}} \quad , \quad \sigma_z = \frac{0.06\cdot x}{\sqrt{1+0.0015\cdot x}} \qquad \text{(A-4)}$$

The larger denominator in σ_z relative to σ_y means that spreading in the vertical direction is smaller on average than spreading in the cross-stream direction (the plume is wider than it is tall).

The dose received is simply the breathing rate (R_{breath}) multiplied by the local concentration:

$$dose = R_{breath} \cdot \langle c(x,y,z,t) \rangle \tag{A-5}$$

The Gaussian slender-plume model can be used to determine the source strength, q, for a given downstream dosage. Solving for q, the Gaussian plume model becomes:

$$q = \frac{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z \cdot (dose / R_{breath})}{\exp\left(\frac{-y^2}{2 \cdot \sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2 \cdot \sigma_z^2}\right) + \exp\left(\frac{-(z+h)^2}{2 \cdot \sigma_z^2}\right)\right]} \tag{A-6}$$

Equation (A-6) gives the source strength in terms of the number of spores. To convert to milligrams, we divide this value by the spores/milligram count listed in Table 4.

Table 5 presents the "source strength" predicted by the Gaussian slender-plume model for the ceramics factory facility investigated by Meselson ($x = 2.8km$, $y = 0$ (centerline), $z = 0$ (ground), and $t = x/\bar{u} = 560$ seconds)[5,7] and for the doses listed in Table 3.

	Log-Normal		Independent Action	
Attack Rate	Source Strength	Grams	Source Strength	Grams
2%	4.16E+09	0.004	6.07E+11	0.607
28%	5.64E+11	0.564	9.86E+12	9.863
50%	3.70E+12	3.700	2.08E+13	20.812

Table 5: Source strength for various doses at the ceramics factory investigated by Meselson *et al*, estimated using a Gaussian slender-plume model. Attack rate is the percentage of deaths in the target population; source strength is the number of spores required at the source predicted to achieve a given attack rate.

As shown in Table 5, Meselson *et al* predicted a source-strength of approximately 4 to 21 grams, depending on the dose-response relation used. This is approximately three orders-of-magnitude less than the estimates made by U.S. intelligence analysts.[6] The reasons for this discrepancy remain unclear. However, it is interesting to note that the by changing the ground total-reflection boundary condition (A.7) to a ground total-absorption condition, the source-strength prediction is on the order of intelligence estimates.

USING A TOTAL ABSORPTION ASSUMPTION

The total reflection model used by Meselson is based on an assumed deposition velocity of less than 0.005 meters per second. Table 6 shows the calculations of the previous section repeated for the case of total absorption of anthrax by the ground. Retaining all other assumptions, the Gaussian slender-plume model becomes (Seinfeld and Pandis,[1] page 925):

$$\langle c(x,y,z,t) \rangle = \frac{q}{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z} \times \exp\left(\frac{-y^2}{2 \cdot \sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2 \cdot \sigma_z^2}\right) - \exp\left(\frac{-(z+h)^2}{2 \cdot \sigma_z^2}\right)\right] \tag{A-7}$$

Once again, solving for source strength:

$$q = \frac{2\pi \cdot \bar{u} \cdot \sigma_y \cdot \sigma_z \cdot \left(dose / R_{breath}\right)}{\exp\left(\frac{-y^2}{2 \cdot \sigma_y^2}\right) \times \left[\exp\left(\frac{-(z-h)^2}{2 \cdot \sigma_z^2}\right) - \exp\left(\frac{-(z+h)^2}{2 \cdot \sigma_z^2}\right)\right]} \tag{A-8}$$

This equation is undefined at the ground ($z = 0$). We instead choose a height slightly above the ground; specifically, we use the average height of a human being (approximately 1.5 meters). The calculations for total reflection are repeated for this height to provide an appropriate comparison to the total absorption case. Once again, we use the ceramics factory facility to estimate the source-strength ($x = 2.8km$, $y = 0$, $t = x/\bar{u} = 560\,seconds$).

	Total Reflection				Total Absorption			
	Log-Normal		Independent Action		Log-Normal		Independent Action	
Attack Rate	Source Strength	Grams	Source Strength	Grams	Source Strength	Grams	Source Strength	Grams
2%	4.16E+09	0.004	6.07E+11	0.607	1.48E+12	1.48	2.16E+14	216.15
28%	5.64E+11	0.564	9.87E+12	9.866	2.01E+14	200.83	3.51E+15	3513.61
50%	3.70E+12	3.701	2.08E+13	20.816	1.32E+15	1318.00	7.41E+15	7413.72

Table 6: Source strength for various doses at the ceramics factory, estimated using a Gaussian slender plume model for total absorption and reflection at the ground. Attack rate is the percentage of deaths in the target population; source strength is the number of spores required at the source predicted to achieve a given attack rate. The two Gaussian models are calculated at the same position in space and time.

Changing this single assumption increases the estimated source-strength by three orders of magnitude, from a range of 3.7 to 20 grams (total reflection) to a range of 1.3 to 7.4 kilograms (total absorption) for an LD_{50} dose. As one might expect, the source-strength estimate is very sensitive to the ground reflection/absorption assumption.

We consider these two value ranges as lower- and upper-bound estimates of the source-strength of the Sverdlovsk incident of 1979. A total-reflection boundary condition assumes that none of the anthrax striking the ground between the source and the ceramics factory remains on the ground – not in puddles, on damp leaves, or inside buildings. This seems overly optimistic about transporting anthrax downstream. Conversely, the total-absorption conditions assumes that all of the anthrax sticks to the ground on contact, significantly increasing the amount of anthrax at the source required for a specified attack rate at the ceramics factory. This seems overly pessimistic. A more accurate model of ground conditions using a Gaussian slender-plume model would estimate the source-strength to be between 3.7 grams and 7.4 kilograms for the assumptions and conditions used.

REFERENCES

[1] Seinfeld, John H. and Pandis, Spyros N., Atmospheric Chemistry and Physics: From Air Pollution to Climate Change, John Wiley & Sons, Incorporated, New York, 1998.

[2] Sugiyama, G., Nasstrom, J. S., and Baskett, R. L., "Operational Systems for Emergency Preparedness and Response", Lawrence Livermore National Laboratory, Symposium on Planning, Nowcasting, and Forecasting in the Urban Zone, 84th American Meteorological Society Annual Meeting, Seattle, WA, January 11-15, 2004, UCRL-CONF-200951, November 13, 2003.

[3] Mitchell, J. A., Molenkamp, C. R., *et al*, "Comparison of Average Transport and Dispersion Among a Gaussian Model, a Two-Dimensional Model and a Three-Dimensional Model," Lawrence Livermore National Laboratory, International Conference on Probabilistic Safety Assessment and Management 7, Berlin, Germany, June 14-18, 2004, UCRL-PROC-204132, May 13, 2004.

[4] Richard Preston, "The Bioweaponeers", *The New Yorker*, March 9, 1998, pages 52-65.

[5] Meselson, Matthew, Guillemin, Jeanne, *et al*, "The Sverdlovsk Anthrax Outbreak of 1979", *Science*, Volume 266, pages 1202-1208, 1994.

[6] "Volume V: Anthrax at Sverdlovsk, 1979. U.S. Intelligence on the Deadliest Modern Outbreak." The National Security Archive, National Security Archive Electronic Briefing Book No. 61, edited by Robert A Wampler and Thomas S. Blanton, November 15, 2001.

[7] Meselson, Matthew, "Note Regarding Source Strength", The ASA Newsletter, Applied Science and Analysis, Incorporated, June 8, 1995.