**National Institutes of Health**
**Office of the Director**
**Office of Biotechnology Activities**

# NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY

**July 13, 2006**
**National Institutes of Health Campus**
**9000 Rockville Pike**
**Building 31, 6C, Room 10**
**Bethesda, Maryland**

## MEETING SUMMARY

**VOTING MEMBERS**

Dennis L. Kasper, M.D., NSABB Chair
Arturo Casadevall, M.D., Ph.D.
Murray L. Cohen, Ph.D., M.P.H., C.I.H.
Susan A. Ehrlich, J.D.
Lynn W. Enquist, Ph.D.
Barry J. Erlick, Ph.D.
David R. Franz, D.V.M., Ph.D.
Claire M. Fraser-Liggett, Ph.D.
Paul S. Keim, Ph.D.
Stanley M. Lemon, M.D.
Stuart B. Levy, M.D.
Adel A.F. Mahmoud, M.D., Ph.D.
Mark W. Nance, J.D.
David A. Relman, M.D.
Harvey Rubin, M.D., Ph.D.
Thomas E. Shenk, Ph.D.
Andrew A. Sorensen, Ph.D.
Admiral William O. Studeman (Ret.)
Anne Vidaver, Ph.D.
Diane W. Wara, M.D.

**EX OFFICIOS and FEDERAL AGENCY REPRESENTATIVES**

Jason Boehm, Ph.D., Executive Office of the President
Kenneth Cole, Ph.D., Department of Defense
Natalia Comella, Ph.D., Department of State
Brenda A. Cuccherini, Ph.D., M.P.H., Department of Veterans' Affairs
Jerome Donlon, M.D. Ph.D., DHHS/Office of Public Health Emergency Preparedness
Maria Giovanni, Ph.D., National Institute of Allergy and Infectious Disease
Peter R. Jutro, Ph.D., Environmental Protection Agency

Norman Kahn, Intelligence Technology Innovation Center
Janet K.A. Nicholson, Ph.D., DHHS/Centers for Disease Control and Prevention
Stuart L. Nightingale, M.D., Department of Health and Human Services
Tunuja Rastogi, Sc.D., Department of State
Caird E. Rexroad, Jr., Ph.D., Department of Agriculture
Scott Steele, Ph.D., Department of Justice
John F. Turner, Department of State
Joanne Tornow, Ph.D., National Science Foundation

**NSABB EXECUTIVE DIRECTOR**

Amy P. Patterson, M.D.

**GUEST SPEAKERS**

Ralph Baric, Ph.D.
Professor, Department of Microbiology and Immunology
University of North Carolina, Chapel Hill

Mark Hemphill, M.S.
Chief of Policy, Select Agents Program
Centers for Disease Control and Prevention

John Mulligan, Ph.D.
President and CEO
Blue Heron Biotechnology

**CALL TO ORDER**
**Dennis L. Kasper, M.D.**
**Amy Patterson, M.D.**

Dr. Dennis Kasper called to order the fifth meeting of the National Science Advisory Board for Biosecurity (NSABB). He welcomed NSABB members, Federal Agency Representatives, members of the public in attendance, and those watching via webcast.

Dr. Amy Patterson, Executive Director of NSABB, described the rules of conduct and conflict of interest considerations that apply to Board members as Special Government Employees. She stated that the rules are explained in the report, "Standards of Ethical Conduct for Employees of the Executive Branch," which was received by each member. Board members are required to recuse themselves in advance of any discussion in which they believe they have a conflict of interest.

Board members stated their names and affiliations. The Board then voted unanimously to approve the March 2006 NSABB meeting minutes that had been distributed in advance of the meeting.

**INTRODUCTION AND AGENDA OVERVIEW**
**Dennis L. Kasper, M.D.**

Dr. Kasper provided an overview of the meeting agenda, stating that the Chairs of the Dual Use Criteria, Communications, and Codes of Conduct Working Groups would present draft products and report on ongoing activities. Discussion and voting for approval of the products would follow. The afternoon schedule included a public comment session and updates on the activities of the Synthetic Genomics and International Working Groups. Dr. Kasper noted that NSABB had established a new Working Group to develop the principles and attributes of a framework for the oversight of dual use research in the life sciences. He stated that the Oversight Framework Working Group would incorporate the current NSABB work products. It would then be forwarded to the U.S. Government for broad input from the scientific community, other stakeholders, and the public. Ultimately, to ensure effective oversight of dual use of research, training and education on the framework would be needed at institutions throughout the country.

**DUAL USE CRITERIA WORKING GROUP: STATUS REPORT**
**Dennis L. Kasper, M.D.**

Dr. Kasper explained that dual use biological research is broadly defined as legitimate research that could be misused to threaten public health or other aspects of national security. This potential for misuse requires the consideration of new biosecurity measures. The challenge is to reduce the likelihood that biological research results could be misapplied, while minimizing the impact on scientific inquiry. Criteria are needed to identify research of concern. Since the term "national security" means different things to

different people, the Working Group defined it in the context of dual use biological research of concern, concluding that the components include public health, agriculture, plants, animals, non-biological resources (materiel) and the environment. Threats in these areas could result in significant economic or public safety consequences.

The draft criteria were developed through discussions at Working Group meetings, consultation with colleagues, and presentations at institutional, regional, and national meetings. The Working Group also held a roundtable on the subject that included researchers, biosecurity experts, research administrators, and research policy experts. Dr. Kasper stated that the comments received on the previous version of the criteria, which were presented at the March 2006 NSABB meeting, were instrumental in the development of the latest draft. Most suggestions related to the clarification or emphasis of certain points. One recurring suggestion was that the criteria should explicitly convey the principles of immediacy and scope. Some comments suggested clarifying that the standard for assessment of dual use is based on a current understanding of the state of the science and a reasonable anticipation that research results could be misapplied.

Based on this, the Working Group refined the wording of the criteria to emphasize that the threshold for dual use research of concern requires that the research results have the potential to be directly misapplied (i.e., immediacy) and to have broad consequences (i.e., scope). Dr. Kasper stressed that the designation of research as dual use does not mean it should not be performed or that its results should not be communicated.

The Working Group felt it is necessary to provide examples of the types of research results that are of concern to aid in evaluating the dual use assessment of research. Although not a part of the criteria for dual use research of concern, the Working Group determined that careful consideration should be given to knowledge, products or technologies that:

- Enhance the harmful consequences of a biological agent or toxin;
- Disrupt immunity or the effectiveness of an immunization without a clinical and/or agricultural justification;
- Confer to a biological agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin, or facilitate their ability to evade detection methodologies;
- Increase the stability, transmissibility, or ability to disseminate a biological agent or toxin;
- Alter the host range or tropism of a biological agent or toxin;
- Enhance the susceptibility of a host population; or
- Generate a novel pathogenic agent or toxin or reconstitute an eradicated or extinct biological agent.

Dr. Kasper said that an assessment of research for dual use potential will require scientific expertise and sound judgment about the probability that its results could be misapplied by others. These assessments should be performed by individuals skilled in the art of biological research, such as principal investigators. He also noted that since

biological research is an extraordinarily dynamic field that encompasses diverse disciplines, it will be important to periodically review the criteria and modify them to ensure their continued relevance.

**Discussion**

Dr. Lemon asked if the term "national security" should be replaced with a phrase that relates to the security of society in general to increase the document's acceptance by the international community. Several participants pointed out that the Board's charter refers specifically to U.S. national security. Admiral Studeman suggested using language that reflects both national security and applications to global biosecurity. Several Board members expressed concern that the word "global" might imply that the intent of the Board was for the document to be accepted globally, which could be misleading and affect international relations. The Board decided that the term "national security" was overused in the criteria and should be replaced in several places with wording that refers to society in general.

Judge Ehrlich commented on footnotes 2 and 3, which explained the terms "biological agent" and "toxin" in the criteria's definition of dual use research of concern. The footnotes incorporated the definitions of these terms from 18 USC 178. She suggested referring readers to the statutory section rather than providing the definitions in footnotes.

The Board voted on approval of the Criteria for Identifying Dual Use Research of Concern, including changes agreed upon by the Board during the discussion. Dr. Kasper took a vote by roll call and the criteria were unanimously approved.

**COMMUNICATIONS WORKING GROUP: STATUS REPORT**
**Paul Keim, Ph.D.**

Dr. Keim, Chair of the Communications Working Group, stated that the Working Group's goal was to develop guidance and tools to facilitate consistent, well-considered decisions on communication, as well as to demonstrate to the public that scientists recognize and are being responsive to concerns about the security implications of dual use research. He presented three products from the Working Group for a vote by the Committee:

- Principles for the responsible communication of research with dual use potential;
- A framework for identifying and assessing the risks and benefits of communicating research information with dual use potential; and
- Considerations in the development of a communication plan for research with dual use potential.

Dr. Keim stated that these communication tools could serve as educational tools to raise awareness of dual use issues and could be used to review research proposals, manuscripts, presentations and Internet postings. Potential users include investigators and

research supervisors, students/postdoctoral candidates, institutional biosecurity review entities, proposal reviewers, research funding agencies, government policymakers and scientific journal editors, reviewers and publishers.

The Working Group conducted outreach with stakeholders to obtain input on product development. In addition to consultations with colleagues by Working Group members, a panel discussion with experts in biosafety, microbiology and security policy and a roundtable discussion with editors of scientific journals, including members of the international scientific publishing community, also provided helpful feedback on the Working Group's efforts.

Dr. Keim reported that, in general, the individuals consulted were positive about tools that can make review processes more consistent. The Working Group's efforts were perceived as helpful in these areas. The idea of using the tools in ethics courses to introduce the concept of communication of dual use at an early stage in education was also well received. Stakeholders strongly agreed that the way in which dual use research information is presented is as important as its substance. Some who commented suggested strategies for engaging the general media similar to those used by the Science Media Center in the United Kingdom.

Some concerns were also expressed, including that every manuscript submitted for publication might be subject to assessment for dual use potential. The questionnaire format for the framework raised concerns about creating a regulatory burden, leading the Working Group to use a "points to consider" format. It was thought that this format would make it especially useful for inclusion as a hyperlink for submitting authors and for manuscript reviewers and editors who conduct biosecurity reviews.

International journal editors indicated that dual use research issues are not a high priority in Europe and Asia; public health issues, specifically infectious diseases, are of more concern. Dr. Keim noted that if dual use research issues were presented as part of public health and global infectious disease concerns, they might have more impact. Framing the issues in terms of well being for society, rather than U.S. national security concerns alone, could promote acceptance by the international community. A U.K. mechanism to ensure that authors have alerted public-health officials when a publication may raise dual use concerns was also described.

Dr. Keim discussed the Working Group's three products in detail, starting with the principles for responsible communication of research with dual use potential. The principles address the following points:

- Communication is vital for scientific progress.
- Communication of research should be done to the fullest extent possible.
- There is a need for balance.
- There is a need to assess the risks and the benefits of communicating information.
- The decision about whether to communicate should not be binary; rather a range of communication options should be considered.

- Communication occurs throughout the research process.
- There is a need to consider both *what* is communicated and the *way* in which it is communicated.

After receiving feedback from stakeholders, the Working Group added the following principle:

- Public trust is essential to the vitality of the life science research enterprise. Life scientists must engage in outreach on a regular basis to raise awareness and to reassure the public that research is being properly conducted and communicated.

Dr. Keim next introduced discussion of the assessment framework or "points to consider" document, which was previously in a questionnaire format. Users should be encouraged to tailor it for their specific purposes. The key features of the document are a general overview of information, a risk assessment, a benefit assessment, a risk versus benefit assessment and a formulation of recommendations for communication, which should address the content, timing and extent of distribution.

Dr. Keim then addressed the elements of a communication plan, noting that this might be the most important part of the communication process because it affords the opportunity to explain the importance of the research in terms that all can understand. The goal is to promote public understanding and trust.

Dr. Keim noted that the Working Group was also developing a statement that will emphasize the importance of communicating findings in the life sciences. It is being designed primarily for the public, but will hopefully be embraced by scientific colleagues.

**Discussion**

Dr. Vidaver suggested removing the word "local" from point eight under principles for communication. She also suggested adding all the categories listed as components in the dual use criteria (public health, agriculture, plants, animals, non-biological resources and the environment) to the language on development of a communication plan. Dr. Lemon felt the wording on risk analysis should mirror the terms used in the dual use subcommittee definitions, e.g., "reasonably anticipated" and "directly misapplied," to make the messages more consistent. The Board discussed the use of the term "responsible" in the products and decided it was acceptable.

The Board voted to approve the three Communications Working Group products. Dr. Kasper took a vote by roll call on the three products with the suggested changes. They were approved unanimously by the Board.

**CODES OF CONDUCT WORKING GROUP: STATUS REPORT**
**Mark Nance, J.D.**

Mr. Nance stated that a draft document had been prepared with recommendations on the development of a code of conduct for scientists and laboratory workers that could be adopted by professional organizations and institutions engaged in life sciences research. It was developed using feedback from focus groups consisting of practicing scientists, leaders of scientific societies, personnel with institutional oversight responsibilities and ethicists. The Working Group identified the issues most relevant to the conduct of dual use research that should be addressed by a code and developed standards and principles that could be incorporated into formal education and training programs. Mr. Nance described the analysis and extensive consultation process that contributed to the document's development.

As the Working Group conducted focus groups, several fundamental operating principles emerged that guided the direction of code development. Mr. Nance said the group recognized that their efforts would not deter future acts of terrorism. He said a code of conduct can make good people better, but has a negligible impact on intentionally malicious behavior. The Working Group therefore focused on identifying principles relevant to the responsible conduct of dual use research with the goal of raising awareness within the scientific community about their responsibilities. It also became apparent during discussions with target audiences that a clear and understandable definition of dual use research is critical for the acceptance and appreciation of a code. Participation by the research community helped define appropriate standards and language and will hopefully encourage broad acceptance of the final document.

The product includes introductory material to educate users about dual use issues and the value of codes of conduct, a set of core principles for life scientists on the responsible conduct of dual use research, general statements of responsibility and a section that provides specific behavioral guidance on funding, reviewing, conducting and communicating research. Mr. Nance said the Working Group was recommending that life science professional societies, as well as institutions that sponsor or conduct life sciences research, use the document as a tool to begin a dialogue about dual use research. They might want to incorporate the concepts expressed in the document into their own codes of conduct. The Working Group was continuing to engage life sciences societies and associations, research institutions, industry, research leadership, individual scientists, technicians and students, funding agencies and journal editors.

Mr. Nance stated that the heart of the document was titled, "The Core Responsibilities of Life Scientists in Regard to Dual Use Research of Concern." It summarizes key duties that all life scientists should assume related to the responsible conduct of dual use research and potential harm that could result from the misuse of their research results. It states that every scientist should assess his or her own research efforts for dual use potential; seek to stay informed of literature, guidance and requirements associated with dual use research; train others to identify and appropriately manage dual use research of concern; serve as a role model for responsible behavior; and identify and report dual use

research of concern through appropriate channels.

The third section of the document expands on the fundamental responsibilities by outlining specific duties associated with various stages of the research process. For example, the draft reflects the following concepts:

- When designing and proposing research, scientists should try to anticipate whether the end products could be deliberately misused; design research to promote scientific advances while minimizing elements associated with dual use research of concern; weigh the benefits of elements of dual use research of concern that cannot be avoided against the potential harm that might result from misuse to ensure that the benefits exceed the risks; and modify the research design, as appropriate, to manage and mitigate potential misuse.

- Managers of research programs should promote the awareness of dual use research and accompanying responsibilities; develop and maintain systems, policies, and training programs to ensure appropriate management and identification of dual use research; and implement all guidelines and regulations specific to dual use research of concern. Those who oversee the research review process, such as funding agencies, institutional review committees and institutional leadership, should ensure that review systems are appropriately prepared to identify and manage dual use research concerns; ensure that researchers and reviewers are knowledgeable and compliant with all ethical, institutional and legal requirements related to dual use research; and periodically reconsider existing review systems to ensure that the systems reflect current knowledge and guidelines related to dual use research.

- Those who review research should stay informed about dual use research of concern and all applicable ethical, legal and institutional requirements; routinely assess research proposals against the criteria established for the identification of dual use research of concern during the review process; and notify appropriate parties when the research under review meets the criteria for dual use research.

- Individuals who are engaged in the conduct of research should observe safe practices and ethical behaviors in the laboratory and ensure that all personnel working in the laboratory do the same; use physical security measures and routinely assess their adequacy; observe applicable guidelines for the responsible conduct of dual use research of concern; be attentive to the dual use potential of knowledge, products and technology associated with all research activities; and alert responsible institutional officials when dual use research of concern is identified and when decisions about its management are being made.

- When individuals collaborate on research activities, they should discuss whether research knowledge, products or technologies meet the criteria for dual use research of concern and understand associated ethical responsibilities; agree on specific responsibilities for the oversight of research with dual use potential;

respect expressions of concern from other individuals that research efforts may have dual use potential and raise these concerns with appropriate oversight officials; use appropriate measures to minimize risks to public health, agriculture, plants, animals, the environment and materiel; and maintain current awareness of national and international policies concerning dual use research.

- When communicating the knowledge, products or technologies associated with dual use research of concern, those involved in sharing the information should be aware of the ethical and legal considerations associated with communication of dual use research; weigh the risks and benefits to public health, agriculture, plants, animals, environment and materiel that could result through research-related communications; and consider options that may reduce or eliminate potential risks associated with research-related communications while clearly identifying the benefits.

- Those in scientific education and professional societies in the life sciences should raise awareness about the meaning and importance of dual use research of concern; inform developing scientists of associated ethical, legal and institutional responsibilities; and encourage collegial discussion of dual use research issues, especially whether specific activities meet the criteria for dual use research of concern.

Mr. Nance explained how this document dovetailed with other NSABB work products. It incorporated the language developed by the Dual Use Criteria Working Group on dual use research of concern, incorporated fundamental principles for the responsible communication of dual use research developed by the Communications Working Group, used language appropriate for international audiences, and referenced the functions that will be associated with efforts by NSABB in the areas of oversight guidelines and outreach and education.

Mr. Nance closed by stating that the Codes of Conduct Working Group was submitting its draft document for evaluation by NSABB, as the members believed it was ready for broader public input and comment. He asked that the Board consider whether the work product should be approved.

**Discussion**

Dr. Sorenson wondered how the code would apply to those across the university with research oversight responsibilities, not just individual scientists, who seemed to be the primary audience. He asked if the group had discussed how a code of conduct could be effectively disseminated throughout an entire institution. Mr. Nance said the group had discussed this. While development of a dissemination plan was beyond the charge of the Working Group, this topic will be addressed by the outreach and education efforts of NSABB and staff. Dr. Kasper added that the Working Group charged with developing an oversight framework group would also address implementation at the administrative level in institutions. In response to a question, Mr. Nance clarified that the codes of conduct

product is meant to apply to both individuals and institutions.

Dr Shenk suggested modifying the third bullet on training under "Core Responsibilities" to state: "train others to identify dual use research of concern and manage it appropriately and communicate it responsibly."

Mr. Nance acknowledged that there are already comprehensive codes in existence governing most of the target audiences of concern. He said the Codes of Conduct Working Group hoped that the authors of those codes would consider incorporating the NSABB Codes of Conduct. He said education and outreach would address this issue.

Dr. Vidaver suggested modifying the point on physical security under "Conducting Research" so that it states: "using appropriate physical and/or biological security." Dr. Fraser-Liggett suggested adding "cybersecurity." Mr. Nance agreed with both points and suggesting deleting the word "physical," saying instead: "use an appropriate security measure." The Board agreed.

The group discussed the bullets under "Core Responsibilities" on identifying and reporting dual use research of concern. The Board wanted to avoid any inference that scientists should be "patrolling the halls" looking for the possible misdeeds of their colleagues. There was extensive discussion of language that would communicate the responsibility of scientists to be aware of their environment but without creating an atmosphere of suspicion. The Board agreed to modify the first bullet on monitoring one's own research to state: "assess their own research efforts for dual use potential and report as appropriate." The last bullet was modified to state: "be alert to potential misuse of research."

Judge Ehrlich suggested adding the phrase "State guidelines" wherever "Federal guidelines" appeared.

Dr. Levy raised a question about use of the word "legal" as it relates to communication of dual use research. After some discussion, the Board agreed that the word referred to the conventional use of "legal" by which researchers have responsibilities both in statute and in common law to prevent communication of information that could foreseeably cause harm. It agreed to retain the word "legal" in the communication products.

The Board voted on approval of the Considerations for Development of Codes of Conduct for Dual Use Research of Concern with the modifications discussed. The document was unanimously approved by the Board.

**INTERNATIONAL WORKING GROUP: STATUS REPORT**
**David Franz, D.V.M, Ph.D.**

The International Working Group was charged with recommending strategies to help foster international collaboration on the development of effective national oversight mechanisms for dual use life sciences research. Dr. Franz stated that the risks and threats

to security are viewed differently in different parts of the world. He said that if the U.S. were to over-regulate without being aware of the implications for the rest of the world, it could be a disservice to the scientific enterprise and affect national security.

Dr. Franz described the recent activities of the Working Group. He stated that Dr. Stuart Levy, who chairs the Alliance for the Prudent Use of Antibiotics (APAU), sent out an informal questionnaire to 56 chapters of that organization and that approximately half responded.

In addition, a diplomatic cable was sent to the science sections of 37 embassies to help develop collaborative international partnerships, share U.S. concerns, identify points-of-contact, capture the diversity of international views and determine how best to achieve international engagement regarding the development of a culture of responsibility. By the time of the NSABB meeting, responses had been received from more than half of the countries, many of which were substantive comments. Most responders indicated some level of awareness of the dual use issue and indicated an interest in working with NSABB. Several noted that they have similar initiatives underway in their countries. A number of countries identified dual use issues as security of laboratories or biosecurity practices. The kinds of government representatives provided as points-of-contact included scientists from universities and other governmental research institutions; various ministries and agencies (e.g., Health, Environment, Agriculture, Foreign Affairs, Education); science and technology ministries; and agencies working on biological weapons-related issues. Non-governmental points-of-contact included the National Academies of Science in these countries and universities/research institutions.

Dr. Franz noted that Working Group members also held discussions with colleagues in U.S. scientific societies with international interests and with international scientific and security organizations. The Working Group is in the process of developing a database of people who expressed interest in collaborating. Dr. Franz said they would be sending out emails to members of the committee asking for help in populating the database. Dr. Levy said that they had to disseminate NSABB documents through the right channels. If the information came directly from NSABB, it could be perceived as pushing a U.S. viewpoint. The Working Group was therefore collaborating with the International Union of Microbiological Societies (IUMS), which oversees 100 professional societies in 100 countries. Dr. Levy noted that the NSABB documents would be presented to the American Society for Microbiology (ASM) Public and Scientific Affairs Board and then to its international subcommittee. Once approved by this group, the documents would be sent to the IUMS with a request for input. Dr. Levy said this is not a new issue for the IUMS, as they developed a brief code of ethics dealing with dual use issues. However, it had nothing substantial to move forward. At this time, the Working Group was helping it in its efforts, and there was great excitement about collaborating with the ASM and other professional organizations.

Dr. Franz said an important initial effort of the Working Group was to build a database of key individuals and societies to serve as international points of contact. He also expressed the hope that some countries will incorporate sections of the NSABB documents into

their work. The health of the world will not be protected if the U.S. is the only country working on these issues, he said. Dr. Franz described this effort as the first routing of NSABB documents, to be followed by additional requests for comments. He said follow-up would come not from the U.S. Government, but from a professional society.

Dr. Franz stated that a small, international 2-day meeting is being planned for the Working Group in Fall 2006 in collaboration with the leadership of the World Health Organization (WHO). The meeting will include approximately 40 to 50 people, and its purpose is to increase awareness among international scientists and policymakers on dual use issues. The Working Group plans to solicit input on principles and concepts for an effective national oversight strategy for dual use life sciences research. Dr. Franz said that approved NSABB work products would be made available during the international meeting, but their dissemination would not be the central focus. Dr. Vidaver suggested having representatives from the Food and Agriculture Organization (FAO) and World Organization for Animal Health (OIE) at this meeting. The joint OIE/FAO Influenza Network (OFFLU) was established to improve worldwide knowledge of H5N1 virus strains between human health and animal health laboratories and OIE/FAO reference laboratories.

Dr. Mahmoud commented that the U.S. National Academy of Sciences (NAS) and the Institute of Medicine (IOM) were spearheading a movement to develop a National Academy Medical Panel and to develop Academies of Science and Institutes of Medicine in almost 50 countries. He suggested that NSABB involve NAS and IOM in their meetings and noted that the scientific communities in the developing world are very weak. Dr. Mahmoud also said there is an International Society for Infectious Diseases that could provide contacts for NSABB. Their meetings in Brussels and Lisbon were attended by 3,000 infectious disease microbiologists from 108 countries.

Admiral Studeman suggested motivating countries that haven't thought about addressing dual use to act on this issue by educating them about the possible global consequences of even a single adverse incident arising from misuse of dual use research findings or technologies. Dr. Franz agreed that more open international communication would be a positive thing.

At the request of Dr. Franz, Dr. Lemon agreed to contact the International Agency for the Red Cross concerning its program on life sciences.

**PUBLIC COMMENT**
**Nancy King**
**Policy, Ethics, and Law Corps of the Southeast Regional Center for Biodefense and Emerging Infections**

Ms. King asked for more information about the code being developed by the Codes of Conduct Working Group. Mr. Nance replied that the product did not constitute a full code but rather, core principles. The core principles could be used by societies and associations to formulate stand-alone codes or could be incorporated into existing codes. Ms. King

stated that section 3 of the product seemed very prescriptive, and she felt there was a risk that societies and universities might misunderstand the purpose and think that they were being "handed" a code. Mr. Nance said that education and outreach would be taking place and more work would be done before the product was published in the Federal Register for public comment.

**OPENING REMARKS AND INTRODUCTION OF PANELISTS FOR THE SYNTHETIC GENOMICS WORKING GROUP SESSION**
**David Relman, M.D.**

Dr. Relman stated that the Synthetic Genomics Working Group was launched in November 2005. He said its charge was two-fold. For Phase 1, the Working Group is tasked to determine whether the current regulatory framework for controlling Select Agents is adequate given recent advancements in synthetic genomics and to recommend strategies to address any biosecurity concerns. For Phase 2, the Working Group is tasked to identify, assess, and recommend strategies that address potential dual use concerns that arise from work being performed in the nascent field of synthetic biology.

Dr. Relman reported that the Working Group was nearing completion of Phase 1 during which it assessed the key controls for Select Agent genetic material and identified potential biosecurity concerns. It was considering various strategies for addressing these concerns. Its goal is to present final recommendations to the Board during the October 2006 NSABB meeting.

In carrying out its charge, the Working Group examined the state of the science and technology used to synthesize a select agent *de novo* and the oversight framework for such activity. Dr. Relman displayed a schematic that depicted the process through which synthetic genomes and their expressed products are created, a key focus of the Working Group's deliberations. The Working Group heard from industry experts about the technical capabilities for synthesizing nucleic acids and DNA and the resources needed to do so. It also engaged in discussions with eminent researchers on the state of the science in several key application areas for deriving infectious agents from synthetic nucleic acids. The Working Group received legal and regulatory briefings on the framework for controlling Select Agents by relevant agencies and held a roundtable with stakeholders to hear their perspectives about biosecurity concerns related to the ability to synthesize Select Agents.

To provide a context for the set of recommendations that were prepared for the Board's consideration, the Working Group invited three speakers to the meeting to present various perspectives. Dr. Relman emphasized that the speakers were not part of the Working Group's discussions, nor did they necessarily endorse the options that would be presented to the Board.

**Select Agent Rules: Intent And Interpretation of Controls for Select Agent Nucleotides**

**Mark Hemphill, M.S.**
**Chief of Policy, Division of Select Agents and Toxins, U.S. Centers for Disease Control and Prevention (CDC)**

Mr. Hemphill said he would explain how the Select Agent list was established, provide an overview of the Select Agent regulations, and discuss how synthetic biology and synthetic genomics fit within the language of the Select Agent regulations. He stated that the terrorism events of September 11, 2001 and the subsequent anthrax letters prompted the U.S. Government to review and strengthen legislation and regulations controlling biological agents and toxins.

The first legislation passed was the USA PATRIOT Act. A provision in the Act directly affects the Select Agent regulations, i.e., if an individual meets one of the criteria listed, he or she is prohibited from having access to a Select Agent or toxin and faces criminal penalties for noncompliance.

The second legislation passed was the Public Health Security Bioterrorism Preparedness and Response Act of 2002. Title 2 of that Act is known in the Department of Agriculture (USDA) as the Agriculture Bioterrorism Protection Act. It significantly changed the regulatory authorities that previously existed for the Department of Health and Human Services (DHHS) in regulating Select Agents and toxins. It broadened those authorities beyond regulating the transfer of these agents to regulating mere possession. It also granted comparable regulatory authorities to USDA to regulate agents and toxins that had the potential to pose a severe threat to plant or animal health or plant or animal products. Close coordination and concurrence is required between USDA and DHHS for agents that appear on both departments' lists of regulated agents. These are referred to in the Act as "overlap" agents and toxins. The Act required that DHHS and USDA maintain a list of each biological agent and toxin that has the potential to pose a severe threat to public health and safety. The USDA list must take into consideration threats to animal and plant health and to animal and plant products. The lists must be reviewed every two years at a minimum. The Act established the criteria to be used in determining which agents and toxins should be on the list. They include:

- The effect of exposure to the agent or toxin on human health, animal and plant health, and animal and plant products;
- The degree of contagiousness or pathogenicity of the agent or toxin and the methods by which they are transferred; and
- The availability and effectiveness of pharmacotherapies and immunizations to treat or prevent illness resulting from infection by the agent or toxin.

The Act also required registration with either DHHS or USDA for the possession, use or transfer of Select Agents and toxins. As part of that registration, both the entity and the individuals that require access to the Select Agents or toxins must undergo an electronic database check by the Department of Justice. This is to determine whether they meet the

criteria for one of the prohibiters listed in the USA PATRIOT Act as a restricted person and to see if there are any indications that the individual is associated with terrorist organizations or activities. USDA and DHHS established requirements for safety to ensure that entities working with Select Agents and toxins have the proper training and the appropriate laboratory facilities. Both departments established requirements for security to prevent access to those agents or toxins for use in terrorism or other criminal activities. Mr. Hemphill said the Act added additional criminal penalties for noncompliance. It mandated that regulations balance regulatory oversight to ensure appropriate availability of the agents and toxins needed for legitimate purposes.

Within DHHS, the CDC was delegated with the responsibility for promulgating and implementing these regulations. Within USDA, the Animal and Plant Health Inspection Service (APHIS) was given similar responsibilities. The agencies worked together to develop the interim regulations that were required by the Act. They met the timeline requirement for publishing an Interim Final Rule within 180 days and then sought public comment. APHIS and CDC collaborated to address the public comments and to make the language of their separate regulations parallel. In October 2005, the reconstructed 1918 influenza virus was added to the DHHS Select Agent list.

Mr. Hemphill addressed how the Select Agent list was established. In anticipation of the Public Health Security Bioterrorism Preparedness and Response Act of 2002, DHHS established an interagency work group comprised of subject-matter experts representing 21 Federal entities. After the signing of the Act, DHHS invited professional organizations to address this interagency work group to present their concerns and state what they would like to see in the regulation. The work group provided DHHS with recommendations for the list of agents and for the genetic elements section of the regulation. In August 2002, the list was published for public comment. The comments went back to the work group for review, which led to the Interim Final Rule published in December 2002. After a 60-day comment period, the interagency work group reviewed the comments received and provided feedback to DHHS. The Final Rule was published in March 2005.

Currently, there are three lists of Select Agents: those under the sole purview of DHHS, those under the sole purview of USDA, and those that overlap. More than 90 percent of the registered entities have as part of their registration at least one agent that is on the "overlap" list. Joint monitoring of these agents requires significant coordination between CDC and APHIS.

The Final Rule genetic-element language was reviewed carefully by the interagency work group. It reads in part: "…nucleic acids that can produce infectious forms of any of the select agent viruses; recombinant nucleic acids that encode for the functional form(s) of any toxins listed if the nucleic acids can be expressed *in vivo* or *in vitro* or are in a vector or recombinant host genome and can be expressed *in vivo* or *in vitro*; Select Agents and toxins that have been genetically modified." Mr. Hemphill said this language was determined based on the criteria specified in the Act, such as the effects of exposure, degree of contagiousness or pathogenecity. The intra governmental work group tried to

articulate which nucleic acids are intrinsically capable of causing disease. For viruses, the focus was on the ability to replicate and produce more viruses. It was more difficult to identify what should be captured in the regulation concerning bacteria and disease production from a genomics point of view. They decided to recommend regulation of bacteria that have the ability to express a fully functional form of the toxin. The language excludes, other than those listed above, whole genomic material and partial genomes.

Mr. Hemphill said nucleic acids that encode for a Select Agent virus, whether synthetic or naturally derived, that are intrinsically infectious are subject to the Select Agent Rules. Examples are positive strand RNA viruses and certain double-stranded DNA viruses. Also subject to the rules are any Select Agents created from nucleic acids, either synthetically or naturally. The 1918 influenza virus is an example. Not subject to the Select Agent Rules are nucleic acids that encode for other Select Agent viruses, bacteria or fungi because these nucleic acids are not intrinsically infectious or replication-competent. Recombinant nucleic acids that encode for and can express a functional Select Agent toxin are subject to the Select Agent Rules, while nucleic acids that encode for individual subunits that are not toxic are not subject to the regulation.

Concerning the regulatory language for Select Agent nucleotides, the interagency work group tried to articulate what nucleic acids encoded "factors associated with disease." However, those factors are often not known and things could be inadvertently regulated, such as one specific gene that in and of itself does not pose a threat to public health. They also looked at terminology such as "full length nucleic acids" and at what percentage of the genome was present. This brought up homology issues that could lead to inadvertent regulation. Mr. Hemphill re-stated that it isn't a public health threat to have a nucleic acid of the toxin itself. The public health threat is posed when that toxin sequence is in an expression system, and that concept went into the regulation.

Mr. Hemphill summarized by stating that the regulation represents a balance of regulatory oversight with the realistic impacts the regulations have on the scientific community. The goal is to minimize the disruption of legitimate research. The regulation provides flexibility when emergent infectious agent threats are identified, such as the addition of the 1918 virus. However, synthetic genomics creates a challenge because it has the potential to produce novel agents that defy current taxonomic classification.

**Gene And Genome Synthesis: Current Methods, Business Practices, and Anticipated Advances**

**John Mulligan, Ph.D.**
**President and CEO, Blue Heron Biotechnology, Inc.**

Dr. Mulligan addressed the current state of commercial gene synthesis technology, its future, and the screening practices for orders for Select Agents. He stated that access to DNA is central to modern biology, including synthetic biology. Acquiring and modifying DNA is costly, as researchers spend from $300 to $500 million a year on the reagents used to clone and modify genes. In addition, for every dollar spent on reagent use, fully

loaded costs add another $300 to $500 million.

Dr. Mulligan stated that the adoption of commercial gene synthesis could potentially substitute for current molecular biology at much less cost. The adoption of gene synthesis as a substitute is a relatively limited practice but is growing rapidly. The business is highly fragmented; the $30 million industry is spread among 30 to 50 different companies, most of which are very small. Dr. Mulligan projected that over the next 5 to 10 years, gene synthesis will become a significant percentage of all the molecular biology activities in the world because demand will drive rapid development. The core technology was first used in the late 1970s, but has been more widely used only recently. Dr. Mulligan said there are three general approaches to gene synthesis: 1) standard PCR-based gene synthesis, 2) array-based PCR synthesis, and 3) solid phase gene assembly.

He explained that PCR-based gene synthesis is simple in concept. Researchers synthesize an overlapping set of oligonucleotides that cover the desired sequence on all or parts of both strands. Those oligos are pooled together and PCR amplified. Some protocols involve a ligation step before the amplification and most use a secondary amplification with outside primers. It's then cloned into a plasmid vector of the new sequence, a correct clone is chosen, and larger fragments are assembled by fusion PCR or other methods. Most commercial synthesis, and essentially all synthesis in individual labs, is based on PCR. There are many published protocols and most will work on a substantial subset of all genes. A large percentage of natural sequences require or benefit from other approaches because of high GC content, because they are repetitive or for other reasons.

Array-based PCR synthesis technology isn't yet commercially viable, but has interesting possibilities. Instead of starting with a pool of oligos synthesized individually on a conventional oligosynthesizer, companies could use one of the technologies developed for measuring gene expression. They could synthesize a large number of oligos on a single surface, release them, and use them as the pool for PCR-based gene assembly. This technology has the potential to make gene synthesis very large-scale and inexpensive, although it is technically very demanding. It is limited by the quality of array-based oligo synthesis.

Solid-phase gene synthesis is conceptually similar to oligonucleotide synthesis except that the monomers for gene assembly are duplexed fragments of DNA rather than individual bases. It works on almost any sequence and is the method used at Blue Heron Biotechnology.

Dr. Mulligan stated that performing gene synthesis economically depends on reducing error rates and most companies have a method for error removal. The technical challenges in converting chemically synthesized oligonucleotides into cloned DNA fragments or genes create an error rate of about 1 in 300. The best synthesis has an error rate of about 1 in 500.

From the point of view of industry, gene synthesis is a complicated manufacturing process. Every order is different and each gene is made from a few dozen to several

thousand parts. Every part is new and is used only once for a specific order. The smallest parts are chemicals, with a mixed population of good and bad parts. The larger parts are biological, which causes unpredictability in the behavior. The final product must be perfect with no errors in the many thousands of bases.

Dr. Mulligan explained that the existing standards of conventional manufacturing tools are inadequate for this kind of manufacturing process so companies must build their own. It is a commodity market with prices dropping at 30 to 50 percent a year, meaning that companies must drop their production costs by at least that rate. Not many tools can be brought in to industrialize the process. Existing manufacturing tools focus on either pure assembly line production or job shop production.

Today, most or all commercial gene synthesis is carried out in sophisticated laboratories with some automation. Currently, it's relatively easy to start up a gene synthesis company for small-scale gene synthesis. However, within a few years, most commercial gene synthesis will be carried out through a highly industrialized process. A small number of companies are investing in converting from semi-automated labs into a robust manufacturing process with largely automated steps. Robots will be used for production and people will be used for process development. There will be highly sophisticated, internally developed process control and scheduling software.

Dr. Mulligan said the future will bring centralized commercial gene synthesis with industrialization and the ability to scale the critical competitive arena for commercial providers. New technologies will include array-based synthesis, new oligonucleotide synthesis technology and new assembly technology for large fragments. He stated that, within several years, there will be only two to four major companies still in business, each with the capacity to produce 20 to 50 million base pairs of synthetic DNA a year. This will substitute for a substantial fraction of the conventional molecular biology taking place today and will enable many new approaches in synthetic biology. A small number of specialized boutique operations may remain, but most production will take place in several centralized areas. Companies will use a mix of technologies to do all DNA sequences. Dr. Mulligan said the move toward centralization will make it easier to monitor the legitimate uses of gene synthesis.

He stated that there is a robust worldwide market for used equipment, such as oligo synthesizers and PCR machines. In addition, many countries have the industrial capacity to build every piece of equipment needed for gene synthesis from scratch. Oligonucleotide chemistry is feasible for companies or laboratories in nearly all countries, and molecular biology and bacteriology kits are readily available for purchase. The protocols and core knowledge are available on the Internet. Therefore, it's within the reach of many governments and non-governmental organizations to assemble all the technology needed for gene synthesis with only a moderate investment.

Dr. Mulligan said he toured a biotech corporation in Korea the previous year. The corporation built everything from scratch, and the core technology for gene synthesis was established in one building. The company has the capacity to produce phosphoramidites

at the multi-ton level where micrograms of material are used to synthesize a particular oligonucleotide.

Controlling synthesis technology will be difficult. Any sophisticated chemistry group could build oligo synthesis capacity from scratch, and it would use materials on such a large scale that controlling access to them would be difficult. Dr. Mulligan noted that PCR-based synthesis works on many sequences and that transforming and growing bacteria is a low-tech process.

Dr. Mulligan quoted from a number of publications to make the point that new methods are available that extend synthesis capabilities. For example, the Internet makes available all the engineering drawings needed to convert a Canon ink jet printer into an array-based oligonucleotide synthesizer.

Centralization will simplify monitoring and regulation of gene synthesis, but dispersion of the technology makes complete control implausible. Therefore, screening the orders in commercial facilities will be increasingly important to reduce the potential for nefarious uses. Dr. Mulligan said that not all companies screen their orders. Those that do not screen are concerned with the costs of screening, liability, and the effort required. Even the simplest screening technology requires that a Ph.D. examine the sequences and determine whether they violate a Select Agent Rule. Screening inefficiently actually exposes companies to greater potential liability than not screening at all.

In Dr. Mulligan's company, all orders are screened against a database of Select Agents. A Ph.D. reviews the positive hits, but most of these hits are not Select Agent genes. Those that are receive a second level of review. However, in some cases, the company reviews the literature and discusses an order with the customer to decide whether the company should make a particular sequence. They have never had an order for a sequence that they would not be allowed to supply under the Select Agent regulations.

Dr. Mulligan described the current screening tools as very simple. A homology search (BLAST) is used with a very low threshold, causing a high false positive rate. The database has all of the Select Agent sequences, not just those of concern. The Select Agent rules require some interpretation and his company interprets them a bit more broadly than the CDC. Screening is expensive and, as the industry grows, will become progressively more expensive.

Dr. Mulligan closed by describing the International Consortium for Polynucleotide Synthesis that was established in June 2006. Its goals are to pool the efforts of the gene synthesis companies to improve screening software and other tools, make them more economical to use, ensure that they're responsive to all relevant regulations, and encourage widespread use of the tools. They also want to provide an industry point-of-contact for the Government in the gene synthesis industry. Each of the member companies is investing effort to understand the government regulations in their home countries and the countries that they export to.

**Synthetic DNA to Select Agent Virus: Current Capabilities**

**Ralph Baric, Ph.D.**
**Professor, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill**

Dr. Baric stated that the objectives of his talk were to review the biothreat list, to describe a simple classification scheme that allows simplification of the number of strategies used by reverse genetics to recover an infectious genome from a DNA molecule, to discuss reverse genetics and synthetic genomes, to address technical barriers involved in resurrecting a virus genome from a synthetic DNA molecule, and to talk about technical chimeras and synthetic viruses using coronaviruses as an example.

Dr. Baric said there are several lists of biothreat viruses from various Federal agencies, including DHHS/CDC; USDA; the Department of Commerce, which has a list regulating the shipment of specific organisms internationally; and the NIH/NIAID categories A through C list, which focuses on various pathogens. Dr. Baric said these are very heterogeneous groups of viruses, with different genome organizations and different replication strategies, so the reverse genetic strategies developed to recover an infectious genome from a DNA molecule are very different. The genomes range from double-stranded DNA genomes, such as pox and herpesvirus, to single-stranded plus polarity RNA genomes, such as polio and foot and mouth disease virus. There are also single-stranded RNA negative genomes, such as influenza, and double-stranded RNA genomes. Several approaches are used to recover viral genomes using reverse genetics. Virus reverse genetics is defined as the ability to produce infectious virus from recombinant or synthetic DNA genomes.

Dr. Baric described the Baltimore classification scheme, which classifies viruses based on their ability to synthesize messenger RNA. He stated that if a viral genome is capable of synthesizing messenger RNA that can be translated into proteins essential for genome replication, it is infectious. Depending on the nature of the genome, all viruses are clustered into seven fundamentally different groups that utilize different strategies to synthesize messenger RNA from the input genome. Group I viruses include the double-stranded DNA viruses that use host transcriptase machinery to drive messages that can be translated into protein. The herpesvirus is one example. In contrast, the poxvirus genome, which is also double-stranded DNA, is not infectious because it requires one or more viral proteins to initiate messenger RNA transcription and boot (jump start) infectivity. Dr. Baric noted that there are no biothreat viruses in Groups II and VI. Group III viruses include double-stranded RNA viruses. They are not infectious in isolation and the components for booting genome infectivity in these viruses remain unresolved. Group IV viruses contain a single-stranded positive polarity RNA genome. Genome infectivity usually requires viral proteins or transcripts in trans (in addition to the genome itself) to be infectious, although some exceptions have been reported. Group V viruses contain a single-stranded RNA negative polarity RNA genome and include the Ebola and Marburg viruses. Genome infectivity requires the presence of full length RNA and a set of virally encoded replicase proteins that function as a transcriptional complex to express

messenger RNA. If messenger RNAs encoding the transcription complex are provided in trans, Group V genomes become infectious.

For the Group I double-stranded DNA viruses, technologies for virus recovery have been developed for both herpesviruses and poxviruses. Dr. Baric said it's easier with herpesviruses because the genome is infectious by itself. In the case of poxviruses, it is more complex because of the large genome size. The ends of the molecule form covalent hairpin loops that are essential for virus replication and it is difficult to produce them in cells to be able to boot infectivity. The genome is not infectious, so helper products must be provided in trans. Dr. Baric noted that a molecular clone has been described for vaccinia virus, providing a theoretical template for guiding similar technology with other members in the double-stranded DNA virus family.

Recovery technology has not been developed for the Group III double-stranded RNA viruses, although Dr. Baric said there was one report suggesting that it was. There is skepticism about this in the field.

For the Group IV viruses, i.e., the positive -stranded RNA viruses, the virus recovery technology has been well known for about 25 years. The Select Agents are the coronaviruses, foot and mouth disease virus, swine vesicular disease virus, plum pox, the alphaviruses, Venezuelan equine encephalitis and Eastern equine encephalitis, several tick-borne encephalitis viruses, and the flavivirus group. The noroviruses are the only Group IV viruses for which the recovery technology has not been solved.

For the Group V viruses, the virus reverse technology has been solved, in many cases with prototype members of the various families. The Select Agents include the myxovirus, such as the 1918 flu and the H5 avian flu; the paramyxoviruses; the filoviruses, such as Ebola and Marburg; the rhabdoviruses; the bunyaviruses, including Rift Valley Fever; and the arenaviruses. Dr. Baric stated that although there are no reverse genetic systems for many of the Select Agents, the basic mechanisms have been solved for the virus families in general.

He gave an example using the Group I herpesvirus. He said the basic strategy is to transfer the herpesvirus genome into a cell in combination with a bacterial artificial chromosome vector that contains flanking sequences that allow for homologous recombination into the herpesvirus genome. It doesn't knock out infectivity and few viral herpes viruses are produced that contain bacterial artificial chromosome sequences. They allow the viral genome to be maintained in E. coli. When the virus particle infects cells early on in herpesvirus replication, there's a circular double-stranded intermediate that can be purified out of the cell and that can be transfected into E. coli for the back sequences. The genome can be pulled out, modified, and put back into E. coli, eventually re-transfecting it into cells to recover virus. He said the basic strategy with poxvirus is similar, but much more complex because of the covalently linked ends of the molecule.

For the positive strand viruses, the strategy is basically the same and evolved from early work with poliovirus. The genome is infectious, so if the researcher purifies the RNA and

transfects it into cells, the researcher can recover virus. If the researcher clones a copy of that viral genome and puts it into a plasmid vector, even without expression systems, the researcher can occasionally, but rarely, get the virus out. The efficiency of this process can be increased by using either a DNA or an RNA launch system. In the case of a DNA launch, a eukaryotic promoter is used to drive transcripts; transfect the plasmid into the cells, the DNA promoter drives transcription, and virus infection takes place. Alternatively, the researcher can drive transcripts inside a test tube and make positive-stranded RNA transcripts, electroporate those into cells, and recover viruses.

The negative-stranded viruses are more complex. The genomes fall into linear and segmented categories. The linear negative-stranded RNA viruses include paramyxoviruses, filoviruses, and rhabdoviruses. The segmented genomes include arenaviruses, bunyaviruses, and myxoviruses, such as influenza. The basic strategy is to infect cells with vaccinia expressing the T7 RNA polymerase and co-transfect in plasmid DNA encoding a T7 RNA promoter that expresses helper messenger RNAs that encode the accessory functions needed to boot the infectivity. Negative-stranded RNA genomes are not infectious unless accessory factors are present. If the RNA genome is expressed in cells, the accessory proteins must be provided in trans to boot infectivity. The plus-stranded full-length RNA genome is transcribed into negative-stranded RNA, which serves as a template for message and then the infection kicks off to make virus. Dr. Baric stated that a major difference between the linear and segmented negative-stranded RNA viruses is that multiple genome segments have to be co-transfected into cells simultaneously.

Dr. Baric stated that if the genome is infectious, the researcher can directly recover the genome and the positive-strand RNA viruses. If the genome is non-infectious, the researcher must boot infectivity. These methods have been well determined.

There are several ways to incorporate synthetic DNA into the genomes of reverse-engineered viruses, including introducing synthetic genes into the molecular clones, making synthetic full-length genomes, and making chimeric viruses (blends of different viruses) with the purpose of creating designer vaccines or designer pathogens. In most cases, classic recombinant DNA approaches can be used to produce similar constructs. The major differences are reduced speed and mutagenesis capacity.

There are approximately 50 companies that synthesize DNA, and full-length genomes can be synthesized for most viruses. The most important issue is infectivity and the barriers to acquiring biodefense pathogens. Dr. Baric acknowledged that synthetic biology will increase the availability of biothreat viruses. Currently, almost all viruses are available in nature or in the laboratory. However, it's not always easy to obtain them from nature. In many cases, viruses in the laboratory must be passed in cell culture, so there could be cell culture adaptation mutations that would allow for efficient growth in cell culture, but simultaneously attenuate pathogenesis in humans. Viruses that are extinct in the wild include the 1918 flu, the 1957 flu, smallpox, the 2002-2003 epidemic SARs, and hopefully, in the future, poliovirus. In each case, these viruses are available in laboratories around the world. Dr. Baric added that he didn't know of any systematic way

to “clean up” after an outbreak of a virus such as SARS. He said genome-length sequences have been reported for almost all of the biodefense pathogens and they can be downloaded from GenBank.

Dr. Baric stated that one of the biggest issues in making synthetic pathogens *de novo* is having an accurate sequence and the stability of that sequence in plasmid vectors. He said that just because the sequence has been reported doesn’t make it infectious and the error rate in GenBank has been predicted at about 1-500 to 1-10,000, with small groups that do sequencing of pathogens having a higher error rate than large DNA synthesis consortiums. He said they have made four molecular clones of coronaviruses to date and none of the public sequences that came out was correct initially. He stated that a mistake in a sequence can be lethal or can attenuate pathogenesis. Another problem with synthetic DNA is size. Most synthetic DNA companies can handle from about one to about five kb in length, the size of an easy PCR-based approach. Viral genomes greater than 10 kb become progressively more difficult to make.

Dr. Baric walked through the steps involved in producing an infectious virus. He said the first step would be to pick a pathogen and obtain the sequence, taking into account size considerations and the fact that the plus polarity RNA viruses would probably be easier to launch than the negative polarity RNA viruses. Next would be a sequence validation, which is easiest if the infectious sequence has been reported in the literature. If not, then the individual would have to use phylogenetic comparisons within the family to try to estimate the correct sequence. Once a candidate sequence is chosen, the next step would be to synthesize the sequence and decide on a DNA launch system or an RNA launch system. The individual would have to know whether accessory factors are needed to boot infectivity and think about covert operations, i.e., hiding what is being done. One easy way to do that is to purchase DNA from multiple companies rather than a single company, perhaps from across the globe. Sequence variation of from 30 to 40 percent could be incorporated into the sequence. Sometimes a nucleotide sequence can be made to look like a benign virus within the family rather than one of the pathogenic members. Misdirection approaches could leave sequence tracks within the genome that could point the finger of blame toward others in the field and misdirect legal efforts to a specific lab. In addition, making gene fragments instead of full-length genomes would help get around the Select Agent rules. The individual could decide whether to make designer pathogens by synthetically blending in virulence genes from other viruses.

The next step is to assemble the full-length clone. If the sequence is less than 10 kb, it could be built by a company or built in pieces that could be easily assembled by the individual. If it were larger than 10 kb, the individual would have to build it. Next is the recovery of the recombinant or synthetic virus from a cell culture system, which is not difficult. Cell culture facilities and transfection techniques can be done fairly easily with a small, trained staff, although as the pathogen becomes more virulent, the staff would need protection.

Dr. Baric stated that the small, positive-strand RNA viruses would be the easiest pathogens to synthesize (e.g., polioviruses, alphaviruses and flaviviruses). Their

sequences have been reported and the molecular clones exist. He said that if the price of DNA were to go down by 20 percent per base, a high school student could buy the foot and mouth disease genome with about 1,500 base pairs and a DNA launch system.

Dr. Baric then described how to scale up, i.e., build larger genomes. He used the SARS coronavirus as an example, which has a 30 kb genome and is stable in plasmid cloning vectors. The SARS genome has been broken down into five or six pieces that have unique linker sequences at the end that allow for systematic and directional assembly. It uses Class IIS restriction endonucleases, such as BsmB1. It recognizes asymmetric seven nucleotide sequence and cleaves and leaves a four nucleotide asymmetric end. In the traditional cloning strategy, there would be two pieces of DNA to join. There's a four-nucleotide overhang generated at each piece. In the case of the Class IIS restriction enzymes, this is an asymmetric sequence, CCAG. Dr. Baric said Esp3I can leave more than 260 different ends, which give directionality to the assembly process. The asymmetric cutter can be placed in either strand. The orientation of the two Esp3I sites can be flipped, so that the variable end projects back into the sequence of each viral fragment. After cutting away the accessory sequences, they are joined back to form a seamless junction composed of the viral sequence. This means that one can break a viral genome at any four-nucleotide fragment and systematically reassemble it at any four-nucleotide fragment. There are no genetic signatures in those molecules. With a seven-nucleotide recognition sequence, one can assemble DNA molecules of over 1 million base pairs in length. Dr. Baric said that in 5 years it will probably be possible to resurrect microbial genomes *de novo* by DNA synthesis from synthetic DNA.

Dr. Baric showed a diagram of a phylogenetic tree of the 2002-2003 SARS coronavirus epidemic. It consisted of early phase, middle phase, and late phase epidemic strains associated with human infection. Most of the world was exposed to the Urbani strain or another late phase strain. Most middle and early phase isolates were only present in China and are not available to the rest of the world. Dr. Baric said that in China and Southeast Asia, there are a variety of zoonotic SARS isolates in civets, raccoon dogs, and bats that have been sequenced. These viruses have never been isolated in culture and exist only as computer viruses in sequence databases. Of the SARS isolates, the zoonotic strains are most likely to be re-introduced into the human population and it would be good to know if vaccines based on other strains, which Dr. Baric and his colleagues are studying, would protect against them. He noted that because of their work, the greatest number of divergent strains of SARS coronavirus exist at Chapel Hill in North Carolina. The vaccines that they've developed against SARS probably won't protect the elderly, who are most vulnerable to SARS.

Dr. Baric gave an example of synthesizing a molecular clone of a virus using reported sequences. He described the human coronavirus, NL63, which is a BL2 pathogen that causes croup and a lower respiratory tract disease. Two sequences had been published and they differed in 64 positions. Bioinformatic analysis and comparisons within the group reduced the number to 10 sites of concern. They synthesized this genome and transfected cultures, but were not able to get virus out. It turned out that the published sequence was incorrect. In addition, they found additional changes that couldn't be

predicted by bioinformatics. The errors in the sequence weren't necessarily errors made by the sequencers but could be caused by accumulated mutations. Dr. Baric made the point that having a limited number of sequences reported in the database makes virus reverse genomics difficult to do, especially with large genomes.

He also noted the risk of designer pathogens, which are increasing in number daily. These viral and microbial virulence genes can affect cell signaling and cell death pathways, antigen processing and presentation, acquired immune responses, and innate immunity. He explained that the ability to blend genes into virulent pathogens is complex and gave three examples from the literature in which genes had been dropped into viruses resulting in increased virulence rather than attenuation.

In summarizing the advantages of synthetic genomics, Dr. Baric mentioned speed of synthesis, mutagenic superiority, ease of genome construction, and low cost. Disadvantages include the fact that there's no guarantee that the synthetic genome will function as intended and in some cases, a sophisticated design requires extensive technical expertise and state-of-the-art research facilities.

**PANEL DISCUSSION WITH INVITED GUESTS**
**David Relman, M.D.**

Dr. Relman led a discussion with the panelists who presented on synthetic genomics. In response to a question concerning whether certain nucleic acids qualify as Select Agents, Mr. Hemphill said that a criterion is whether the mixture has, by classic scientific definitions, infectious nucleic acids. If the raw material is not infectious and would have to be manipulated to become infectious, it is not considered a Select Agent.

A Board member asked what the process is for requesting that an agent be removed from the Select Agent list. Mr. Hemphill explained that a request can be submitted to either USDA/APHIS or DHHS/CDC, depending on the agent, stating the reasons why the agent or toxin does not meet the criteria for inclusion outlined in the Act, i.e., posing a severe threat to public health and safety. Mr. Hemphill said the list is reviewed every two years, however, no agent or toxin has been removed from the DHHS/CDC Select Agent list since the publication of the Interim Final Rule.[1]

Judge Ehrlich asked Dr. Baric his opinion on the recent National Academies Report (Globalization, Biosecurity, and the Future of the Life Sciences) that recommended broadening the awareness of threats beyond the classic special agents to include synthetic organisms. Dr. Baric had not read the report and did not feel that he could recommend a policy, although he recognized that the report was accurate in pointing out a potential problem.

Dr. Relman asked Dr. Mulligan about the instructions given to his Ph.D.s for screening

[1] Note: Prior to publication of the Final Rule, plum pox *potyvirus* and *phakospora pachyrhizi* (Asian soybean rust) were removed from the USDA/APHIS Select Agent list, and *Clostridium botulinum* was removed from the USDA/APHIS-DHHS/CDC "overlap" Select Agents list.

orders. Dr. Mulligan said the primary issue is whether the gene being requested is identical to a gene in a Select Agent genome or whether the degree of homology yields a false conclusion in that regard. The threshold is set very low so that all Select Agent genes are captured in the screening process. Dr. Mulligan's company is also concerned that individuals might assemble pieces to create a viral genome, so the company considers whether the order has a legitimate use in vaccine or drug development. Dr. Casadevall commented that a company cannot always determine malicious intent by looking at what has been ordered, because virulence can only be expressed in a susceptible host. The screener would therefore have to know the immunity of the intended host. He acknowledged that the science does not exist to guard against this problem. Dr. Mulligan said he was in favor of the Government maintaining centralized information about those who order a broad range of restricted sequences. He said that eventually his company hopes to have the ability to recognize designer pathogens.

Mr. Nance asked how Dr. Mulligan's company could compete with companies that start up overseas without investing the time and money to screen orders. Dr. Mulligan replied that they currently compete with overseas companies that have fewer costs because they are not screening. Dr. Mulligan is bringing together an industry organization, described previously, to improve screening tools and drive costs down. Dr. Levy asked for more information on the group consortium. Dr. Mulligan said the initial goal is to improve screening software and communication in the industry, but he noted that the organization is still in the early stages.

To respond to a question about the difficulty of making a zoonotic strain highly pathogenic in humans, Dr. Baric explained the sequence of SARS from the civet to the epidemic strain. He said that many changes must occur to make a strain highly pathogenic in humans, not simply changing the tropism of the virus by engineering and dropping in a couple of changes. He said it is not as simple as switching a single codon to change the host range. Dr. Baric emphasized that pathogenesis is very complex in the case of SARS and this is probably true for many other viruses as well.

Dr. Casadevall noted that there are legitimate reasons to change the specificity of a virus, such as to create vaccines. Dr. Baric added that the treatment of some human diseases also rely on this technology.

**RECOMMENDATIONS OF THE SYNTHETIC GENOMICS WORKING GROUP**
**David Relman, M.D.**

Dr. Relman led a discussion of the findings and concerns identified by the Working Group on Synthetic Genomics. He stated that the following components of the regulatory framework were identified as most relevant to the control of synthetic Select Agents:

- Select Agent Rules;
- Export Controls (Commerce Control List);
- Title 18 USC, Section 175c (the variola Amendment); and
- Title 18 USC, Section 175 (prohibitions with respect to biological weapons).

He said a fundamental problem is defining the criteria for Select Agents. A 100 percent sequence match with a known strain of a Select Agent is a clear match, but anything less than that is uncertain. It is also relevant which segments of the genome and over what length of the segments there is sequence similarity. Dr. Relman said these are difficult issues for which there is no scientific consensus or complete clarity in existing rules and regulations. The Working Group therefore identified two major biosecurity concerns:

- Synthetic genomics enables the synthesis and production of a SA by nontraditional means, perhaps bypassing HHS/USDA review.
- It is possible to develop and produce agents that resemble, and have the attributes of specific Select Agent(s), without being clearly identifiable as SA based on their sequence.

These two concerns highlighted five issues and practices of concern to the Working Group. First is the ease of acquisition of synthetic Select Agent nucleic acids. Individuals who are versed in and equipped for routine methods in molecular biology can use readily available starting materials and procedures to derive some Select Agents *de novo*. This is facilitated by the fact that screening of orders is not standard practice among all vendors.

Second is the need for additional regulatory clarity. While the preamble of the Select Agent regulations notes that it is incumbent on entities that manufacture "substances," i.e., polynucleotides, to know what they are manufacturing and comply with the regulations, the regulations do not contain provisions that explicitly require genome service providers to screen orders. This could allow orders for regulated agents to evade detection.

Third is the difficulty in developing a suitable regulatory framework. The Select Agent Rules do not provide precise definitions for nucleic acids covered under the Rules. However, developing precise definitions will be challenging, given that there are many possible genetic alterations to the sequence of a Select Agent that would lead to expression of an agent with similar properties to that of the natural agent. In addition, pathogens can be engineered *de novo* with features of known Select Agents that might not be easily identified as Select Agents. The ability to predict the function and behavior of the expressed agents based on their genetic sequence is currently inadequate.

Fourth is the need for scientific consensus. Although some DNA synthesis providers screen orders against known sequences, including those of pathogens, there is no optimized, standardized, or agreed-upon method for screening.

The fifth issue is the construction of new pathogens. Synthetic genomics allows expression of agents that resemble and have the attributes of Select Agents without being clearly identifiable as Select Agents based on their sequence. This provides the capability for producing novel agents that pose risks equal to or greater than those of naturally occurring Select Agents.

The Working Group came to the conclusion that the language and requirements of existing controls for Select Agents will become increasingly ambiguous because of developments in the field of synthetic genomics. Therefore, relevant agencies should consider options for refining existing oversight mechanisms and reevaluate reliance on a finite list of specific agents as the foundation for the oversight framework.

Based on this conclusion, the Working Group developed a set of possible recommendations. The first stated that the Government should promote outreach and education for users and providers of synthetically derived nucleic acids and contribute to the development of best practices, such as standard procedures for ordering, screening, transferring, or using synthetic genomes. In addition, the Government should consider the international implications of any proposed changes to the current oversight framework and foster an international dialogue on these issues.

Second, the Government should provide additional guidance to users and providers of synthetically derived polynucleotide nucleic acids on the interpretation of the Select Agent Rules, especially on the definition of the agents. The Select Agent list and the Commerce Control List should be reconciled to allow for a coordinated oversight system by all involved agencies. Lawmakers should reexamine the language of Title 18, USC 175c. It currently allows for multiple interpretations.

Third, the relevant Government agencies should establish a group of experts from the gene synthesis industry and research communities to clarify the purview of the Select Agent Rules and develop guidance on genetic elements, recombinant nucleic acids, and recombinant organisms. Advances in technology are outpacing list-based regulations.

Fourth, Government agencies should reevaluate their reliance on an oversight framework that is predicated on a finite list of agents. It is now feasible to produce synthetic genomes that encode novel and taxonomically unclassified agents that have properties equivalent to or worse than those on the Select Agent list.

Dr. Relman said the next steps for the Working Group would be to continue to engage relevant groups within and outside the U.S. Government to develop policy options related to synthetic genomics. The Group also planned to finalize their recommendations and write a report for NSABB to review at the next meeting. Dr. Relman asked the Board for additional suggestions on recommendations and appropriate parties with whom the Working Group should engage as they move forward.

**Discussion**

Board members agreed that the Working Group had accurately identified the issues to be addressed and noted that it would be a long-term process to devise new systems to address these evolving issues.

Dr. Cohen asked what is on the Commerce Control List. Dr. Rexroad explained that it is a list of biological agents that must be accounted for when shipped and transported across

State boundaries or internationally.

Judge Ehrlich suggested that the recommendation to examine the language of Title 18, USC 175c on the variola virus ought to be changed to recommend repealing this section of the code. Judge Ehrlich also asked for clarification of the National Academies Report she alluded to earlier. Dr. Lemon explained that the Select Agent list is largely based on agents that were used in the weapons development programs of the 1960s, 1970s, and 1980s. The possibilities for weaponry using biotechnology are now much more complex. He said paradigms should be developed that work for synthetic genomics, as well as the older agents. He said the current Select Agent list will eventually become an anachronism.

Dr. Levy said he would like to hear more on activities that can be used to control the release of Select Agents by the synthetic industry. He hoped the Working Group would help define some cooperative movements in that industry.

**PUBLIC COMMENT**
**Edward Hammond**
**The Sunshine Project**

Mr. Hammond stated that variola is of particular interest to his organization, which finds the language of Title 18, USC 175c confusing. He expressed concern that the day's discussion did not address the variola virus. It resides in only one place in the U.S., at the CDC. The transfer of its DNA can only take place with the explicit authorization of the World Health Organization (WHO). He said the U.S. had recently been chastised for transferring variola virus DNA without obtaining WHO approval. Mr. Hammond said he learned recently that fully functional variola genes had been synthesized and inserted into other organisms at a national laboratory. He suggested that the language of the statute should state that this DNA should not be found anywhere other than the CDC and that there is an intergovernmental body for overseeing this type of research and the U.S. Government has made commitments to this body. Use of the variola virus should not be subject only to the law that applies to any other pathogenic agent in the U.S.

Dr. Relman agreed that variola is a special case and falls under the purview of the WHO guidelines. He said, however, that they do allow possession of less than 20 percent of the genome outside the CDC and a number of labs have asked for permission and been given less than 20 percent. He said this illustrates the complexity of the issue, including the question of the legal standing of the World Health Assembly Resolution in the United States.

Dr. Nicholson commented that CDC will transfer only a certain portion of the variola virus and that CDC keeps track of who has received it. But nobody can have anymore than 20 percent.

**NEXT STEPS AND ADJOURNMENT**
**Dennis L. Kasper, M.D.**

Dr. Kasper reviewed the next steps for the Board over the coming months. He stated that the Board must lay the foundation for education and outreach. When NSABB products are ready for public comment, an educational campaign will be necessary to provide the public with the proper context for understanding the materials. In addition, when NSABB products are adopted by the Government, a vigorous program of outreach will be essential to their effective implementation by the research community. The Chairs of the Working Groups planned to work with NSABB staff to strategize about appropriate outreach programs. They would report on those plans at a future NSABB meeting and also welcome comments from key stakeholders. Dr. Kasper said comments could be forwarded to the NSABB staff at nsabb@od.nih.gov.

The next major step was to make progress on the development of a proposed oversight framework. NSABB formed a Working Group to recommend the features of the proposed framework, including the attributes of review and oversight entities, processes for local and Federal review and oversight, and tools and guidance to facilitate these processes. Dr. Kasper said the Working Group would engage other NSABB members and consult various stakeholder groups, and he encouraged all the Federal agencies represented on the committee to participate. He reported that the Working Group met for the first time the previous day to discuss their charge. They planned to examine research oversight systems that could serve as models, such as the system for the oversight of recombinant DNA.

The major issues that must be addressed concerning oversight include: How do you review research for dual use potential within an institution? How do you assess risk? How do you manage risk once you assess it? How do you educate the institutional administration and ensure that the institutional level and individual investigator level are both involved? What types of issues should be reviewed at the national level by a different group or by NSABB?

The Working Group plans to discuss the purpose of each activity, the process for carrying it out, roles and responsibilities, a possible appeals process, the necessary expertise of the individuals and entities involved, and possible candidates within an institution. The Group will also discuss the timing of reviews and possible tools to facilitate oversight.

Dr. Kasper then thanked all those who attended the proceedings and concluded the meeting.