Recommendations for Listing Dangerous Biological Agents and Toxins and for Establishing Standards and Procedures Governing Their Possession and Use

INTRODUCTION

This paper presents a discussion and recommendations on the recently enacted Public Health Security and Bioterrorism Preparedness and Response Act of 2002, specifically Title II—Enhancing Controls on Dangerous Biological Agents and Toxins. These comments are a synthesis of consensus viewpoints that emerged at the 2002 annual Environmental Health and Safety Conference sponsored by the Howard Hughes Medical Institute (HHMI). The purpose of these comments is to aid the Secretary in preparing a Final Rule that reflects the collective expertise and knowledge of environmental health and safety professionals who are actively involved in promoting safety in academic research institutions.

The Hughes Institute is a nonprofit scientific and philanthropic organization whose principal purpose is the direct conduct of biomedical research. HHMI employs more than 325 independent researchers, along with their scientific staffs numbering roughly 3,000, who do biomedical research in laboratories located at 71 academic medical centers, universities, and other research institutions throughout the United States. Annually, HHMI sponsors a conference that brings together the directors of the environmental health and safety programs of the 71 host institutions to explore ideas for developing programs that are relevant to actual research risks, practical, and efficient to carry out. The goal is to ensure that environmental health and safety is a positive and integral part of biomedical research.

The 2002 Conference, “A Changed World—A Changing Role for EH&S,” focused on bioterrorism, the HHS Select Agent Rule, and the USA Patriot Act. Fifty-six environmental health and safety directors and 24 biological safety officers from the HHMI host institutions participated in the meeting. Two officials from the Centers for Disease Control and Prevention, Office of Health and Safety—Jonathan Y. Richmond, Ph.D., Director, and Shanna Nesby-O’Dell, D.V.M., Chief, External Activities Branch—shared their insights on the current HHS Select Agent Rule and the potential impact on biomedical research of proposed legislation. Breaking into small work groups, the participants developed consensus viewpoints on select agent security; interpretation of bona fide research exemptions; community outreach for public health preparedness; inventory control and select agent transfer practices; and emergency response.

Several Conference participants prepared these comments: W. Emmett Barkley, Ph.D., Director, HHMI Office of Laboratory Safety; Mary J. Corrigan, C.I.H., Associate Director, Environmental Health and Safety, Harvard Medical School; David W. Drummond, Ph.D., C.I.H., Director, Safety Department, University of Wisconsin-Madison; Claudia A. Mickelson, Ph.D., Deputy Director, Biosafety Program, Massachusetts Institute of Technology; David H. Silberman, Director, Health and Safety Programs, Stanford University School of Medicine; Cheryl A. Warfield, Senior Program Manager, HHMI Office of Laboratory Safety, and Amy Wilkerson, Associate Vice President for Research Support, The Rockefeller University.

The comments that follow are headed “List of Biological Agents and Toxins” and “Possession and Use of Listed Agents and Toxins.”
The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the Secretary to establish and maintain by regulation a list of each biological agent and each toxin that has the potential to pose a severe threat to public health and safety. In determining whether to list a biological agent or toxin, the Secretary is required to consider four criteria:

1. The effect on human health of exposure to the agent or toxin.
2. The degree of contagiousness and the methods by which transfer of the agent or toxin to humans can occur.
3. The availability and effectiveness of pharmacotherapies and immunizations to treat and prevent any illness resulting from exposure.
4. Any other criteria, including the needs of children and other vulnerable populations that the Secretary considers appropriate.

**Biological Agents:** These criteria are sound and appropriate for assessing the risks to public health and safety in the transfer, possession and use of biological agents. It is standard practice for scientists, laboratory managers, and environmental health and safety professionals to consider these criteria in assessing occupational and public health risks associated with the possession and use of infectious agents in research and clinical laboratories. For well over five decades, such assessments have provided the basis for the selection of appropriate practices, containment equipment, and facility safeguards that protect laboratory workers, other persons not directly involved in the laboratory work, and the community from exposure to infectious agents that cause human disease.

**Toxins:** The four criteria generally apply to toxins with one significant exception. The ability to replicate and the degree of contagiousness are not relevant factors in assessing risks from exposure to toxins. Toxins do not replicate in the laboratory or in an exposed individual, and a person ill from exposure to a toxin cannot transmit that illness to another person. **It is the quantity and concentration of a toxin during transfer, possession and use that limits the potential public health risk from exposure.** Quantities and concentrations of an infectious agent during transfer, possession and use do not impose similar limits. Individuals who may unlawfully acquire an infectious agent for terrorism purposes can grow a significant quantity from a small amount with conventional methods and equipment.

The differences in the amounts used between infectious agents and toxins in research, teaching, and other legitimate purposes also vary considerably. These differences impact risk assessments and the severity of a threat to public health and safety. For example, in a research laboratory it is typical to grow liter quantities of infectious virus in tissue cultures at concentrations of $10^6$ to $10^9$ viruses per ml. Standard practices like centrifugation can increase these concentrations by several logs. Such preparations can contain significant numbers of infective doses. For example, 1 ml of a laboratory preparation of Venezuelan equine encephalitis virus can contain $10^9$ theoretical minimal human infective doses for transmission by subcutaneous inoculation. By contrast, typical laboratory stock quantities for toxins seldom exceed 1 mg. The following table shows for several toxins that the number of theoretical lethal doses contained in the typical order size used in a research laboratory are relatively low. The severity of a threat to public health and safety is likely to be greater from exposure to a dangerous infectious agent than from exposure to a hazardous toxin. **The greatest threat may be the efficient dissemination by terrorism methods of a virulent infectious agent that causes a human contagious disease with high mortality.**
<table>
<thead>
<tr>
<th>Toxin</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Order Size</th>
<th>Number of LD&lt;sub&gt;50&lt;/sub&gt;s</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridium botulinum</em> neurotoxin</td>
<td>200 pg/kg</td>
<td>10 µg per vial</td>
<td>700</td>
</tr>
<tr>
<td>Ricin</td>
<td>2 µg/kg</td>
<td>1 mg – 1 g</td>
<td>7-7,000</td>
</tr>
<tr>
<td>Shiga toxin</td>
<td>250 ng/kg</td>
<td>10 µg</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Shigella shiga neurotoxin</td>
<td>1350 ng/kg</td>
<td>10 µg</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Staphylococcus enterotoxin F</td>
<td>2 µg/kg</td>
<td>10 µg</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>8 µg/kg</td>
<td>1 mg</td>
<td>2</td>
</tr>
</tbody>
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**Recombinant DNA:** The criteria for effect, contagiousness, availability of therapies and preventive treatments, and vulnerability are difficult to apply to recombinant DNA molecules because there is no evidence that recombinant DNA molecules are dangerous. However, there is an impressive body of knowledge available for making informed judgments about the potential of recombinant DNA molecules to pose a threat to public health and safety. The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) have been applicable to most recombinant DNA research conducted in the United States since 1976. This experience in the assessment of potential risks of recombinant DNA molecules, the actual conduct of research involving recombinant DNA molecules, and more recent safety testing for gene transfer studies are helpful in assessing whether certain recombinant DNA molecules have potential to pose a threat to public health and safety. This experience suggests that the potential risk associated with experiments using human pathogens as host-vector systems, or experiments in which the DNA from human pathogens is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems, is either less than or no more than the risk associated with the actual human pathogen. A further conclusion from this experience is that the potential risks are substantially lower where only a totally and irreversibly defective fraction of a pathogen’s genome is present in a given recombinant. This observation has made it possible to increase safety in infectious disease research by conducting experiments with clones of subgenomic segments from human or animal pathogens.

The NIH Guidelines consider certain experiments involving the deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally to present a higher order of potential risk. Also included in this group are experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD<sub>50</sub> of less than 100 nanograms per kilogram body weight. These types of experiments require approval of NIH and the investigator’s institution before initiation. The potential risks are evident and the general description of the recombinant organism is clear. A laboratory-derived recombinant of *Bacillus anthracis* that is genetically modified to be resistant to Cipro® would clearly be a candidate for listing.

It is important to base the determination for listing recombinant DNA molecules on the best available science, experience, and judgment and not on perceived or hypothetical risks. In addition, the characteristics of a recombinant molecule or organism that make it a dangerous biological agent must be clear. **Inaccurate risk perception or ambiguous hazard definitions will result in the unnecessary regulatory control of many recombinants that are possessed and used in thousands of research laboratories.** For example, the current definition of a hazardous genetic element from a select agent, which is, a genetic element “shown to produce or encode for a factor associated with a disease,” could apply to any genetic element of the select agent. Application of this
Comments on Select Agents

The definition would lead to an unwarranted overly broad inclusion of materials. This would constrain the availability of these recombinants for research, encumber important research in disease prevention and treatment designed to safeguard the public health from bioterrorism, and divert the important enforcement initiatives of the federal government from their proper focus on control of dangerous biological agents and toxins.

In determining whether to list a biological agent or toxin, application of the four criteria by the Secretary should substantiate how the biological agent or toxin meets the principal requirement of the Law, which is that a listed biological agent or toxin has the potential to pose a severe threat to public health and safety. This will require a careful examination of the possible means of agent dissemination for mass destruction purposes, means that may differ from an agent’s natural route of exposure. This is in addition to the normal risk assessment strategies that are necessary for the prevention of occupational disease and protection of public health from legitimate uses of biological agents and toxins. The potential for aerosol transmission of an agent is likely to present the greatest risk to public health and safety. Other means of dissemination are likely to present lower risks because usual hygiene and public health practices in the United States can better control such exposures. The risk of use of an agent in domestic or international terrorism is a significant component of the necessary risk assessment strategy for evaluating whether a select agent has the potential to pose a severe threat to public health and safety.

Recommendations for Listing Dangerous Biological Agents and Toxins:

1. The Secretary should identify candidate biological agents using the risk assessment paradigm described in the HHS Publication No. (CDC) 93-8395, Biosafety in Microbiological and Biomedical Laboratories, 4th Edition, 1999. The Secretary should limit consideration for listing biological agents to those agents that the risk assessment concludes require BSL-3 or BSL-4 practices, safety equipment, and containment facilities for the protection of laboratory workers, other persons not directly involved in the laboratory work, and the community from exposure to infectious agents. Final selection should involve careful consideration of the level of danger the agent poses to public health and safety based on an assessment of the risk of use of a candidate agent in domestic or international terrorism.

   An agent that poses the highest level of danger would have all of the following characteristics: high infectivity (low infectious dose); high mortality for the general public; causes contagious disease; stable in the environment; efficient aerosol transmission; no effective prophylaxis or therapeutic intervention; easy to produce in large quantities; easy to prepare for dissemination as a bioterrorism weapon.

   An agent that poses a low level of danger would have any of the following characteristics: low infectivity (high infectious dose); moderate morbidity for the general public, low mortality for children and other vulnerable populations; no person-to-person transmission; moderate stability in the environment; inefficient aerosol transmission; available effective prophylaxis and therapeutic intervention; difficult to produce in large quantities; difficult to prepare for dissemination as a bioterrorism weapon.

2. The Secretary should identify candidate biological toxins using the risk assessment paradigm described in the National Research Council publication Prudent Practices in the Laboratory – Handling and Disposal of Chemicals, National Academy Press, 1995. The Secretary should limit consideration for listing biological toxins to those
with an LD$_{50}$ for vertebrates less than 100 nanograms per kilogram body weight. Final selection should involve careful consideration of the level of danger the toxin poses to public health and safety based on an assessment of the risk of use of a candidate agent in domestic or international terrorism. The level of danger should be analogous to corresponding levels of danger set for listed biological agents. This correspondence should lead to setting a quantity threshold for biological toxins below which the toxin would not meet the requirements for listing as a toxic agent.

3. The Secretary should identify candidate recombinant DNA molecules by considering major actions and decisions under the NIH Guidelines regarding:

   a. The deliberate transfer of a drug resistance trait to listed dangerous biological agents not known to acquire the trait naturally if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture. (Section III-A-1-a of the NIH Guidelines)

   b. Experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD$_{50}$ of less than 100 nanograms per kilogram body weight. (Section III-B-1 of the NIH Guidelines)

   c. Experiments involving the introduction of recombinant DNA into listed dangerous biological agents. (Section III-D-1-d of the NIH Guidelines)

   d. Experiments involving the introduction of full-length nucleic acids of a listed dangerous biological agent or a segment of the agent’s genome that encodes for either a functional toxin or virulence factor sufficient to cause disease into any Risk Group 2, 3, or 4 agent. (Section III-D-1-a, b, and c, and Appendix B of the NIH Guidelines)

4. The Secretary should establish a formal mechanism for consultation with the NIH Recombinant DNA Committee (RAC) for guidance on determining whether a recombinant DNA molecule or recombinant organism has the potential to cause a severe threat to public health and safety. An appropriate mechanism would be a subcommittee of the RAC, with consultants as necessary, to ensure the subcommittee has the necessary expertise to identify recombinant DNA experiments that might create such an agent and to conduct an appropriate risk assessment to determine if that agent has the potential to pose a severe threat to public health and safety.

5. The Secretary should summarize, as an Appendix to the regulation, the risk assessment data that supports the Secretary’s determination for listing a biological agent or toxin. These data will heighten the awareness of individuals who possess and use a listed agent to the most important risk characteristics of the listed agents. This knowledge will promote safe practices and proficiency in the handling of a listed agent.

**POSSSESSION AND USE OF LISTED AGENTS AND TOXINS**

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 requires the Secretary to establish and enforce by regulation standards and procedures governing the possession and use of listed biological agents and toxins. The standards and procedures must address four provisions in the Law:
1. Safety procedures, including measures to ensure proper training and appropriate skills to handle listed biological agents and toxins; and proper laboratory facilities to contain and dispose of listed biological agents and toxins.

2. Safeguard and security measures to prevent access to listed biological agents and toxins for use in domestic and international terrorism or for any other criminal purpose.

3. The requirement to protect the public safety in the event of a possession or use in violation of established safety procedures and security measures.

4. The requirement to provide for appropriate availability of listed biological agents and toxins for research, education, and other legitimate purposes.

The nation is facing a new challenge in safeguarding the public health from the risk of use of dangerous biological agents in domestic or international terrorism. In meeting this challenge, it is important and prudent to examine the nation’s decades-old experience in developing effective, sensible control measures for the safe conduct of research. This experience is vital for establishing appropriate standards and practices to control the risk of use of dangerous biological agents and toxins in domestic and international terrorism.

Over the years, scientists, laboratory technicians, occupational physicians, epidemiologists, toxicologists, public health officials, biological safety experts, ethicists, health and safety professionals, and other health, safety and medical experts, often in partnership with the federal government, professional societies, and the National Academy of Sciences, have contributed to the development of guidelines and practices that ensure the safe possession and use of hazardous and potentially hazardous biological agents and toxins, including biological agents known to cause disease in humans, and recombinant DNA molecules. Collaborative initiatives in the United States that led to improvements in safety in both research and clinical laboratories intensified with the increased awareness to the health risks of occupational exposures to infectious agents that was evident in the results of surveys on laboratory-acquired disease published in the American Journal of Public Health in 1949 and 1951. Significant improvements in safe practices, equipment, and facilities appropriate for the possession and use of dangerous biological agents were pioneered during the 1950s and 1960s at Fort Detrick, the U.S. Army’s biological warfare defense facility. Scientific and public health journals published safety research and guidance from Fort Detrick without restriction. The health and safety professional staff openly consulted with academic, industrial, and medical communities to promote safety in the possession and use of infectious agents.

This experience laid the foundation for the standards and practices in place today to ensure safe possession and use of dangerous biological agents and to achieve compliance voluntarily. The NIH Guidelines, first published in 1976, and the publication Biosafety in Microbiological and Biomedical Laboratories, first prepared under the auspices of the CDC and NIH in 1984, reflect this experience. These were major collaborative initiatives. The recommended practices, safety equipment, and facility safeguards in these guidelines are advisory in most circumstances. The intent of the guidelines is to establish a voluntary code of practice, one that all members of a laboratory community can together embrace to safeguard their colleagues, and to protect the public. The results are rewarding. Laboratory-acquired infections from exposure to biological agents known to cause disease are infrequent. There are no reports of laboratory-acquired illness associated with the possession and use of recombinant DNA molecules. There are no reports that the possession and use of biological agents and toxins in research, education, and other legitimate purposes endangers the public health.
Today, existing standards and practices may require adaptation to ensure an appropriate level of vigilance and safety necessary for protecting the public health from the risk of use of dangerous biological agents in domestic or international terrorism. The nation’s experience in developing safeguards for protecting the health of laboratory workers, and the public health and safety from risks associated with the use of hazardous biological agents, hazardous chemicals, and radioactive materials in research demonstrates that the active involvement of the scientific community is essential. In addition, history has shown that peer review of compliance with performance-based standards promotes safety awareness and appropriate use of safe practices, equipment, and facility safeguards in biomedical research. A regulatory approach that builds upon peer review of compliance with performance-based standards is appropriate for governing the safe possession and use of listed biological agents and toxins, and for protecting the public health from bioterrorism. This approach will also ensure the appropriate availability of biological agents and toxins for research, education, and for other legitimate purposes, which is a requirement of the law.

Recommendations for Possession and Use of Listed Agents and Toxins

1. The Secretary should develop a performance-based regulation to control the possession and use of listed biological agents and toxins. The establishment and enforcement of performance standards and procedures would build upon the strengths developed within the scientific community for risk assessment, selection and use of appropriate practices, and compliance to prevent occupational disease and to protect the public health from legitimate uses of dangerous biological agents. This approach gives an institution the flexibility necessary to implement a system that is consistent with its specific operational procedures. There should be two core elements in this regulatory approach:

   a. The requirement for a registered facility to prepare and comply with the provisions of a Safety and Security Plan that will protect occupants of the facility and the public health from risks associated the facility’s possession, use, transfer, and disposal of listed biological agents and toxins, including the risk of unlawful access to these agents.

   b. A Code of Practice for standards and procedures for the possession, use, transfer, and disposal of listed biological agents and toxins.

2. The Secretary should require the Safety and Security Plan to include provisions to:

   a. Ensure institutional responsibility and accountability for preparing and carrying out the plan.

   b. Establish institutional procedures, including peer review, for review of safeguards appropriate for the possession, use, transfer, and disposal of listed agents.

   c. Ensure proper training and appropriate skills to handle listed agents.

   d. Ensure proper laboratory facilities to contain and dispose of listed agents.

   e. Prevent access to listed biological agents and toxins for use in domestic and international terrorism or for any other criminal purpose.

   f. Protect the public safety in the event of a possession or use in violation of established safety procedures and security measures, including contingency procedures for theft or loss, accidental release into the facility, or accidental release during external transfer of a listed agent.

3. The Secretary should promote the guidelines and recommendations in the U.S. Department of Health and Human Services’ publication, *Biosafety in the Microbiological and Biomedical Laboratories*, as a Code of Practice for the possession and use of listed biological agents. The Secretary should promote the guidelines and recommendations in the National Research Council’s publication, *Prudent Practices in the Laboratory—Handling and Disposal of Chemicals*, as a Code of Practice for the possession and use of
listed toxins. The Secretary should promote the guidelines and recommendations in the U.S. Department of Health and Human Services’ publication, NIH Guidelines for Research Involving Recombinant DNA Molecules, as a Code of Practice for the possession and use of listed genetically modified agents. These Codes of Practice allow discretionary decisions by safety committees, safety professionals, and laboratory managers to select practices, safety equipment, and facility safeguards appropriate for and relevant to an actual research program. The regulation should reference these Codes of Practice.

4. The Secretary should recommend that a registered facility establish an Institutional Biosafety Committee (IBC) or augment responsibilities of an existing IBC to aid the institution in developing a Safety and Security Plan; to carry out, as necessary, an independent risk assessment, oversight and review; and to assess institutional compliance with the provisions of the Plan. The membership of the IBC should include an expert in facility security.

5. The Secretary should provide guidance for a registered facility to use in establishing the appropriate security requirements for its Safety and Security Plan. The Secretary should define three levels of security, based on an evaluation of the level of threat to the public health. The levels are likely to correspond to Biosafety Levels 2, 3, and 4, since an agent assigned to Biosafety Level 1 would not be a dangerous agent, which is the requirement for listing. Minimum security (analogous to Biosafety Level 2) as provided to protect the registered facility from routine operational risks is appropriate for providing security for low risk listed agents. A moderate level of security (Level 3) would require safeguards within the laboratory, such as, locking mechanisms for containers that store stock amounts of listed agents. The highest level of security (Level 4) would additionally require safeguards that restrict unescorted entry into the laboratory to authorized persons. At all levels of security, a registered facility should maintain inventories of stock quantities with appropriate source characterization data, and records of transfer of a registered agent within the facility or to another registered facility.

6. The Secretary should encourage registered facilities and individuals who possess and use listed agents to share information on risk assessments, practices, safe equipment, and facility safeguards; theft or loss, accidental release into the facility, or accidental release during external transfer of a listed agent; security practices, as well as identified vulnerabilities; illness and other adverse effects from occupational exposure; and corrective actions that improve compliance for safe possession, use, transfer, and disposal of listed agents.