Implementation of the U.S. Government Policy for Institutional Oversight of Life Sciences DURC: Case Studies

Prepared by the National Institutes of Health on behalf of the United States Government

September 2014

Implementation of the *USG Policy for Institutional Oversight of Life Sciences DURC*: Illustrative Case Studies

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The purpose of the *USG Policy for Institutional Oversight of Life Sciences DURC* is to preserve the benefits of life sciences research while minimizing the risk of misuse of the knowledge, information, products, and technologies provided by such research. The Policy requires research institutions to establish a process for identifying dual use research of concern (DURC) and to implement risk mitigation measures for conducting and communicating such research. In the first step in this process, the principal investigator (PI) identifies research involving one or more of the 15 agents or toxins listed in the policy, and assesses whether the research can be reasonably anticipated to produce any of the seven listed experimental effects. The PI then refers the relevant research, including his/her assessment, to an Institutional Review Entity (IRE). The IRE reviews the PI's assessment and determines whether indeed the research involves any of the seven experimental effects, and if so, the IRE determines whether the research meets the definition of DURC, as defined in the policy. The IRE then works with the PI and the USG funding agency to develop a risk mitigation plan for responsibly conducting and communicating the DURC.

The case studies demonstrate the type of analysis that should be brought to bear during institutional reviews and highlight important administrative steps in the review process (e.g., the role of the PI, the role of the IRE, and the points at which institutions should notify the USG funding agency). The case studies range from research that falls outside the scope of the policy to research that is considered DURC under the policy, and also include points in between. It is important to note that the cases do not exemplify the only types of research that are covered under (or exempt from) the policy. Further, these cases provide *general* examples and analysis, but in practice, IREs should consider in-depth all of the scientific details as well as the specific risks and benefits associated with the research at hand.

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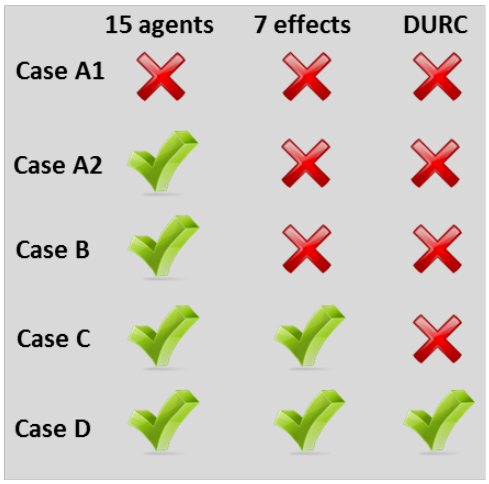
Figure 1. Overview of the Case Studies



Purposes of the Case Studies:

- Provide a range of examples of research that is subject to the *USG Policy for Institutional Oversight of Life Sciences DURC*
- Demonstrate the type of analysis that should be brought to bear during institutional DURC reviews
- Highlight important administrative steps in the review process
- Provide examples of risk mitigation measures
- Serve as an educational tool

Box 1. Reference information for the *USG Policy for Institutional Oversight of Life Sciences DURC*

Agents and toxins subject to the Policy

Avian influenza virus (highly pathogenic)
Bacillus anthracis
Botulinum neurotoxin (any quantity)
Burkholderia mallei
Burkholderia pseudomallei
Ebola virus
Foot-and-mouth disease virus
Francisella tularensis
Marburg virus
Reconstructed 1918 Influenza virus
Rinderpest virus
Toxin-producing strains of *Clostridium botulinum*
Variola major virus
Variola minor virus
Yersinia pestis

Categories of experimental effects

1. Enhances the harmful consequences of the agent or toxin;
2. Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification;
3. Confers to the agent or toxin resistance to clinically or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies;
4. Increases the stability, transmissibility, or the ability to disseminate the agent or toxin;
5. Alters the host range or tropism of the agent or toxin;
6. Enhances the susceptibility of a host population to the agent or toxin; or
7. Generates or reconstitutes an eradicated or extinct agent or toxin listed above.

Definition of dual use research of concern

Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security.

Case Study A1: The research is outside the scope of the Policy

Case Study A1: Description

Project title: Characterizing the immune response to *Burkholderia pseudomallei*

Agent/toxin: *B. pseudomallei* strain Bp82

Research aim/purpose: The investigators are characterizing the immune response to infection using an attenuated strain of *B. pseudomallei* to further understand pathogenesis and guide the development of novel therapeutics against *Burkholderia* infection. The researchers aim to overcome some of the major challenges in developing an effective *Burkholderia* vaccine, such as identifying broadly protective antibodies and understanding the correlations between acute or chronic infection with host innate or adaptive immunity.

Experimental manipulation: The research involves an attenuated strain of *B. pseudomallei* (strain Bp82), which will be used to infect murine macrophages deficient in various signaling molecules involved in the host immune response. Cells will be analyzed for changes in phenotype upon infection, and microarray analysis will be used to determine the transcription factors and signaling molecules that are activated in response to infection or by specific *Burkholderia* virulence factors.

Anticipated outcomes: Using an avirulent strain of *B. pseudomallei*, the researchers anticipate identifying transcription factors and transcriptional networks that are activated by the innate immune system in response to infection. These findings are expected to have significant relevance in understanding host immunity against *Burkholderia* that may lead to better treatment options.

Case Study A1: Analysis

Does the research involve one of the 15 listed agents?

No. The PI identifies B. pseudomallei as one of the 15 agents listed in the policy but determines that the strain he will be using—strain B0011—is listed as an attenuated strain under the Select Agents and Toxins Exclusions. Therefore, the research is not subject to oversight under the USG Policy for Institutional Oversight of DURC.

Consequently: The research does not require review by the IRE.

Case Study A2: The research involves one of the 15 agents, but not one of the 7 categories of experiments

Case Study A2: Description

Project title: Characterizing the immune response to *B. pseudomallei*

Agent/toxin: *B. pseudomallei*

Research aim/purpose: The investigators are characterizing the immune response to infection using wild type *B. pseudomallei* to further understand pathogenesis and guide the development of novel therapeutics against *Burkholderia* infection in humans. The researchers aim to overcome some of the major challenges in developing an effective *Burkholderia* vaccine, such as identifying broadly protective antibodies and understanding better the relationship between acute or chronic infection and host innate or adaptive immunity.

Experimental manipulation: The research involves a virulent, wild-type strain of *B. pseudomallei*, which will be used to infect murine macrophages deficient in various signaling molecules involved in the host immune response. Cells will be analyzed for changes in phenotype upon infection, and microarray analysis will be used to determine the transcription factors and signaling molecules that are activated in response to infection or by specific *Burkholderia* virulence factors.

Anticipated outcomes: Using a virulent strain of *B. pseudomallei*, the researchers anticipate identifying transcription factors and transcriptional networks that are activated by the innate immune system in response to infection. These findings are expected to have significant relevance in understanding host immunity against *Burkholderia* that may lead to better treatment options.

Case Study A2: Analysis

Does the research involve one of the 15 listed agents?

Yes. The PI identifies Burkholderia pseudomallei as one of the 15 listed agents and determines that he will be using a non-attenuated strain—that is, a strain that is regulated under the Select Agent Regulations and not listed under the Select Agents and Toxins Exclusions. Therefore, his research requires review under the USG Policy for Institutional Oversight of DURC.

Consequently: The PI submits the research to be reviewed by the Institutional Review Entity (IRE). The PI also considers whether the research involves any of the 7 categories of experimental effects and provides that rationale to the IRE.

Does the research involve any of the 7 categories of experimental effects?

PI Perspective: *The PI considers the range and nature of results that can be reasonably anticipated from this research and concludes that none of the 7 categories of experimental effects applies. He gave particular consideration to whether his proposed experiments might disrupt the host's immunity or render an immunization ineffective. However, he determined that the agent is not being modified and will be used for the purposes of characterizing changes in host-cell phenotype and cell-signaling pathways upon infection. While the research will shed light on signaling pathways that are important for host immunity, the proposed studies are not anticipated to disrupt the host's immunity or render an immunization ineffective. The PI reports this research along with his assessment to the IRE.*

IRE Perspective: *The IRE agrees with the PI's assessment that the research does not aim to produce, nor is reasonably anticipated to produce, any of the 7 listed experimental effects.*

Consequently: The research is not subject to additional oversight under *USG Policy for Institutional Oversight of DURC* and is not reported to the USG funding agency. The PI will report to the IRE any results or changes in the research such that one or more of the 7 categories of experimental effects may apply, or if the PI feels that the research may be DURC.

Case Study B: The research involves one of the 15 agents but not one of the 7 categories of experiments

Case Study B: Description

Project title: Comparative genomics and gene expression analysis of *Yersinia pestis*

Agent/toxin: *Y. pestis*

Research aim/purpose: The investigators are using comparative genomics and microarray analysis to understand the biology and pathogenesis of *Y. pestis*. Through gene expression profiling, the investigators aim to identify significant genes and expression patterns involved in various cellular mechanisms and to compare the genes and expression patterns of different *Y. pestis* strains.

Experimental manipulation: The investigators are using virulent strains of *Y. pestis*. The strains will be cultured in various growth media in the absence or presence of antibiotics and harvested at desired time points for RNA isolation. *Y. pestis* RNA will be extracted and used to synthesize cDNA for microarray analysis. Gene expression profiles will be generated over various growth stages of the bacteria and upon exposure to antibiotics.

Anticipated outcomes: Strains of *Y. pestis* will be cultivated under certain growth conditions (e.g., presence/absence of antibiotics, oxygen levels, acidity) and RNA will be extracted and used in microarray analysis. Gene expression profiles will be used for comparative genomics and computational studies with different strains of *Y. pestis*. By growing *Y. pestis* under different growth conditions and in the presence or absence of antibiotics, the investigators will likely identify *Y. pestis* genes that are activated or repressed under such conditions.

Case Study B: Analysis

Does the research involve one of the 15 listed agents?

Yes. The PI identifies Yersinia pestis as one of the 15 listed agents and determines that she will be using a non-attenuated strain—that is, a strain that is regulated under the Select Agent Regulations and not listed under the Select Agents and Toxins Exclusions. Therefore, her research requires review under the USG Policy for Institutional Oversight of DURC.

Consequently: The PI submits the research to be reviewed by the Institutional Review Entity (IRE). The PI also considers whether the research involves any of the 7 categories of experimental effects and provides that rationale to the IRE.

Does the research involve any of the 7 categories of experimental effects?

PI Perspective: *The PI considers the range and nature of results that can be reasonably anticipated from this research and concludes that none of the 7 categories of experimental effects applies. She gave particular consideration to whether her research might generate strains that would be resistant exisiting therapeutics. She will characterize how Y. pestis responds to antibiotic treatments but will not modify the agent or select for drug resistant strains, therefore, she concluded that it is unlikely that she will generate strains that are resistant to therapeutic interventions. The PI reports this research along with her assessment to the IRE.*

IRE Perspective: *The IRE agrees with the PI's assessment that the research does not aim to produce, nor is reasonably anticipated to produce, any of the 7 listed experimental effects.*

Consequently: The research is not subject to additional oversight under the *USG Policy for Institutional Oversight of DURC* and is not reported to the USG funding agency. The PI will report to the IRE any results or changes in the research such that one or more of the 7 categories of experimental effects may apply, or if the PI feels that the research may be DURC.

Case Study C: The research involves one of the 15 agents and one of the 7 categories of experiments, but is not DURC

Case Study C: Description

Project title: Developing novel antimicrobial compounds against *Francisella tularensis*

Agent/toxin: *F. tularensis*

Research aim/purpose: Investigators at a pharmaceutical company are developing new members of an existing class of antimicrobial compounds that target RNA and RNA/protein complexes in *F. tularensis*, the causative agent of tularemia. The researchers will seek FDA approval for successful antimicrobial compounds.

Experimental manipulation: Researchers will synthesize numerous antimicrobial compounds predicted to bind tertiary structures of bacterial RNA or RNA/protein complexes, thus blocking the essential functions of these molecules/complexes in *F. tularensis*. The researchers will test the efficacy of these compounds *in vitro* against wild-type *F. tularensis* as well as *F. tularensis* strains that have developed resistance to extant antimicrobials. The dose responsiveness of successful candidate compounds will be tested in animal models. Compounds that are safe and effective in animal models will move into clinical trials. As part of the FDA approval process, the company will be required to provide information about the frequency of resistance associated with each compound. This will include assays whereby *F. tularensis* strains are grown in the presence of the novel antimicrobial compound and step selection assays to determine the frequency of resistance associated with the compounds. Resistant strains will be destroyed and not characterized further.

Anticipated outcomes: The company expects to identify novel, safe, and effective antimicrobial compounds to treat tularemia. To comply with FDA requirements, the company will perform step selection of *F. tularensis* strains to generate information on the frequency of resistance associated with the compounds. Since the company is developing new members of an existing class of drugs, it is possible that they could generate a strain of *F. tularensis* that is resistant to compounds that have previously been approved for treatment.

Case Study C: Analysis

Does the research involve one of the 15 listed agents?

Yes. The PI identifies F. tularensis as one of the 15 listed agents and determines that she will be using a non-attenuated strain—that is, a strain that is regulated under the Select Agent Regulations and not listed under the Select Agents and Toxins Exclusions. Therefore, her research requires review under the USG Policy for Institutional Oversight of DURC.

Consequently: The PI submits the research to be reviewed by the Institutional Review Entity (IRE). The PI also considers whether the research involves any of the 7 categories of experimental effects and provides that rationale to the IRE.

Does the research involve any of the 7 categories of experimental effects?

PI Perspective: *The research involves testing various candidate antimicrobial compounds, both in vitro and in animal models, for their effectiveness against various strains of F. tularensis. In determining the rate and frequency of resistance associated with these novel compounds, the researchers will generate F. tularensis strains with varying levels of antimicrobial resistance. Since the candidate antimicrobial compounds are in the same class as existing antimicrobials against this agent (and may function through similar mechanisms as existing drugs in the same class) the PI recognizes the possibility that the F. tularensis strains generated from this research may also exhibit resistance to existing antimicrobials approved for treatment of tularemia. The PI concludes, therefore, that 1 of the 7 categories of experimental effects may apply to this research: "Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies." The PI reports this research along with her assessment to the IRE.*

IRE Perspective: *The IRE agrees with the PI's assessment that these experiments can be reasonably anticipated to generate new strains of F. tularensis that may be resistant to existing and approved treatments. The IRE also notes that, although the researchers are focusing their efforts on developing new antimicrobial compounds, and any F. tularensis strains with increased antimicrobial resistance will not be directly used for further studies, the information regarding such strains could potentially be used to cause harm. The IRE concludes, therefore, that 1 of the 7 categories of experimental effects may apply to this research: "Confers to the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitates their ability to evade detection methodologies."*

Consequently: Since the research involves 1 of the 15 listed agents and the IRE determined that 1 of the 7 listed experimental effects applies, the research requires further review. Toward this end, the IRE considers whether the research meets the definition of dual use research of concern (DURC).

Is the research DURC?

The IRE conducts an assessment of the research for DURC potential using the "Companion Guide, Section C: Points to Consider in Risk Assessment of DURC" as a framework for their discussion. After thorough deliberation, the IRE determines that, although the research could potentially generate strains of F. tularensis that are resistant to certain antibiotics, the risk of generating a strain for which there is no existing therapeutic interventions is particularly low. Specifically, the strains of F. tularensis generated from this research will not necessarily be resistant to other compounds of the same class of antimicrobials and are unlikely to be resistant to those in different classes of antimicrobials. The IRE also notes that any drug resistant strains are to be destroyed and will not be further characterized nor shared broadly. Therefore, the IRE determines that the research is unlikely to provide information about how similar drug resistant strains could be engineered or enhanced for harmful purposes. As a result, the likelihood of misuse of the agents that might be generated from this research, as well as the potential consequences of such misuse, are considered to be low. Therefore, the IRE concludes that this research does not meet the definition of DURC.

Consequently: Since the research involves 1 of the 15 listed agents and 1 of the 7 listed experimental effects, the IRE reports its findings to the USG funding agency, including the IRE's rationale as to why the research was determined not to be DURC. The PI will report to the IRE any results or changes in the research involving any of the 7 categories of experimental effects, or if the PI feels that the research may be DURC. Specifically, the IRE requested that the PI consult the IRE in situations where she plans to characterize further or publish information on any drug resistant strains that are generated from the research.

Case Study D: The research is determined to be DURC

Case Study D: Description

Project Title: A novel expression system for characterizing botulinum neurotoxin subtypes

Agent/toxin: Botulinum neurotoxin, Toxin-producing strain of *Clostridium botulinum*

Research aims/purpose: The investigators seek to characterize the specific biological properties of recently discovered botulinum neurotoxins (BoNT/X) and to elucidate structure-function relationships and enhance understanding of the molecular mechanisms of BoNT intoxication. A better understanding of these properties will contribute to improved treatments for botulism as well as other clinical applications using BoNT-based pharmaceuticals. One of the hurdles to this research is that the new BoNTs are only found in a strain of *C. botulinum* that co-expresses one other BoNT (BoNT/B1). Investigation of the new BoNTs requires significant quantities of purified, stable toxin, and this has been a major challenge using extant toxin-producing *Clostridium* strains. The investigators have developed a novel expression system to overcome co-expression and produce a single BoNT protein at very high yields for the purposes of toxin purification and *in vitro* characterization.

Experimental manipulation: The investigators have created a novel expression system using a *Clostridium* host to overexpress and purify large quantities of the desired BoNT/X. The host strain contains a BoNT gene, which has been disrupted, allowing the desired, plasmid-borne, BoNT/X to be the sole toxin expressed. In addition, the toxin gene has been genetically modified such that the purified neurotoxins are more stable and have a longer shelf-life, making them more useful for subsequent studies. This system overcomes poor expression levels and produces greater yields of stable BoNT/X for the purposes of toxin purification and *in vitro* characterization.

Anticipated results: It is anticipated that significant quantities of BoNT/X will be generated under controlled plasmid expression in a *Clostridium* strain. Toxins will be purified and used for further study and biochemical characterization. These toxins are expected to maintain potency and have greater stability than naturally produced BoNT. Moreover, the newly discovered BoNT/X to be overexpressed is poorly characterized and may not be neutralized by existing countermeasures.

Case Study D: Analysis

Does the research involve one of the 15 listed agents?

Yes. The PI identifies toxin-producing strains of C. botulinum and botulinum neurotoxin as two of the 15 listed agents. He determines that he will be using non-attenuated forms of the Clostridium strain and of Botulinum neurotoxin—that is, both agents are regulated under the Select Agent Regulations and not listed under the Select Agents and Toxins Exclusions. Further, he notes that the USG Policy for Institutional Oversight of DURC applies to research involving any quantity of botulinum neurotoxin. Therefore, his research requires review under the USG Policy for Institutional Oversight of DURC.

Consequently: The PI submits the research to be reviewed by the Institutional Review Entity (IRE). The PI also considers whether the research involves any of the 7 categories of experimental effects and provides that rationale to the IRE.

Does the research involve any of the 7 categories of experimental effects?

PI Perspective: *The research involves the production of significant quantities of a new BoNT via controlled plasmid expression in a strain of C. botulinum. The PI recognizes the possibility of a genome-plasmid recombination leading to the generation of a novel toxin-producing strain with unknown properties, such as producing toxin in greater quantities and/or with greater toxicity or stability. However, due to the rarity of such a recombination event and the unlikelihood of generating such a strain, the PI does not consider this result to be reasonably anticipated. In contrast, the genetic modifications resulting in the increased stability of the BoNT/X toxins leads the PI to conclude that the research is reasonably anticipated to produce 2 of the 7 categories of experimental effects: "Enhances the harmful consequences of the agent or toxin," and "Increases the stability, transmissibility, or the ability to disseminate the agent or toxin." The PI reports this research along with his assessment to the IRE.*

IRE Perspective: *The IRE agrees with the PI's assessment that: 1) a recombination event that might generate a novel strain of Clostridium that produces BoNT in greater yields and/or with greater toxicity is unlikely and, therefore, not of significant concern; and 2) the research involves modifications of BoNT/X genes that are expected to increase toxin stability. The IRE also notes that 3) the research will likely generate information about how to fairly easily produce quantities of pure and stable BoNT that is sufficient for use against populations; and 4) the investigators plan to overexpress a toxin that has not been fully tested against existing countermeasures. The IRE concludes that the research is reasonably anticipated to produce 2 of the 7 categories of experimental effects: "Enhances the harmful consequences of the agent or toxin," and "Increases the stability, transmissibility, or the ability to disseminate the agent or toxin."*

Consequently: Since the research involves 2 of the 15 listed agents and the IRE determined that 2 of the 7 listed experimental effects apply, the research requires further review. Toward this end, the IRE considers whether the research meets the definition of dual use research of concern (DURC).

Is the research DURC?

The IRE conducts an assessment of the research for DURC potential using the "Companion Guide, Section C: Points to Consider in Risk Assessment of DURC" as a framework for their discussion. After thorough deliberation, the IRE concludes that, considering the high potency and stability of overexpressed BoNT and the potential inadequacy of countermeasures against the new BoNT/Xs, the research is reasonably anticipated to provide products, information, and technology that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, animals, or national security. In other words, the research is considered to be dual use research of concern.

Consequently: The IRE reports its findings to the USG funding agency, including its rationale for why the research meets the definition of DURC. The IRE begins drafting a risk mitigation plan for the research.

What risk mitigation measures are appropriate?

The IRE assesses the risks and benefits ("Companion Guide, Section C") of the research and begins drafting a risk mitigation plan ("Companion Guide, Section D;" "March 2012 DURC Policy, Section IV") for conducting and communicating the research. The IRE identifies the following risk mitigation measures for this research:

- *In accordance with institutional policies, all laboratory personnel involved in this research will be required to be up-to-date with DURC training.*
- *All toxins generated from this research will be tested for their susceptibility to BoNT antitoxin, provided by the Centers for Disease Control and Prevention.*
- *Extant biosafety and biosecurity measures for this research are sufficient. However, during the annual review of this plan, the IRE will evaluate the research to determine whether additional or enhanced biosafety or biosecurity measures are needed.*
- *Prior to any form of public communication of research results, the PI, the institution's Director of Research Communications, and other relevant entities, as deemed appropriate by the institution, will convene and discuss the information to be shared, the risks of sharing that information, and whether the intended venue or mode of communication (e.g., content, timing, and extent of distribution) is appropriate. The institution will use the tool provided in the Companion Guide to the DURC Policies, Section F: Guidance for Responsible Communication of DURC for guidance in making these determinations.*
- *In consultation with the funding agency, the researchers are required to submit to the USG funding agency for pre-publication review all manuscripts regarding this research.*
- *The risk mitigation plan will be reviewed annually to determine whether it needs to be revised in light of new research findings.*

Consequently: The IRE works with the USG funding agency to finalize the risk mitigation plan and the PI agrees to conduct and communicate the research in accordance with that plan.