



Restricted Experiment Guidance Document

Preface

The information in this guidance document is meant to provide additional information to regulated entities in meeting the requirements for conducting a restricted experiment or possess the products resulting from a restricted experiment.

Revisions: This is a living document subject to ongoing improvement. Feedback or suggestions for improvement from registered Select Agent entities or the public are welcomed. Submit comments directly to the Federal Select Agent Program at:

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Revision History:

August 30, 2013: Initial posting

March 27, 2014 (Revision 1): Added note that the restricted experiment provision dealing with toxins currently only applies to Botulinum neurotoxin. Other changes are editorial errors from previous version.

August 28, 2014 (Revision 2): Added language describing the types of experiments that may fall under the restricted experiment provisions described in Section 13. Modified restricted experiment definition to reflect technical amendment published in the Federal Register on May 12, 2014. Other changes are editorial errors from previous version.

February 27, 2015 (Revision 3): Added language under subheading “Request to conduct a restricted experiment” describing the criteria reviewed and considered by the Federal Select Agent Program when determining if gradient selection experiments or drug efficacy experiments meet the definition of a restricted experiment.

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Introduction

The Federal Select Agent Program (FSAP) is a joint program consisting of the United States Department of Health and Human Services' (HHS) Centers for Disease Control and Prevention (CDC) Division of Select Agents and Toxins (DSAT) and the United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Services (APHIS) Agricultural Select Agent Program (ASAP-Veterinary Services and ASAP-Plant Protection and Quarantine). The Federal Select Agent Program oversees the use, possession, and transfer of select agents and toxins at registered entities throughout the United States. Select agents or toxins can be regulated as HHS only, APHIS only (Veterinary Services (VS) or Plant Protection and Quarantine (PPQ)), or Overlap Agents (regulated by both agencies).

The use of select agents and toxins in "restricted experiments" are regulated by the following Code of Federal Regulations (CFR): 42 CFR § 73.13 (HHS), 7 CFR § 331.13 (APHIS-agriculture), and 9 CFR § 121.13 (APHIS-animals and animal products).

Definition of Restricted Experiments

Select agent and toxin "Restricted experiment" provisions are found in Section 13 of the HHS and USDA Select Agent Regulations (42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121)

(a) An individual or entity may not conduct or possess products resulting from the following experiments unless approved by and conducted in accordance with the conditions prescribed by the HHS Secretary and/or Administrator

(1) Experiments that involve the deliberate transfer of, or selection for, a drug (or chemical §331.13) resistance trait to select agents that are not known to acquire the trait naturally, if such acquisition could compromise the control of disease agents in humans, veterinary medicine, or agriculture.

(2) Experiments involving the deliberate formation of synthetic or recombinant DNA containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD₅₀ < 100 ng/kg body weight.*

**Note: Currently, only nucleic acids containing genes for the biosynthesis of Botulinum neurotoxin meet the definition of 42 CFR 73.13 (b)(2).*

(b) The HHS Secretary or USDA Administrator may revoke approval to conduct any of the experiments in paragraph (a) of this section, or revoke or suspend a certificate of registration, if the individual or entity fails to comply with the requirements of this part.

(c) To apply for approval to conduct any of the experiments in paragraph (a) of this section, an individual or entity must submit a written request and supporting scientific information. A written decision granting or denying the request will be issued.

Examples of Potential Restricted Experiments

Examples of experiments that involve the transfer of, or selection for, drug resistance traits include, but are not limited to, alterations of the target site of the antibiotic, transfer of a genetic element that encodes for enzymes that inactivate antibiotics, induction of efflux pumps responsible for the active transport of the antibiotic out of the bacterial cell, introduction of genetic changes that decrease permeability of the bacterial cell wall to the antibiotic, etc. These mechanisms of action may be induced through recombinant or synthetic technology or passive, step-wise selection pressure methods.

Request to Conduct a Restricted Experiment

A request to conduct a restricted experiment should be submitted by the entity's Responsible Official and sent to the entity's Federal Select Agent Program file manager. The request will be reviewed by the Federal Select Agent Program. HHS only (42 CFR 73.3) or overlap (42 CFR 73.4 and 9 CFR 121.4) select agents may be further reviewed by the Intragovernmental Select Agents and Toxins Technical Advisory committee (ISATTAC). For VS only agents (9 CFR 121.3) and PPQ agents [7CFR 331.3], federal subject matter experts from APHIS, Agriculture Research Service (ARS), and Center for Veterinary Biologics (CVB) will review the request. It should not be submitted as part of an amendment to an entity's registration, as doing so may delay approval of other changes proposed in the amendment while the restricted experiment request is under review. An entity should submit the following information when requesting to conduct a restricted experiment.

A cover letter (on official entity letterhead) requesting to conduct the experiment along with the following relevant information:

- Synopsis of the proposed experiment(s) and the intended objective(s).
- Description of the nucleic acid insert (if applicable, complete sequence information is not required) and the predicted biological characteristics of the recombinant product.
- Description of the cloning/expression vector.
- Identification and characteristics of the host organism used for molecular cloning (if applicable).
- Description of the methods used for selection (e.g. recombinant or passive selection) to include all potential drug resistant traits (if applicable, **including intermediate variants**).
- Address whether there are alternative drug resistance markers that could be used, i.e., to avoid the acquisition of drug resistance that could compromise the use of the drug to

control disease agents in humans, veterinary medicine, or agriculture (if applicable). Provide an explanation as to why these alternative marker genes are not being considered.

- Description of biosafety level including facility containment, equipment and special practices to be utilized for the proposed experiment(s).
- Description of the mechanism and specificity of the antimicrobial resistance (antibiotic, antifungal and antiviral resistance) being conferred to include any cross-resistance to other therapeutically useful antimicrobials (if applicable).
- Estimated amount of toxin (recombinant or synthetic) to be produced (if applicable).
- Synopsis of any planned animal or plant experiments (if applicable) or other relevant animal or plant work.
- Scientific references or supporting documentation, particularly with respect to the biosafety aspects of the proposed experimental product.

In addition, the following criteria are reviewed and considered by the Federal Select Agent Program when determining if a) gradient selection experiments (*in vitro* or *in vivo*) or b) drug efficacy experiments (*in vitro* or *in vivo*) with the intention of evaluating any resistant strains that result from failed therapy with drugs that control disease agents in humans, veterinary medicine, or agriculture in select agents meet the definition of a restricted experiment. For these types of experiments, please address the following:

- Whether isolates less susceptible to the drugs that control disease agents in humans, veterinary medicine, or agriculture will be retained
- Whether drugs that control disease agents in humans, veterinary medicine, or agriculture will be used at therapeutic doses to limit the growth of a less susceptible population
- Whether the experimental model will exert pressure to create resistant strains
- Whether agar plates or broth media will be supplemented with drugs that control disease agents in humans, veterinary medicine, or agriculture to determine if resistant isolates were generated.
- Whether purposeful selection for resistant strains will be conducted.

Request to Conduct a Restricted Experiment Using Synthetic Nucleic Acids or Passive Selection

If an experiment using synthetic nucleic acids or passive selection (e.g. transfer of drug resistant traits through gradient or step-wise selective pressure as a method to select for drug resistance traits in select agents) was conducted prior to December 4, 2012, the experiment is not subject to the requirements of section 13 of the regulations. However, all requests to conduct a restricted experiment using synthetic nucleic acids or passive selection on or after December 4, 2012 are subject to the select agent regulations under Section 13.

A request to conduct a restricted experiment should be submitted by the entity's Responsible Official and sent to the entity's Federal Select Agent Program file manager requesting permission to conduct the experiment. If the request to perform the experiment is approved, the entity must update the relevant information as an amendment to their select agent registration.

Please refer to Table 1 for more information regarding the regulation status to conduct restricted experiments using synthetic nucleic acids, passive selection, or utilizing recombinant DNA.

Request to Generate Intermediate Variants

A request to conduct restricted experiments that involve the use of intermediate selection markers to generate intermediate variants should be submitted by the entity's Responsible Official and submitted to the entity's Federal Select Agent Program file manager. A description of the methods used for selection (e.g. recombinant or passive selection) to include all potential drug resistant traits, including intermediate selection markers not retained in the final construct, should be described in the request.

As a general rule, long-term storage materials include select agents and toxins (including viral genetic elements, recombinant and/or synthetic nucleic acids, recombinant and/or synthetic organisms, and products resulting from restricted experiments) which are not part of an ongoing experiment or have not been accessed for a significant period of time (e.g., 30 calendar days). All intermediate variants that contain restricted selection markers in long term storage must be added to the select agent inventory and managed in accordance with the select agent regulations. Please refer to the "Guidance on the Inventory Requirements for Select Agents and Toxins" document for more information.

Request to Possess the Product resulting from a Restricted Experiment

If the product was created prior to December 4, 2012, the product resulting from a restricted experiment does not require special approval for possession under Section 13. However, if the product is a select agent or toxin, approval to possess, use, and transfer is required under Section 7 of the regulations. If the product was created on or after December 4, 2012, the possession of the product from a restricted experiment is regulated under Section 13 and Section 7 of the regulations. Therefore, the entity must submit a written request to the FSAP asking permission to possess the product. If the possession of the product from a restricted experiment is approved, the entity must update the relevant information as an amendment to

their select agent registration. In addition, the subsequent Principal Investigator (PI)'s work objective should be submitted for review, and the entity could expect that any approval would be subject to the same restrictions and biosafety requirements as were imposed by the initial restricted experiment approval.

Please note that the requirements outlined above apply to products resulting from a restricted experiment conducted inside of the United States as well as outside of the United States. Therefore, if a restricted experiment was performed outside of the U.S. after December 4, 2012, the possession of the product resulting from a restricted experiment is regulated under Section 13 and Section 7 of the regulations. If the experiment was conducted prior to December 4, 2012, the possession of the product resulting from a restricted experiment does not require special approval under Section 13. However, the possession, use, and transfer of the product are required under Section 7 of the regulations.

Please refer to Table 1 for more information regarding the regulation status of the possession of products resulting from a restricted experiment.

Transfer of a Product resulting from a Restricted Experiment

Depending on when the product was generated, the possession of a product resulting from a restricted experiment may require pre-approval from the FSAP before a transfer request is approved. The potential receiver must submit the following documentation to the FSAP for review prior to transfer:

- Synopsis of the proposed experiment(s) and the intended objective(s)
- Description of biosafety level including facility containment, equipment and special practices to be utilized for the proposed experiment(s).
- Synopsis of any planned animal or plant experiments (if applicable) or other relevant animal or plant work.
- Description of the transferor (e.g. entity, name of person initiating transfer, point of contact information, etc.)
- Description of occupational health and medical surveillance procedures (applicable to Tier 1 select agents only)
- Scientific references or supporting documentation, particularly with respect to the biosafety aspects of the proposed experimental product

In addition, the APHIS/CDC Form 2, Request to Transfer Select Agents and Toxins, must be completed and submitted to the Federal Select Agent Program prior to transferring any select agent(s) or any toxin(s) over the exclusion amount as indicated in 73.3 (d)(3). Please refer to the instructions and guidance documents for Form 2 found at <http://www.selectagents.gov/TransferForm.html>. If approved, please note that an amendment to the recipient entity's select agent registration must be submitted to the Federal Select Agent Program.

Table 1: Restricted Experiments with Select Agents and Toxins Requiring Federal Select Agent Program Approval

	Experiment conducted prior to February 7, 2003	Experiment conducted between February 7, 2003 – December 3, 2012	Experiment conducted on or after December 4, 2012
Conducted a restricted experiment utilizing recombinant nucleic acids and/or recombinant organisms	NO	YES	YES
Conducted a restricted experiment using passive selection methods or synthetic nucleic acids	NO	NO	YES ¹
Possession of a product of a restricted experiment (includes experiments involving recombinant and/or synthetic nucleic acids, passive selection methods)	NO	NO	YES ²

¹Only passive selection or synthetic nucleic acid experiments conducted on or after December 4, 2012 are regulated under Section 13.

²Products that result from restricted experiments conducted on or after December 4, 2012 are regulated under Section 13.

Useful Links and Resources

Federal Select Agent Program: www.selectagents.gov

NIH OBA guidelines: http://oba.od.nih.gov/oba/rac/Guidelines/NIH_Guidelines.htm.

BMBL 5th edition: <http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf>