

## SELECT AGENTS AND TOXINS

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Some biological agents are capable of causing substantial harm to human, animal, or plant health and are high-risk agents for illegitimate use. Hence, the United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have established regulatory requirements for the possession, receipt, or transfer of such agents. USDA regulations are administered by the Animal and Plant Health Inspection Service (APHIS). HHS regulations are administered by the Centers for Disease Control (CDC). These regulations can be found at 42 CFR 73 (human/overlap select agents and toxins), 9 CFR 121 (animal/overlap select agents and toxins), and 7 CFR 331 (plant select agents). The term “overlap select agents and toxins” refers to those regulated select agents that are pathogenic to both humans and animals. Biological agents subject to these regulations are listed at the end of this document, along with key definitions from the regulations.

In accordance with regulatory requirements, all facilities that possess, receive, or transfer regulated agents must be registered, except diagnostic laboratories. However, certain conditions must be met to take advantage of the diagnostic laboratory exclusion (see EHS SOP, **Select Agents and Toxins – Clinical and/or Diagnostic Laboratory Activities**). Registration is specific to location and type of work. **Hence, Principal Investigators (PIs) who wish to possess, receive, or transfer select agents must gain approval from their department head/chair and notify EHS at least twelve weeks prior to such action to allow adequate time to complete the registration process.**

Projects involving the use of regulated select agents are subject to prior IBC review and approval, regardless of whether such projects involve recombinant or synthetic nucleic acids. Major provisions of the regulations for such projects are described below. Consult the actual regulations or contact EHS for a full description of regulatory requirements.

- The United States Departments of Health and Human Services or Agriculture must approve personnel who work with and/or have access to regulated select agents. No person should possess or have access to select agents without first having obtained approval. This process is referred to as Security Risk Assessments. Approvals are valid for a maximum of three years.
- The registration process with CDC/APHIS must be completed prior to possessing or commencing work with select agents. Registration is valid for a maximum of three years.

- Entities (i.e., UNL) that possess, receive, or transfer regulated agents must designate a Responsible Official (RO). At UNL, the Director of EHS serves in this capacity. The EHS Biosafety Officer serves as the Alternate Responsible Official. The RO is responsible for all official correspondence with federal agencies, including coordination of the registration and security risk assessment processes, oversight and inspection of laboratories, reporting and recordkeeping, and training. PIs also have similar responsibilities with respect to their individual laboratories.
- Work with select agents is subject to the following requirements:
  - Development and implementation of a written safety plan that is consistent with BMBL, NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, 29 CFR 1910.1450 (OSHA's Lab Standard), 29 CFR 1910.1200 (OSHA's Hazard Communication Standard).
  - Development and implementation of a security plan. Some elements of the plan will be covered by institutional procedures and policies. Work/project specific elements will include: IT (information technology) security, barriers (i.e., locks, video surveillance, maintenance and custodial activities, passwords, etc.), etc.
  - Separation of agent use and storage locations from public areas of the building.
  - Establishment of an emergency response plan.
  - Initial and annual refresher training for all workers.
  - Maintenance of an accurate inventory.
- All transfers (off-site and intra-facility) must be managed through EHS with appropriate documentation/records.
- In some cases, destructions must be managed through EHS with appropriate documentation/records.
- Immediate notification of theft, loss, or release must be made to the Responsible Official, who in turn is responsible to notify appropriate federal agencies.

## Regulated Agent List

### HHS SELECT AGENTS AND TOXINS

Abrin

Botulinum neurotoxins\*

Botulinum neurotoxin producing species of Clostridium\*

Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence X<sub>1</sub>CCX<sub>2</sub>PACGX<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub>CX<sub>7</sub>)

Coxiella burnetii

Crimean-Congo haemorrhagic fever virus

Diacetoxyscirpenol

Eastern Equine Encephalitis virus<sup>2</sup>

Ebola virus\*

**Francisella tularensis**\*<sup>a</sup>

Lassa fever virus

Lujo virus

**Marburg virus**\*

Monkeypox virus<sup>2</sup>

Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments  
(Reconstructed 1918 Influenza virus)

Ricin

Rickettsia prowazekii

SARS-associated coronavirus (SARS-CoV)

Saxitoxin

South American Haemorrhagic Fever viruses: Chapare, Guanarito, Junin, Machupo, Sabia

Staphylococcal enterotoxins A,B,C,D,E subtypes

T-2 toxin

Tetrodotoxin

Tick-borne encephalitis complex (flavi) viruses: Far Eastern subtype, Siberian subtype

Kyasanur Forest disease virus

Omsk hemorrhagic fever virus

**Variola major virus (Smallpox virus)**\*

**Variola minor virus (Alastrim)**\*

**Yersinia pestis**\*

## **OVERLAP SELECT AGENTS AND TOXINS**

**Bacillus anthracis**\*

Bacillus anthracis Pasteur strain

**Bacillus cereus biovar anthracis**\*

Brucella abortus

Brucella melitensis

Brucella suis

**Burkholderia mallei**\*

**Burkholderia pseudomallei**\*

Hendra virus

Nipah virus

Rift Valley fever virus

Venezuelan equine encephalitis virus<sup>2</sup>

## **USDA SELECT AGENTS AND TOXINS**

African horse sickness virus

African swine fever virus

Avian influenza virus<sup>2</sup>

Classical swine fever virus

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<sup>a</sup> The following strains are excluded: *Francisella tularensis* subspecies *tularensis*, SCHU S4Δ*clpB* strain (effective November 10, 2014) and all *Francisella tularensis* subspecies *novicida* (also referred to as *Francisella novicida*) strains and *F. novicida*-like strains (effective November 10, 2014)

**Foot-and-mouth disease virus\***

Goat pox virus

Lumpy skin disease virus

Mycoplasma capricolum

Mycoplasma mycoides

Newcastle disease virus<sup>1, 2</sup>

Peste des petits ruminants virus

**Rinderpest virus\***

Sheep pox virus

Swine vesicular disease virus

**USDA PLANT PROTECTION AND QUARANTINE (PPQ) SELECT AGENTS AND TOXINS**

Peronosclerospora philippinensis (Peronosclerospora sacchari)

Phoma glycinicola (formerly Pyrenochaeta glycines)

Ralstonia solanacearum

Rathayibacter toxicus

Sclerophthora rayssiae

Synchytrium endobioticum

Xanthomonas oryzae

**\*Denotes Tier 1 Agent**

<sup>1</sup> A virulent Newcastle disease virus (avian paramyxovirus serotype 1) has an intracerebral pathogenicity index in day-old chicks (*Gallus gallus*) of 0.7 or greater or has an amino acid sequence at the fusion (F) protein cleavage site that is consistent with virulent strains of Newcastle disease virus. A failure to detect a cleavage site that is consistent with virulent strains does not confirm the absence of a virulent virus.

<sup>2</sup>Select agents that meet any of the following criteria are excluded from the requirements of this part: Any low pathogenic strains of avian influenza virus, South American genotype of eastern equine encephalitis virus, west African clade of Monkeypox viruses, any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus, all subspecies *Mycoplasma capricolum* except subspecies *capripneumoniae* (contagious caprine pleuropneumonia), all subspecies *Mycoplasma mycoides* except subspecies *mycoides* small colony (Mmm SC) (contagious bovine pleuropneumonia), and any subtypes of Venezuelan equine encephalitis virus except for Subtypes IAB or IC, provided that the individual or entity can verify that the agent is within the exclusion category. 9/10/13

## Key Definitions

**Biological Agent:** Any microorganism (including, but not limited to, bacteria, viruses, fungi, or protozoa), or infectious substance, or any naturally occurring, bioengineered, or synthesized component of any such microorganism or infectious substance, capable of causing:

- (1) Death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism;
- (2) Deterioration of food, water, equipment, supplies, or material of any kind; or
- (3) Deleterious alteration of the environment

**Toxin:** The toxic material or product of plants, animals, microorganisms (including, but not limited to, bacteria, viruses, fungi, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes:

- (1) Any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or
- (2) Any poisonous isomer or biological product, homolog, or derivative of such a substance.

## Clarifications

### **Genetic Elements**

The following genetic elements, recombinant and/or synthetic nucleic acids, and recombinant and/or synthetic organisms are regulated as select agents (See sections 3(c) and 4(c) of 42 CFR Part 73, 9 CFR Part 121, and 7 CFR Part 331):

- Nucleic acids that can produce infectious forms of any of the select agent viruses.
- Recombinant and/or synthetic nucleic acids that encode for the functional form(s) of select toxins 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.

Currently, only nucleic acids that encode for the functional forms of Botulinum neurotoxins, or infectious forms of Foot-and-Mouth Disease virus, are regulated as a Tier 1 agent and therefore all Tier 1 regulatory requirements apply to these nucleic acids.

### **Exclusions**

The following select agents and toxins are excluded from the regulations if they meet any of the following criteria:

- (1) Any select agent or toxin that is in its naturally occurring environment provided the select agent or toxin has not been intentionally introduced, cultivated, collected, or otherwise extracted from its natural source.

- (2) Non-viable select agents or nonfunctional select toxins.
- (3) Low pathogenic strains of avian influenza virus
- (4) Any strain of Newcastle disease virus which does not meet the criteria for virulent Newcastle disease virus
- (5) All subspecies of *Mycoplasma capricolum* except subspecies *capripneumoniae* (contagious caprine pleuropneumonia) and all subspecies *Mycoplasma mycoides* except subspecies *mycoides* small colony (Mmm SC) (contagious bovine pleuropneumonia)
- (6) South America genotypes of Eastern Equine Encephalitis virus (EEEV)
- (7) Any subtypes of Venezuelan Equine Encephalitis virus (VEEV) except for subtypes IAB or IC.
- (8) The West African clade of Monkeypox virus
- (9) An animal inoculated with or exposed to an HHS select toxin
- (10) HHS toxins under the control of a principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor, if the aggregate amount does not, at any time, exceed the following amounts:

Abrin	100 mg
Botulinum toxin	0.5 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	1,000 mg
Ricin	100 mg
Saxitoxin	100 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	5 mg
T-2 toxin	1,000 mg
Tetrodotoxin	100 mg

### Exclusions/Attenuated Strains

HHS and/or USDA have excluded some attenuated strains of listed agents. These exclusions are published on the Federal Select Agent Program web site ([www.selectagents.gov](http://www.selectagents.gov)) and are limited to stated purpose/activities.

Genetic modifications to excluded attenuated strains may require submission, review, and approval of a separate exclusion request. Consult with EHS to determine applicability of any published exclusion to an attenuated, and/or genetically modified strain. In general, the following types of activities require registration or application for specific exclusion:

- Genetic manipulations of excluded, attenuated strains that enhance or restore virulence are subject to registration. Generally, genetic manipulations that delete or inactivate genes of excluded, attenuated strains would not reasonably be

expected to increase virulence and are therefore not subject to registration. However, registration must be sought if the PI later determines that the modification has enhanced virulence.

- Introduction of antibiotic resistance markers **may** require registration or application for a specific exclusion. However, introduction of sequences encoding reporter genes (e.g., GFP or beta-galactosidase) are not subject to registration or separate exclusion. A determination is generally based on whether the antibiotic resistance could compromise the use of the drug to control disease agents used in humans, veterinary medicine, or agriculture.

All provisions of the regulations remain in full-force until such time as the Agency provides positive, written consideration of an exemption request.

### **Prohibitions**

The following experiments require express prior approval from the Secretary of HHS/USDA:

- Experiments utilizing recombinant DNA that involve the deliberate transfer of a drug resistance trait to select agents that are 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.
- Experiments involving the deliberate formation of recombinant DNA containing genes for the biosynthesis of toxins lethal for vertebrates at an LD<sub>50</sub> of less than 100 ng/kg body weight.

Generally, HHS and USDA require Agency review and approval of any protocol or project involving the transfer of an antibiotic resistance trait to a listed agent, regardless of whether the antibiotic is used to treat infections in humans or animals.