SELECT AGENTS AND TOXINS

Introduction

The United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) have established regulatory requirements for certain microorganisms and biological toxins that have the potential to pose a severe threat to public health, animal health, plant health and safety or to the safety of animal or plant products. Federal law has been implemented for the possession, receipt, or transfer of these agents and the program is known as the Federal Select Agent Program.

Regulatory Information

USDA regulations are administered by the Animal and Plant Health Inspection Service (APHIS). HHS regulations are administered by the Centers for Disease Control and Prevention (CDC). These regulations can be found in 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. The Select Agents and Toxins list is found at this link: https://www.selectagents.gov/sat/list.htm and below in Appendix A.

In accordance with regulatory requirements, all facilities that possess, receive, or transfer regulated agents or toxins must be registered, except diagnostic/ clinical laboratories. See EHS SOP, ***Select Agents and Toxins – Clinical and/or Diagnostic Laboratory Activities*** for more information regarding the diagnostic/ clinical lab requirements under the law. Registration is specific to location and type of work. Hence, Principal Investigators (PIs) who wish to possess, receive, or transfer select agents or toxins must gain approval from their department head/chair and notify EHS at least **six (6) months** prior to such action to allow adequate time to complete the registration process.

In accordance with UNL Biosafety Guidelines, projects involving use of select agents or toxins are subject to UNL Institutional Biosafety Committee (IBC) review and approval prior to initiation of the work. 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 select agents or toxins. No person should possess or have access to select agents or toxins without first having obtained approval. This process is referred to as a Security Risk Assessment (SRA) and approvals are valid for a maximum of three years. Renewal of the SRA is required before the 3-year expiration date.

- The registration and amendment process with CDC/APHIS must be completed prior to possessing or commencing any work with select agents or toxins. Registration is valid for a maximum of three years. Each work objective and agent or toxin has to go through an amendment process and be approved by the Federal Select Agent Program before work can commence.

Registration Requirements

Entities (i.e., UNL) that possess, receive, or transfer select agents or toxins must designate a Responsible Official (RO). At UNL, the RO is in the Environmental Health & Safety (EHS) Department. 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, developing, reviewing and updating biosafety, security and incident response plans, reporting and recordkeeping, and training. Alternate Responsible Officials (AROs) are also responsible for these duties when the Responsible Official is not available/ on site. 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 biosafety plan that is consistent with Biosafety in Microbiological and Biomedical Laboratories (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 regulated select agent use and storage locations from public areas of the building.
    - Development and implementation of an incident response plan. This plan includes preplanned responses for incidents that may occur within a facility or to the facility. Examples include spills, natural disasters, fire, etc.
    - Initial and annual refresher training for all workers pertinent to the containment level of the work being conducted, the select agent regulations and developed plans.
    - Maintenance of an accurate inventory.

- All select agent transfers (off-site and intra-facility) must be managed through the RO with appropriate documentation/records.
- In some cases, destruction of select agents must be managed through the RO with appropriate documentation/records.
- Immediate notification of theft, loss, or release of select agents must be made to the RO, who in turn is responsible for notifying appropriate federal agencies.

Key Definitions

Select Agent and/or Toxin

All the agents or toxins listed in the regulations, unless specifically exempted. See Appendix A of this document.

Tier 1 Select Agent or Toxin

A subset of select agents and toxins that pose severe threat and therefore are subject to additional regulatory requirements beyond that of other select agents and toxins. See Appendix A of this document.

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 which 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.

Additional information is available in the Guidance on the Regulation of Select Agent and Toxin Nucleic Acids.

Select Agent and Toxins Exclusions

Based upon consultations with subject matter experts and a review of relevant published studies and information provided by the entities requesting the exclusions, the Federal Select Agent Program has determined that certain attenuated select agent strains or less toxic select toxins are not subject to the requirements of the select agent regulations. These exclusions are published on the Federal Select Agent Program web site (www.selectagents.gov) and are limited to stated purpose/activities.

An excluded select agent strain or modified toxin will be subject to the regulations if there is a reintroduction of factor(s) associated with virulence, toxic activity, or other manipulations that modify the attenuation such that virulence or toxic activity is restored or enhanced. In addition, excluded select agent strains or modified toxins are not exempt from the requirements of other applicable regulations or guidelines (e.g., NIH guidelines, USDA/APHIS permits, etc.).

Genetic modifications to exclude attenuated strains may require submission, review, and approval of a separate exclusion request. Consult with the RO 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 the Federal Select Agent Program provides positive, written consideration of an exemption request.

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

Toxins

Toxins under the control of a principal investigator, treating physician or veterinarian are exempt if the aggregate amount does not exceed, at any time, the amounts indicated in the table below:

Toxin	Amount
Abrin	1000 mg
Botulinum toxin	1 mg
Short, paralytic alpha conotoxins	200 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Possession of the above toxins in amounts less than that indicated in the table does not exempt the possessor from the requirement of UNL IBC review and approval of work with the toxin.

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_{50} 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.

Select Agents and Toxins List – Appendix A

Refer to the Federal Select Agent Program website, www.selectagents.gov, for a complete and current list.

HHS SELECT AGENTS AND TOXINS

HHS Select Agents and Toxins

1. Abrin [6]
2. *Bacillus cereus* Biovar *anthracis* [1]
3. Botulinum neurotoxins [1][6]
4. Botulinum neurotoxin producing species of *Clostridium* [1]
5. Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$) [6]
6. *Coxiella burnetii* [8]
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol [6]
9. Eastern equine encephalitis virus [4][5]
10. *Ebolavirus* [1]
11. *Francisella tularensis* [1]
12. Lassa fever virus
13. Lujo virus
14. Marburg virus [1]
15. Monkeypox virus [4]
16. 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)
17. Ricin [6]
18. *Rickettsia prowazekii*
19. Severe acute respiratory syndrome coronavirus (SARS-CoV) [5]
20. SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors
21. Saxitoxin [6]

South American Haemorrhagic Fever viruses:

22. Chapare
23. Guanarito
24. Junín
25. Machupo
26. Sabia

27. Staphylococcal enterotoxins (subtypes A,B,C,D,E) [6]
28. T-2 toxin [6]
29. Tetrodotoxin [6]

Tick-borne encephalitis complex (flavi) viruses:

30. Far Eastern subtype [5]

31. Siberian subtype [5]

32. Kyasanur Forest disease virus [5]
33. Omsk hemorrhagic fever virus [5]
34. Variola major virus (Smallpox virus) [1]
35. Variola minor virus (Alastrim) [1]
36. *Yersinia pestis* [1]

Overlap Select Agents and Toxins

37. *Bacillus anthracis* [1]
38. *Bacillus anthracis* Pasteur strain
39. *Burkholderia mallei* [1]
40. *Burkholderia pseudomallei* [1]
41. Hendra virus
42. Nipah virus [1]
43. Rift Valley fever virus
44. Venezuelan equine encephalitis virus [4][5]

USDA Veterinary Services (VS)
Select Agents and Toxins

45. African swine fever virus
46. Avian influenza virus [4]
47. Classical swine fever virus [5]
48. Foot-and-mouth disease virus [1][5]
49. Goat pox virus
50. Lumpy skin disease virus
51. *Mycoplasma capricolum* [4]
52. *Mycoplasma mycoides* [4]
53. Newcastle disease virus [3][4]
54. Peste des petits ruminants virus
55. Rinderpest virus [1]
56. Sheep pox virus
57. Swine vesicular disease virus [5]

USDA Plant Protection And Quarantine (PPQ)
Select Agents and Toxins

1. *Coniothyrium glycines*
   (formerly *Phoma glycinicola* and *Pyrenochaeta glycines*)
2. *Ralstonia solanacearum* [7]
3. *Rathayibacter toxicus*
4. *Sclerophthora rayssiae* [7]
5. *Synchytrium endobioticum*
6. *Xanthomonas oryzae*

[1] Denotes Tier 1 Agent

[2] C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins a-MI and a-GI (shown above) as well as a-GIA, Ac1.1a, a-CnIA, a-CnIB; X1 = any amino acid(s) or Des-X; X2 = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X3 = Arginine or Lysine; X4 = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X5 = Tyrosine, Phenylalanine, or Tryptophan; X6 = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X7 = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example, if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

[3] 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

USDA Veterinary Services (VS)
Select Agents and Toxins

58. African swine fever virus
59. Avian influenza virus [4]
60. Classical swine fever virus [5]
61. Foot-and-mouth disease virus [1][5]
62. Goat pox virus
63. Lumpy skin disease virus
64. *Mycoplasma capricolum* [4]
65. *Mycoplasma mycoides* [4]
66. Newcastle disease virus [3][4]
67. Peste des petits ruminants virus
68. Rinderpest virus [1]
69. Sheep pox virus
70. Swine vesicular disease virus [5]

USDA Plant Protection And Quarantine (PPQ)
Select Agents and Toxins

7. *Coniothyrium glycines*
   (formerly *Phoma glycinicola* and *Pyrenochaeta glycines*)
8. *Ralstonia solanacearum* [7]
9. *Rathayibacter toxicus*
10. *Sclerophthora rayssiae* [7]
11. *Synchytrium endobioticum*
12. *Xanthomonas oryzae*

A key follows:

[1] Denotes Tier 1 Agent

[2] C = Cysteine residues are all present as disulfides, with the 1st and 3rd Cysteine, and the 2nd and 4th Cysteine forming specific disulfide bridges; The consensus sequence includes known toxins a-MI and a-GI (shown above) as well as a-GIA, Ac1.1a, a-CnIA, a-CnIB; X1 = any amino acid(s) or Des-X; X2 = Asparagine or Histidine; P = Proline; A = Alanine; G = Glycine; X3 = Arginine or Lysine; X4 = Asparagine, Histidine, Lysine, Arginine, Tyrosine, Phenylalanine or Tryptophan; X5 = Tyrosine, Phenylalanine, or Tryptophan; X6 = Serine, Threonine, Glutamate, Aspartate, Glutamine, or Asparagine; X7 = Any amino acid(s) or Des X and; "Des X" = "an amino acid does not have to be present at this position." For example, if a peptide sequence were XCCHPA then the related peptide CCHPA would be designated as Des-X.

[3] 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.

[4] 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, Madariaga virus, Clade II Monkeypox, 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.

[5] For determining the regulatory status of nucleic acids that are capable of producing infectious forms of select agent viruses, please reference guidance here.

[6] For determining the regulatory status of Recombinant and/or Synthetic nucleic acids that encode for the toxic form(s) of any select toxins if the nucleic acids (i) can be expressed in vivo or in vitro, or (ii) are in a vector or recombinant host genome and can be expressed in vivo or in vitro; please reference guidance here.

[7] Select agents or toxins that meet any of the following criteria are excluded from the requirements of this part: Any subspecies of *Ralstonia solanacearum* except race 3, biovar 2 and all subspecies of *Sclerophthora rayssiae* except var. *zeae,* provided that the individual or entity can identify that the agent is within the exclusion category.

[8] *Coxiella burnetii* Phase II, Nine Mile Strain, plaque purified clone 4 with reversion to wildtype *cbu0533* is a select agent.

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