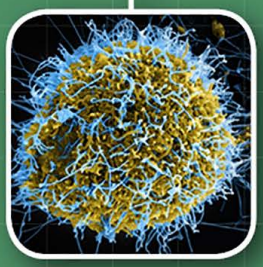

SELECT TOXIN GUIDANCE

MAY 2017

Centers for Disease Control and Prevention
Division of Select Agents and Toxins

Animal and Plant Health Inspection Service (APHIS)
Division of Agricultural Select Agents and Toxins

Preface

The information in this guidance document is meant to provide additional information to regulated entities to assist them in meeting the requirements for select toxins.

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:

CDC: LRSAT@cdc.gov
APHIS: DASAT@usda.gov

Revision History:
June 23, 2016: Initial posting
March 21, 2017: Updates include new regulatory requirements, revised List of Select Toxins and Non-Regulated Amounts, added section on food and clinical samples, revised section on toxin as a byproduct, revised section on documenting due diligence, and revised chart in Appendix A.
May 17, 2017: Updates to Identification of a select toxin requirements (APHIS/CDC Form 4) section.

Table of Contents

Introduction

The Federal Select Agent Program (FSAP) oversees the possession, use, 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)), Plant Protection and Quarantine (PPQ)), or Overlap Agents (regulated by both agencies). Currently select toxins are only regulated by HHS (42 CFR Part 73). This guidance document is intended to assist those entities that work with select toxins in meeting the requirements of the regulations. The majority of the guidance is intended for those entities that are registered to possess, use, or transfer a select toxin, regardless of whether they actually possess any amount of a select toxin. The guidance on exclusions, exemptions, and due diligence is pertinent for unregistered entities that work with select toxins below the regulatory threshold.

Regulatory Definitions (§ 73.1)

Toxin – the toxic material or product of:

- Plants, animals, or microorganisms (including, but not limited to: bacteria, viruses, fungi, rickettsia, or protozoa), or
- Infectious substances, or
- A recombinant or synthesized molecule, whatever their origin and method of production, and includes:
    - Any poisonous substance or biological product that may be engineered as a result of biotechnology, produced by a living organism, or
    - Any poisonous isomer or biological product, homolog, or derivative of such a substance.

Principal Investigator (PI) – the individual designated by the entity to direct a project or program who is responsible to the entity for the scientific and technical direction of that project or program.

List of Select Toxins and Non-Regulated Amounts

The following toxins are not regulated if the amount under the control of a principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor does not exceed, at any time, the amounts indicated in the table below[1].

HHS Select Toxins [§73.3(d)(3)]	Amount
Abrin	1000 mg
Botulinum neurotoxins (BoNT)	1 mg
Short, paralytic alpha conotoxins containing the following amino acid sequence X_1**CC**X_2PA**C**G$X_3X_4X_5X_6$**C**X_7*	100 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin (STX)	500 mg
Staphylococcal Enterotoxins (SE) (Subtypes A, B, C, D, E)	100 mg
T-2 toxin	10,000 mg
Tetrodotoxin (TTX)	500 mg

* C = Cysteine residues (indicated in bold) 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 α-MI and α-GI (shown above) as well as α-GIA, Ac1.1a, α-CnIA, α-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.

Note: Recombinant and/or synthetic nucleic acids, that encode for the toxic form(s) of select toxins if the nucleic acids can be expressed, are regulated. The aggregate toxin amounts stated above do not apply to regulated nucleic acids as any amount of these nucleic acids would be regulated.

With respect to a Principal investigator (PI), (defined as an individual who is designated by the entity to direct a project or program and who is responsible to the entity for the scientific and technical direction of that project or program) an entity is not required to register with FSAP as long as each PI possesses less than the regulated amount of select toxin.

If one PI transfers select toxin to another PI such that the receiving PI now possesses an amount of toxin above the regulatory amount, then the receiving PI and entity are both in violation of the SAR. The aggregate amount pertains to the PI and not the entity.

Regulated select toxin derivatives

A select toxin derivative is a select toxin that possesses modifications, such as the addition of a hydroxyl group, that make them different from the parent molecule in nomenclature but still retain

[1] Currently, USDA does not regulate any toxins.

toxicity similar to the parent. An example of a derivative that is regulated is the **select toxin derivative HT-2.** HT-2 has similar toxic properties as T-2.[2].

List of examples of unregulated non-toxic select toxins

- Ricin immunotoxin
- T-2 glucoside
- Toxin subunits such as the light chain of BoNT or the Ricin subunit A only. (However, any reconstitution of the holotoxin with the heavy chain of BoNT or Ricin subunit B respectively would be regulated).
- Toxoids (A toxoid is a toxin whose toxicity has been inactivated or suppressed either by chemical or heat treatment. Other properties, typically immunogenicity, are maintained).

Select Toxin Genetic Elements, Recombinant and/or Synthetic Nucleic Acids, and Recombinant and/or Synthetic Organisms (Sections 3(c), 4(c))

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

- Recombinant and/or synthetic nucleic acids that encode for the toxic 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 toxins that have been genetically modified.

Go to Guidance on the Regulation of Select Agent and Toxin Nucleic Acids for additional information.

Restricted Experiments for Select Toxins

The "restricted experiment" provisions are found in section 13 of 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 or APHIS Administrator:

(2) Experiments involving the deliberate formation of synthetic or recombinant DNA containing genes for the biosynthesis of select toxins lethal for vertebrates at an LD[50] < 100 ng/kg body weight.

[2] PLoS One. 2013;8(3):e60484. doi: 10.1371/journal.pone.0060484. Epub 2013 Mar 27. Influence of T-2 and HT-2 toxin on the blood-brain barrier in vitro: new experimental hints for neurotoxic effects. Weidner M1, Hüwel S, Ebert F, Schwerdtle T, Galla HJ, Humpf HU.

Note: Currently, only nucleic acids containing genes for the biosynthesis of native Botulinum neurotoxin meet the definition of 42 CFR § 73.13(a)(2). Any genetic modifications of other select toxins to increase their LD [50] to <100 ng/kg body weight would be subject to this provision.

For more information regarding restricted experiments, please see the Restricted Experiment Guidance.

Tier 1 requirements for Botulinum neurotoxin and Botulinum neurotoxin producing species of *Clostridium*

A subset (Tier 1) of select agents and toxins have been identified as presenting the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects to the economy, critical infrastructure, or public confidence. In addition to Botulinum neurotoxins (BoNT) and BoNT-producing species of *Clostridium* being regulated as Tier 1, the nucleic acids that encode for the toxic forms of Botulinum neurotoxins and can be expressed are also regulated as Tier 1 agents. See the Guidance on the Regulation of Select Agent and Toxin Nucleic Acids for more information. Additional guidance regarding the Tier 1 requirements can be found in the guidance documents pertaining to security, occupational health, incident response, and training on the Compliance Assistance page.

Regulatory Exclusions (Section 3(d))

Nontoxic select toxins

The Select Agent Regulations (SAR) state that nontoxic select toxins are excluded from these regulations [7 CFR §331.3(d)(2); 9 CFR §121.3(d)(2) and 9 CFR §121.4(d)(2); 42 CFR §73.3(d)(2) and 42 CFR §73.4(d)(2)]. A "nontoxic" select toxin is no longer capable of exerting its toxic effect. For regulated nucleic acids, the term "nontoxic" means that the nucleic acids are no longer capable of producing a toxic select toxin without further genetic manipulation. In general, the methods used to render a select toxin nontoxic may fall into one of the following categories:

- Physical, like heat or irradiation
- Enzymatic, like a lysozyme
- Chemical

Different processes (e.g. heat, radiation, or chemicals) work by different mechanisms. For a select toxin to be properly characterized as nontoxic after treatment, exposure to the select toxin must not result in toxicity or express a toxic select toxin from regulated nucleic acids. A select toxin shall be considered nontoxic only after it has been subjected to a method that has been validated to be effective on a specific toxin. The individual or entity possessing the select toxin or regulated nucleic acid is responsible for validating non-toxicity.

Additional information on nontoxic toxins and rendering samples free of select toxins is available on the FSAP website.

Animals Exposed to a Select Toxin

42 CFR §73.3(d)(4) of the SAR addresses animals inoculated with or exposed to an HHS select toxin. Even after an animal has been inoculated with or exposed to a select toxin (for example, by inhalation, dermal absorption, or ingestion), the animal is not considered a "select toxin" and does not need to be housed in a registered space.

For dermal exposure, any residual select toxin on the animal must be removed before the animal can be transferred to an unregistered space. The residual select toxin wiped off the animal should be treated similarly to select toxin material not used during injection of an animal.

If the excess select toxin used for inoculation or exposure is retained and not destroyed as waste, it must be accounted for. The number of animals inoculated with or exposed to a select toxin does not need to be recorded for long-term storage.

However, the select toxin is regulated under the SAR until it is injected into or exposed to the animal. This includes storage or use of the select toxin (e.g., injection or exposure procedure). The room where the inoculation or exposure of animals with an HHS select toxin occurs may be assessed using laboratory biosafety level criteria instead of animal laboratory biosafety level criteria. These rooms must be included on an entity's registration. Once the inoculation or exposure has occurred, the animals can be moved to an unregistered room.

If the toxin preparation used to inoculate or expose animals contains viable Botulinum neurotoxin producing species of *Clostridium*, the material and animal is regulated unless a validated method to remove the organism from the toxin preparation is used.

Toxins in their Natural Environment

Sections 3(d)(1), 4(d)(1) of Part 73 and Part 121 provide for exclusion of select toxins in their natural environment:

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

Please see the natural environment table below for a description of the natural environments for each regulated toxin.

The chart below provides the natural environment for select toxins which would not be subject to the SAR.

Select Toxin	Natural Environment
Abrin	Seeds of the plant *Abrus precatorius* (rosary peas) including rosary pea mash.
BoNT	Botulinum neurotoxin producing species of *Clostridium* for which no additional procedures have been done to collect, extract, amplify, or produce BoNT.
Alpha-conotoxins	Cone snails *(Conus* spp.)
DAS	1) *Fusarium sambucinum* cultures that produce DAS, or 2) Food (e.g., potatoes) that was naturally contaminated by the fungi.
Ricin	Castor beans (*Ricinus communis*) including castor bean mash, which is the by-product of castor oil production that contains crushed plant material.
STX	Species of cyanobacteria and marine dinoflagellates and filter feeding shellfish that have concentrated STX from these sources.
SE subtypes A, B, C, D, E	*Staphylococcus aureus* strains that produce SE A, B, C, D, E subtypes for which no additional procedures have been done to collect, extract, amplify, or produce SE A, B, C, D, E subtypes.
T-2 toxin	1) *Fusarium sporotrichioides* cultures that produce T-2 toxin, or 2) Food (e.g., oats) that was naturally contaminated by the fungi.
TTX	Aquatic animals and amphibians and organs that contain TTX so long as no additional extraction of TTX occurs.

Regulation point for each select toxin

The chart below provides the regulatory starting point for select toxins extracted or collected from the natural environment. Synthesized toxic select toxins in excess of the regulatory threshold are regulated at all times.

Select Toxin	Regulatory Starting Point (so long as the quantity under the control of a principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor is in excess of the regulatory threshold)
Abrin	When crushed *Abrus precatorius* (rosary peas) mash is further processed, resulting in the extraction or concentration of abrin, the abrin containing product of this procedure is regulated.
BoNT	Intentional BoNT collection, extraction, amplification, or production. For licensed products that contain BoNT (e.g. BOTOX), once BoNT is vialled in its final formulation and to be used for medical purposes as stipulated by its license, it is no longer regulated.
Alpha-conotoxins	Soluble peptides of the appropriate amino acid sequence extracted from the venom bulb of cone snails that have been treated with proteases to properly fold and activate alpha-conotoxins.
DAS	Extraction from culture supernatant or contaminated food in organic solvent.
Ricin	When castor bean mash is further processed, resulting in the extraction or concentration of ricin, the ricin-containing product of this procedure is regulated.
STX	Dinoflagellate or cyanobacterial pellet, contaminated fish or shellfish that is sonicated or otherwise disrupted and acidified water is added to extract STX.
SE subtypes A, B, C, D, E	Intentional SE subtypes A, B, C, D, E collection, extraction, amplification, or production.
T-2 toxin	Extraction from culture supernatant or contaminated food in organic solvent.
TTX	Extraction from aquatic animals and amphibians and organs that contain TTX in acidified water or organic solvent.

Toxins Identified in an Original Food or Clinical Sample

Section 3(d)(9) of Part 73, Part 121, and Part 331 of the SAR provides for exclusion of select toxins identified in an original food sample or clinical sample.

Original food samples and clinical samples are those specimens that are submitted to laboratories for diagnosis or verification purposes to identify or verify a biological toxin. For example, an original food sample could be a container of potato salad or juice. An original clinical sample could be serum or stool from a patient. Laboratories that test food and clinical samples for the presence of toxins generally do not know the level of toxin in a sample and do not extract and purify a toxin as part of their studies. This exclusion does not pertain to samples (food or otherwise) spiked with known select toxin.

Toxins as a byproduct

For those laboratories that are not exempt under §73.5(a) and §73.6(a), Botulinum neurotoxin that is produced as a byproduct in the study of Botulinum neurotoxin producing species of *Clostridium* is not regulated so long as the toxin has not been intentionally cultivated, collected, purified, or otherwise extracted, and the material containing the toxin is rendered non-toxic and disposed of within 30 days of the initiation of the culture. Botulinum neurotoxin producing species of *Clostridium* are regulated and the byproduct exclusion only pertains to the toxin. Further, if the material containing the toxin is retained past 30 days the toxin in the material will be subject to the regulations, including inventory requirements.

Select Toxins modified to be less potent or toxic

An entity or individual may submit a written request that FSAP exclude a select toxin modified to be less potent or toxic from the select agent regulations. In general the methods that render a select toxin less potent or toxic are genetic modifications (recombinant or synthetic) such as deletions, point mutations, and chimeras. Typically a select toxin needs to be tested in relevant animal models before their reduction in potency or toxicity can be validated.

The request should contain the rationale for the exclusion of the select toxin and scientific references or supporting documentation that demonstrates the modified select toxin does not pose a severe threat to public health and safety. The request should include:

- Documented history of not causing toxicity in humans, or relevant animal models, including quantitative measures demonstrating a reduction in potency or toxicity.
- Defined genetic mutations or alterations known to reduce potency or toxicity in humans or relevant animal models.
- Data showing the mutations have a low frequency of reversion to wild-type potency or toxicity.
- Level of difficulty in engineering the modified toxin to restore wild-type potency or toxicity.

FSAP will issue a written decision granting or denying the request. The exclusion will be effective upon notification to the applicant.

If an excluded modified select toxin is subjected to any manipulation that restores or enhances its toxic activity, the resulting select toxin will be subject to the select agent regulations [See 42 CFR §§ 3(e)(2), 4(e)(2)].

For more information on exclusions and how to apply please see the Select Agents and Toxins Exclusion list and the Exclusion Guidance Document.

Regulatory Exemptions

Product Exemptions

Select agents or toxins or products containing or bearing BSAT are exempt from the SAR provisions if they are cleared, approved, licensed, or registered under certain laws, unless the HHS Secretary issues an order making provisions to protect public health and safety, according to 42 CFR §5(c). These laws include:

The Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 301 et seq.)

Products that are covered under this act that are most likely to involve select toxins are:

1. Drugs
    a. Prescription drugs (both brand-name and generic)
    b. Non-prescription (over-the-counter drugs)
2. Biologics
    a. Vaccines
    b. Blood and blood products
    c. Cellular and gene therapy products
    d. Tissue and tissue products
    e. Allergenics
3. Veterinary products, including:
    a. Livestock feeds
    b. Pet foods
    c. Veterinary drugs and devices

Section 351 of the Public Health Service Act pertaining to biological products (42 U.S.C. § 262)

Products that are covered under this act are:

1. Any biological product (General Licensing Requirements: The biological product must be safe, pure, and potent; the biological product must comply with applicable Federal Food, Drug, and Cosmetic Act requirements; the manufacturing facility must ensure that the biological product is safe, pure, and potent as determined through inspection of the facility). The term 'biological product' includes the following:
    a. Viruses
    b. Therapeutic serums
    c. Toxins
    d. Antitoxins
    e. Vaccines
    f. Blood
    g. Blood component or derivative
    h. Allergenic products, proteins (except any chemically synthesized polypeptide), or analogous products

i. Arsphenamine or derivatives of arsphenamine (or any other trivalent organic arsenic compound) applicable to the prevention, treatment, or cure of a disease or condition of human beings

BOTOX® is licensed under section 351 of the Public Health Service Act pertaining to biological products (42 U.S.C. 262). Currently there are three approved Type A botulinum toxins on the US Market (Botox®/Botox Cosmetic, Dysport®, and Xeomin®) and one Type B Botulinum toxin (Myobloc®). FDA approves the drug making process from the seed stock through the toxin production (from fermenters to purification, formulation, packaging, and labeling) to the final packaged product. FDA approval is based on a medical use. All other product uses (e.g. research or off-label use) fall outside of FDA's jurisdiction. Many entities will ship the substance to multiple facilities (in multiple states or countries) for manufacturing, processing, and packaging. This is all part of the license and is approved by FDA. The basic research to generate an innovator product that could receive investigational new drug (IND) approval is outside of FDA's purview. INDs are only applicable for products (both biosimilar and innovator) that will be used in clinical trials and not the basic research that led to the development of the product. The Biologics Price Competition and Innovation Act established a legal pathway for abbreviated development of a biologic based on its similarity to an already marketed product, referred to as biosimilars. The approval pathway for a biosimilar product is under section 351(k) of the Public Health Service act (for an innovator product it is the 351(a) pathway). A product to be licensed using the 351(k) pathway must establish biosimilarity to a product licensed using the 351(a) pathway, (i.e. the reference product must be a US licensed product). Any 351(a) product is eligible to be a reference product for a biosimilar, as long as there is no patent infringement and the reference product is outside the exclusivity period for the 351(a) licensed product.

Competitor products could be part of the biosimilars process; however, if they are doing basic research and development with the competitor product then it would fall outside of FDA purview. For example, if it is part of their process detailed in their license application as their quality assurance/quality control, then it would fall under the FDA's purview. Any off label use (research and development, practice of medicine) of BOTOX, biosimilars, or competitor products is not regulated by FDA. The manufacturing process from seed stock until BOTOX® is aliquoted into vials is regulated by both FDA and DSAT for different purposes. DSAT regulates BOTOX® as a select agent until it is in its final formulation aliquoted in vials, and ready to be used for medical purposes.

The Virus-Serum-Toxin Act (21 U.S.C. 151-159)

Exemption from the select agent regulations does not amount to exemption from any other USDA law or regulation. Under the Virus-Serum-Toxin Act, the importation or movement of any organism or vector requires a permit issued by the Secretary of Agriculture. Contact the Organism and Vector staff for current regulatory requirements at OV@aphis.usda.gov or 301-851-2070.

See the Virus-Serum-Toxin Act (21 U.S.C. §§ 151 - 159) for more information.

The Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. § 136 et seq.)

Please see the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. § 136 et seq.) for more information.

Investigational Product Exemption Requirements for Select Toxins

An investigational product that is, bears, or contains a select toxin may be exempted from the select agent regulation requirements when such product is being used in an investigation authorized under any Federal Act. The exemption process is as follows:

(1) To apply for an exemption, an individual or entity must submit a completed APHIS/CDC Form 5.
(2) A determination regarding the application will be made within 14 calendar days after receipt, provided the application meets all of the requirements of the select agent regulations and the application establishes that the investigation has been properly authorized. A written decision granting or denying the request will be issued.
(3) The applicant must notify CDC or APHIS (depending on who the applicant is registered with) when an authorization for an investigation no longer exists. This exemption automatically terminates when such authorization is no longer in effect.

More information on investigational product exemption requirements can be found at Request for Exemption of Select Agents and Toxins for an Investigational Product on the FSAP website.

Inventory requirements

Individuals and entities[3] that possess aggregate amounts of select toxins that exceed the amounts listed in 42 CFR § 73.3(d)(3) of the SAR must maintain records containing all of the information required in section 17(a)(3) for all regulated select toxin materials[4].

All regulated select toxin material must be entered into inventory records. All regulated select toxin material, including working stock and material in long-term storage, must be entered into the inventory records. The current quantity of each vial must be documented for select toxins following each use. The current quantity of each vial that is recorded following the last usage may be examined during inspection. All personnel with access to select toxin materials must have FSAP approval to access select agents and toxins and be in compliance with the SAR. Clinical or diagnostic laboratories and other entities that possess, use, or transfer an HHS select toxin that is contained in a specimen presented for diagnosis or verification will be exempt from the SAR requirements **including inventory requirements** for seven calendar days after identification of the select toxin (except for Botulinum neurotoxin and/or *Staphylococcal* enterotoxin (Subtypes A-E)), or within 30 calendar days after identification of Botulinum neurotoxin and/or *Staphylococcal* enterotoxin (Subtypes A-E). The select toxin must be transferred in accordance with § 73.16 or destroyed on-site by a recognized sterilization or inactivation process. The toxin must be secured against theft, loss, or release during the period between identification of the select toxin and either the transfer or destruction of the toxin. Any theft, loss, or release of such toxin must be reported, and the identification of the select toxin must be reported to CDC or APHIS either immediately for BoNT or within seven days after identification for the other select toxins.

See Guidance on the Inventory of Select Agents and Toxins on the FSAP website for more information.

Regulation if registered for a select toxin regardless of the quantity possessed

The Select Agent Regulations state that a registered entity must comply with all SAR requirements for each select toxin listed on the entity's registration, regardless of the amounts of select toxin in possession.

Consider the following situation. An entity has a PI that is registered to possess, use, and transfer a select toxin, but the PI does not currently possess any of the select toxin for which the PI is registered.

[3] It is of course possible that an entity could be in possession of an aggregate amount of select toxin that exceeds the amounts listed in 42 CFR §73.3(d)(3) of the SAR and not be required to be registered with the FSAP if the aggregate amount of the select toxin is distributed across multiple individual PIs at the entity, each of whom possess below the regulatory threshold for the toxin.

[4] Select toxin material is material that contains select toxin (serum, stool, culture, etc.).

The PI must still be in compliance with all the SAR for that select toxin since the PI has been approved to obtain and possess that select toxin at any time.

A person who meets the SAR definition of a PI, but is not listed as a PI on the entity's registration, is excluded from the SAR as long as the aggregate amount of select toxin under their control is below the regulatory threshold.

Registration and Biosafety level

If an entity chooses to register for select toxin work at BSL3 based upon their risk assessment then they must use BSL3 practices, containment equipment, and facilities.

- General biosafety guidance for work with select toxins
- Toxin specific biosafety guidance

Transfer requirements

Information on shipping requirements can be found at:

- Guidance for Completing the Shipper's Declaration for Dangerous Goods
- APHIS/CDC Form 2 Transfers FAQs

Due diligence

"Due diligence" is a measure of prudence, activity, or assiduity exercised by a reasonable and prudent person under the particular circumstances. It is not measured by any absolute standard, but depends on the relative facts of the specific case.

It is the entity's responsibility to document how it has conducted its due diligence.

Sections 42 CFR §§ 73.3(d)(3)(i) and 73.16(l) require the transferor to use "due diligence" when transferring an amount of a HHS select toxin otherwise excluded under the provisions of §73.3(d). This provision requires a transferor to take reasonable actions to ensure that the recipient:

- Is eligible to receive the select toxin (principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor).
- Has a legitimate need (i.e., reasonably justified by a prophylactic, protective, bona fide research, or other peaceful purpose) to handle or use such select toxins.

The SAR also requires the transferor to report to FSAP immediately if they detect a known or suspected violation of Federal law or become aware of suspicious activity related to the select toxin. FSAP developed this provision to address the concern that someone might stockpile select toxins by receiving multiple orders below the excluded amount.

Documenting Due Diligence

The transferor can document how they have determined that an individual has a legitimate purpose to handle and use such select toxins in a few ways:

- The transferor can require the recipient to complete documentation stating their intended use of the select toxin.
- The transferor can document their own knowledge of the recipient's legitimate need for the select toxin.

Information pertinent to the person requesting and using the select toxins must include, but is not limited to:

- The recipient identity information, including the recipient's name, institution name, address, telephone number, and email address.
- The name of the toxin and total amount transferred.
- The legitimate need claimed by the recipient.

Reporting Suspected Violation of Federal Law or Suspicious Activity

If the transferor detects a known or suspected violation of Federal law or becomes aware of suspicious activity related to the shipped select toxin, the transferor should report to FSAP the requested select toxin and the pertinent information of the person requesting and using the select toxins (e.g., name, institution name, address, telephone number, and email address). A transferor can contact FSAP either by emailing to CDC: LRSAT@cdc.gov or APHIS: DASAT@usda.gov or calling FSAP (CDC: 404-718-2000 or APHIS: 301-851-2070).

Toxin Due Diligence FAQs

1. **Question:** My entity has prepared three vials that each contain less than the regulated amount of select toxin but in combination contain greater than the regulated amount of select toxin, and packaged them into one transfer. Can this transfer occur without the use of an APHIS/CDC Form 2?

   Answer: No. The total amount transferred, regardless of how many vials it is distributed into, has exceeded the regulatory threshold amount and must be shipped via an APHIS/CDC Form 2 to a registered recipient.

2. **Question:** My entity has prepared three vials that each contain less than the regulated amount of select toxin and packaged them into three separate transfers that are shipped on the same day to the same recipient. Can these transfers occur without the use of an APHIS/CDC Form 2?

   Answer: No. The total amount transferred, regardless of how many vials or packages are separately shipped, will result in the recipient possessing greater than the regulatory threshold amount. Therefore, these transfers must be shipped using an APHIS/CDC Form 2 to a registered recipient.

3. **Question:** My entity sent a transfer that contains select toxin below the regulatory threshold amount after performing due diligence. A week later the same recipient requests another shipment for an amount of select toxin below the regulatory threshold and states they have used up the select toxin from the first transfer. Can my entity send this second transfer?

   Answer: It depends. If the transferor detects a known or suspected violation of Federal law or becomes aware of suspicious activity related to the shipped select toxin, the transferor should report to FSAP and not ship the select toxin. However, if the transferor believes the recipient has a legitimate need to handle or use such select toxins within a week of sending the first shipment and does not have concerns about the recipient stockpiling select toxin then the entity can send the second transfer.

4. **Question:** How do the select agent regulations apply to a PI possessing a regulated amount of a select toxin who transfers an “unregulated amount” of a select toxin to another person? Note: An “unregulated amount” of a select toxin would be those amounts listed in 42 CFR § 73.3(d)(3) and the exclusion is limited to select toxin under the control of a bona fide principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor.

   Answer: A PI transferring (meaning the PI will no longer have control of the select toxin) an unregulated amount of a select toxin to the control of a bona fide principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor is not required to obtain prior approval for the transfer using the APHIS/CDC Form 2. However, the PI will be required to exercise due diligence prior to the transfer. The PI will be required to note the

amount of the transfer on the PI's select toxin inventory. Please note that transfer of any amount of select toxins outside of the U.S. requires an export license from the Department of Commerce, Bureau of Industry and Security. See 15 CFR Supplement #1 to Part 774, Export Control Classification Number 1C351 for more detailed information.

NOTE: If a PI moves any amount of a select toxin to someone the PI supervises at the same entity (e.g., a research assistant), such that the toxin is still under the control of the PI, then that movement is not considered a transfer. Therefore, the select toxin would not be excluded from the regulations and the individual and room where the toxin is used or stored would need to be listed on the registration and must meet all requirements of the select agent regulations. For example, the PI maintains two laboratories. One laboratory stores above the excluded amount of the toxin. The second laboratory is where the PI's research assistant works with excluded amount of toxin. Since the PI maintains control over both portions of the select toxin, both rooms must be listed on the registration and must meet all requirements of the select agent regulations.

More information on due diligence requirements can be found in the Due Diligence FAQs.

Importation requirements

The importation of a select toxin is not subject to the "Import Regulations for Infectious Biological Agents, Infectious Substances, and Vectors" (42 CFR 71.54) and therefore does not require an import permit from the CDC Import Permit Program but does require an APHIS/CDC Form 2.

See the CDC Import Permit FAQs for more information.

Identification of a select toxin requirements (APHIS/CDC Form 4)

Any identification of a select toxin contained in a specimen presented for diagnosis or verification (regardless of whether a laboratory is registered with FSAP) must be reported regardless of the amount of select toxin identified. The identification must be reported immediately (for BoNT) or within seven calendar days (for all other toxins) after identification, using the APHIS/CDC Form 4 (Reporting the Identification of a Select Agent or Toxin from a Clinical/Diagnostic Specimen) and include the final disposition of the select toxin.

The select toxin (except original sample) must be transferred in accordance with § 73.16 or destroyed on-site by a recognized sterilization or inactivation process within seven calendar days after identification of the select agent or toxin (except for BoNT and/or SE (Subtypes A–E)), or within 30 calendar days after identification of BoNT and/or SE (Subtypes A–E). The 30 day time frame only applies to the 1) transfer, 2) destruction, or 3) movement into select agent inventory (for a registered entity) after identification and reporting of BoNT and SE (Subtypes A–E). It does not apply to the APHIS/CDC Form 4 reporting requirement. Note: The remaining original food and clinical samples which had portions removed and identified to contain select toxin are excluded. The transfer, destruction, or movement of select toxin positive samples into inventory (for a registered entity) applies only to the portions removed from the original sample and were subsequently used to perform testing. The remainder of the original food sample or clinical sample that was not subjected to the testing protocol is excluded.

Since a registered or reference laboratory typically confirms the identification of a select toxin for public health and agriculture, clinical and diagnostic laboratories, the registered or reference laboratory must inform the specimen provider of the identification as a condition for the reference laboratory to maintain their exemption. It is important that the specimen provider is aware that they are in possession of the toxin and must meet the requirements outlined in 42 CFR §§ 73.5, 73.6, including but not limited to:

- Must secure the toxin against theft, loss, or release.
- Cannot maintain possession of the select agent or toxin.
- Must destroy or get approval for a transfer.
- Must report a theft, loss, or release regardless of the amount.

More information on the theft, loss, release reporting requirements can be found at:

- APHIS/CDC Form 3 Guidance Document

Proficiency Testing Identification

Any person or entity, including any clinical or diagnostic laboratory, having identified a select agent or toxin contained in a specimen or sample presented for proficiency testing, is required by the select agent regulations (9 CFR Part 121 and 42 CFR Part 73) to report this identification by submitting a APHIS/CDC Form 4 to either APHIS or CDC within 90 calendar days of receipt of samples. This requirement applies regardless of whether the person or entity is registered with FSAP.

More information on identification of select agents and toxins requirements can be found at:

- Report or Identification of Select Agent or Toxin FAQs
- Guidance Document for the Completion of APHIS/CDC Form 4

For select toxins identified in clinical, diagnostic, or proficiency testing, the select agent or toxin must be secured against theft, loss, or release during the period between identification of the select agent or toxin, and transfer or destruction of such select agent or toxin. Any theft, loss, or release of a select agent or toxin must be reported using the APHIS/CDC Form 3 (Report of Theft, Loss, or Release of Select Agents And Toxins) as required by 42 CFR §73.19.

Appendix A. Summary of select toxin natural environments, methods of production, regulatory and starting points of select toxin in select toxin-containing samples.

Select Toxin	Regulatory Amount	Natural Environment	Method of Production	Regulatory Point (So long as the quantity is in excess of the regulatory threshold)
Abrin	1000 mg	Seeds of the plant *Abrus precatorius* (rosary peas) including rosary pea mash.	Solvent extraction from crushed rosary pea seeds	When crushed *Abrus precatorius* (rosary pea) mash is further processed, resulting in the extraction or concentration of abrin, the abrin containing product of this procedure is regulated.
Botulinum neurotoxins (BoNT)	1 mg	Original food and clinical samples for which no additional procedures have been done to collect or extract BoNT.	BoNT is extracted and purified from food, stool, serum and liquid cultures of Botulinum neurotoxin-producing species of *Clostridium*	Intentional BoNT collection or extraction. For licensed products that contain BoNT (e.g. BOTOX), once BoNT is vialled in its final formulation and to be used for medical purposes as stipulated by its license, it is no longer regulated.
Alpha-conotoxins	100 mg	Cone snails (*Conus* spp.)	1) Recombinant production systems. 2) Chemical synthesis. 3) Milking of snails.	Soluble peptides of the appropriate amino acid sequence extracted from the venom bulb of cone snails that have been treated with proteases to properly fold and activate alpha-conotoxin.
DAS	10000 mg	1) *Fusarium sambucinum* cultures that produce DAS, or 2) Food (e.g. potatoes) that was naturally contaminated by the fungi.	Liquid cultures of *F. sambucinum* grown on yeast extract, peptone, and glucose, or grown on cooked rice	Extraction from culture supernatant or contaminated food in organic solvent.
Ricin	1000 mg	Castor beans (*Ricinus communis*) including castor bean mash, which is the by-product of castor oil production that contains crushed plant material	Solvent extraction from crushed castor beans.	When castor bean mash is further processed, resulting in the extraction or concentration of ricin, the ricin-containing product of this procedure is regulated.
Saxitoxin (STX)	500 mg	Species of cyanobacteria and marine dinoflagellates and filter feeding shellfish that have concentrated STX from these sources	Algal cells are removed from their growing medium (through filtering or centrifugation), rinsed to remove salts, concentrated into a pellet, and sonicated in mildly acidified water, or, contaminated shellfish are extracted with mildly acidified water.	Dinoflagellate or cyanobacterial pellet, or contaminated fish or shellfish, is sonicated or otherwise disrupted and acidified water is added to extract STX.
Staphylococcal enterotoxin (SE) A,B,C,D,E subtypes	100 mg	1) *Staphylococcus aureus* strains that produce SE A,B,C,D,E subtypes, and 2) Original food and clinical samples that contain SE A,B,C,D,E subtypes for which no additional procedures have been done to collect or extract SE A,B,C,D,E subtypes.	SE A, B, C, D, E subtypes can be extracted from food, stool, serum, and liquid cultures of *S. aureus*.	Intentional SE A, B, C, D, E subtype collection or extraction.
T-2 toxin	10000 mg	1) *Fusarium sporotrichioides* cultures that produce T-2 toxin, or	Liquid cultures of *Fusarium sporotrichioides* grown on yeast extract, peptone, and glucose, or gown on cooked rice.	Extraction from culture supernatant or contaminated food in organic solvent.

		2) Food (e.g., oats) that was naturally contaminated by the fungi		
Tetrodotoxin (TTX)	500 mg	Aquatic animals and amphibians and organs that contain TTX so long as no additional extraction of TTX occurs.	Several Japanese companies produce TTX commercially from puffer fish liver and/or ovaries. Toxic organs are homogenized with water and a weak organic acid followed by filtering. Purified by raising the pH with a weak base followed by cation exchange chromatography. Separation from inorganic salts and alkaline amino acids using activated carbon. Crystalized by concentrating the solution at a pH of 8-10.	Extraction from aquatic animals and amphibians and organs that contain TTX in acidified water or organic solvent.