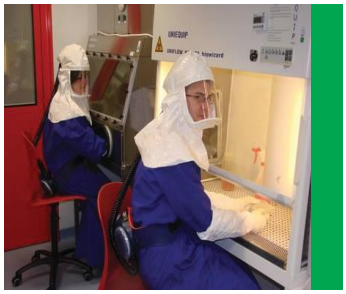

Select Toxin Guidance

(24 May 2016)

Centers for Disease Control and Prevention (CDC)
Division of Select Agents and Toxins
Animal and Plant Health Inspection Service (APHIS)
Agriculture Select Agent Program (AgSAS)

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: AgSAS@aphis.usda.gov

Revision History:
Month, day, year: Initial posting

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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) or Plant Protection and Quarantine (PPQ)), or Overlap Agents (regulated by both agencies). Currently select toxins are 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.

Regulatory Definitions (§ 73.1)

Toxin – the toxic material or product of plants, animals, 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 – 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 Toxins [§73.3(d)(3)]	**Amount**
Abrin	100 mg
Botulinum neurotoxins (BoNT)	0.5 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	1000 mg
Ricin	100 mg
Saxitoxin (STX)	100 mg
Staphylococcal Enterotoxins (SE) (Subtypes A, B, C, D, E)	5 mg
T-2 toxin	1000 mg
Tetrodotoxin (TTX)	100 mg

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

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List of regulated select toxin derivatives

A toxin derivative is a 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 toxicity similar to the parent. Currently only the select toxin derivative H-T2 is regulated.

List of unregulated non-functional 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 (§ 73.3 (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 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.

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

Restricted Experiments for Select Toxins

"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:

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(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.

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 that present the greatest risk of deliberate misuse with most significant potential for mass casualties or devastating effects to the economy, critical infrastructure, or public confidence. For more information on the Tier 1 requirements (security, occupational health, incident response, and training), see the Select Agent Regulations. In addition to Botulinum neurotoxins (BoNT) and BoNT-producing species of *Clostridium* being regulated as Tier 1, the nucleic acids that encode for the functional forms of Botulinum neurotoxins and can be expressed are also regulated as Tier 1 agents. Additional guidance regarding the Tier 1 requirements can be found on the Compliance Assistance page.

Regulatory Exclusions (§ 73.3 (d))

- Nonfunctional select toxins
  The Select Agent Regulations (SAR) state that nonfunctional 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)]. For a select toxin, the term "nonfunctional" means a toxin is no longer capable of exerting its toxic effect. For regulated nucleic acids, the term "non-functional" means that the nucleic acids are no longer capable of producing a functional select toxin without further genetic manipulation. In general, the methods used to render a select toxin nonfunctional fall into one of two broad categories: physical (e.g. heat or radiation) or chemical. Different processes (e.g. heat, radiation, or chemicals) work by different mechanisms. For a toxin to be properly characterized as nonfunctional after treatment, exposure to the toxin must not result in toxicity or express a functional select toxin from regulated nucleic acids. A select toxin must not be treated as nonfunctional until it has been subjected to a method that has

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been validated to be effective on that specific toxin. The burden of validating non-functionality remains on the individual or entity possessing the select toxin or regulated nucleic acid.

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

- HHS toxins
  HHS toxins under the control of a principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor are excluded, if the aggregate amount does not, at any time, exceed the following amounts:
  - 100 mg of Abrin; 0.5 mg of BoNT100 mg of 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[1]
  - 1,000 mg of DAS
  - 100 mg of Ricin
  - 100 mg of STX
  - 5 mg of SE (subtypes A, B, C, D, E); 1,000 mg of T-2 toxin
  - 100 mg of TTX

[1]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.

These thresholds allow a means of balancing the requirements of the Public Health Bioterrorism Preparedness and Response (PHBPR) Act of 2002 to prevent a "severe threat to public health and safety" with the importance of select toxin use in ongoing medical research toward the development of effective medical treatments and vaccines. In such instances, small amounts of toxins may be used which would not rise to the level of presenting the potential to pose a severe threat to public health and safety. Note: the aggregate amounts stated above do not apply to regulated nucleic acids as any amount of this material would be regulated.

An unregistered entity that has Principal Investigators (PI's) with below the regulated amount of toxin are not required to register with FSAP so long as each of the persons in possession meet the regulatory definition of a PI. However, if one PI transfers an amount of a select toxin to another PI such that the receiving PI is now in possession of select toxin above the regulatory amount, then the receiving PI and entity are now both in violation of the SAR.

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- 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 would not be considered a "select toxin" and would not need to be housed in a registered space. For dermal exposure any residual toxin on the animal would have to be removed before the animal could be transferred to unregistered space. The residual toxin wiped off the animal would be considered a select toxin similar to toxin not used during injection of an animal. The number of animals inoculated with or exposed to a select toxin does not need to be recorded for long-term storage.

  However, until the select toxin is injected into or exposed to the animal, the select toxin would be regulated under the SAR. This would include storage or use of the material (e.g., injection or exposure procedure). The room where the inoculation or exposure of animals with an HHS select toxin occurs may be assessed by the entity using laboratory biosafety level criteria instead of animal laboratory biosafety level criteria and 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.

- Toxins in their natural environment
  §73.3(d)(1) of the SAR provide for exclusion of 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.

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

Toxin	Natural Environment
Abrin	Seeds of the plant *Abrus precatorius* (rosary peas) including rosary pea mash.
BoNT	Original food and clinical samples for which no additional procedures have been done to collect or extract the toxin.
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 saxitoxin from these sources.
SE	1) *Staphylococcus aureus* strains that produce those SE subtypes, and 2) Original food and clinical samples that contain the toxin for which no additional procedures have been done to collect or extract the toxin.
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 the toxin so long as no additional extraction of the toxin occurs.

Regulation point for each select toxin

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

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 toxin 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	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 the toxin.
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 the toxin.
SE	Intentional toxin collection or extraction.
T-2 toxin	Extraction from culture supernatant or contaminated food in organic solvent.
TTX	Extraction from aquatic animals and amphibians and organs that contain the toxin in acidified water or organic solvent.

Toxins modified to be less potent or toxic

An entity or individual may request that FSAP exclude a select toxin modified to be less potent or toxic from the select agent regulations by submitting a written request. In general the methods that render a toxin less potent or toxic are genetic modifications (recombinant or synthetic) such as deletions, point mutations, and chimeras. Typically a 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 toxin and scientific references or supporting documentation that demonstrates the modified 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.

A written decision granting or denying the request will be issued. The exclusion will be effective upon notification to the applicant.

If a modified 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

Products that are, bear, or contain listed select agents or toxins that are cleared, approved, licensed, or registered under any of the following laws, are exempt from the provisions of this part insofar as their use meets the requirements of such laws unless the HHS Secretary issues an order making specific provisions of this part applicable to protect public health and safety:

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

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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 (BPCI) 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

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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 Act commonly known as 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) 851-3300 option 3.

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 toxin, when such product is being used in an investigation authorized under any Federal Act may be exempted from the requirements of the select agent regulations.

> (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 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) (See Appendix II) for all toxin materials. All regulated toxin material, including "working stock" and toxins in long-term storage, must be entered into inventory records. The current quantity of each vial must be documented for 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.

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

FSAP policy states that a registered entity must be, within the boundaries of its registration, in compliance with all of the SAR requirements for select toxins listed on the entity's registration regardless of the amounts of 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. 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 toxin under control of the PI is below the regulatory threshold.

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Registration and Biosafety level

If an entity chooses to register for 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

More information on shipping requirements can be found at:

- FSAP Guidance on the Shipment and Receipt of Packages with Select Agents and Toxins
- Guidance for Completing the Shipper's Declaration for Dangerous Goods
- APHIS/CDC Form 2 Transfers FAQs

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Due diligence

Sections 42 CFR §§ 73.3 (d)(3)(i), 73.16(l) require the transferor to use “due diligence” when transferring an amount of a HHS toxin otherwise excluded under the provisions of §73.3(d). The "toxin due diligence" provision requires a transferor to take reasonable actions to ensure that the recipient is eligible to receive the regulated toxin (principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor) and has a legitimate need (i.e., reasonably justified by a prophylactic, protective, bona fide research, or other peaceful purpose) to handle or use such toxins. The SAR also requires the transferor to report to FSAP if they detect a known or suspected violation of Federal law or become aware of suspicious activity related to the toxin. FSAP developed this provision to address the concern that someone might stockpile toxins by receiving multiple orders below the excluded amount.

"Due diligence" is generally understood to be such a measure of prudence, activity, or assiduity, as is properly to be expected from, and ordinarily exercised by, a reasonable and prudent person under the particular circumstances; not measured by any absolute standard, but depending on the relative facts of the specific case.

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

Documenting Due Diligence

The transferor can document how they have determined that an individual has a legitimate purpose to handle and use such toxins in a few ways. The transferor can require the recipient to complete documentation stating their intended use of the toxin. Or, the transferor can document their own knowledge of the recipient's legitimate need for the toxin. Information pertinent to the person requesting and using the toxins should include, but is not limited to:

- The recipient identity information, including the recipient's name, institution name, address, telephone number, and e-mail 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 toxin, the transferor should report to FSAP the requested toxin and the pertinent information of the person requesting and using the toxins (e.g., name, institution name, address, telephone number, and e-mail address). A transferor can contact FSAP either by emailing to CDC: LRSAT@cdc.gov or APHIS: AgSAS@aphis.usda.gov or calling FSAP (CDC: 404-718-2000 or APHIS: 301-851-3300 (option 3) (voice only)).

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Due Diligence FAQs

1. **Question:** My entity has prepared three vials that each contain less than the regulated amount of toxin but in combination contain greater than the regulated amount of 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 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 is 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 toxin below the regulatory threshold amount after performing due diligence. A week later the same recipient requests another shipment for an amount of toxin below the regulatory threshold and states they have used up the 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 toxin, the transferor should report to FSAP and not ship the toxin. However, if the transferor believes the recipient has a legitimate need to handle or use such toxins within a week of sending the first shipment and does not have concerns about the recipient stockpiling toxin then the entity can send the second transfer.

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

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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.

More information on import permit requirements can be found at http://www.cdc.gov/od/eaipp/faq.htm.

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) would need to be reported regardless of the amount of toxin identified. The identification must be reported within seven calendar days 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. Note: The identification of botulinum neurotoxin must be immediately reported and followed up by the submission of the APHIS/CDC form 4.

If your laboratory has identified both BoNT and then after seven days identifies BoNT-producing species of *Clostridium* from the same specimen, you would need to report Botulinum neurotoxin and the BoNT-producing species of *Clostridium* by completing two APHIS/CDC Form 4s reporting these identifications.

FSAP has provided estimates of the amount of select toxins typically found in clinical and environmental samples to help entities determine if the amount of select toxin in their possession exceeds the regulatory threshold. These estimates will be used by FSAP to determine if the amounts of toxin under control of a principal investigator, treating physician or veterinarian, or commercial manufacturer or distributor exceeds the regulatory threshold. Please see Appendix A for toxin estimates in clinical samples.

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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 agent or toxin. Any theft, loss, or release of such agent or toxin is 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.

Theft, loss, and release requirements

The theft, loss, or release of a select toxin, regardless of the quantity, must be reported to FSAP by a registered entity using the using the APHIS/CDC Form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins).

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

- APHIS/CDC Form 3 Guidance Document
- Select Agents and Toxins Theft, Loss, or Release Document

Useful Links and Resources

- Federal Select Agent Program
- NIH OBA guidelines
- BMBL 5th edition

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

Toxin	Regulatory Amount	Natural Environment	Method of Production	Regulatory Point (So long as the quantity is in excess of the regulatory threshold)	Reasonable estimates of toxin in toxin-containing samples
Abrin	100 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.	Typically, *A. precatorius* seeds contain between 0.05 and 0.5 % Abrin.
Botulinum neurotoxins	0.5 mg	Original food and clinical samples for which no additional procedures have been done to collect or extract the toxin.	Toxin is extracted and purified from food, stool, serum and liquid cultures of Botulinum neurotoxin-producing species of *Clostridium*	Intentional toxin 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.	• More than 1,000 5 ml serum samples (200-256 mouse LD [50]/ml) • More than 80 stool samples (About 25 g/sample) • A single food sample (~ 100 g) could have enough BoNT to reach the regulatory threshold • Two samples, each with ten BoNT positive cultures (from enrichment of single colonies selected from agar plates inoculated into ten 8-10 ml broth cultures; 200 milliliters total volume of culture)
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 the toxin.	• A single cone snail would only yield 10 µg or less of active conotoxins • Typical yields of active, substantially pure, conotoxins in research laboratories using synthetic or recombinant systems are in the range of tens of milligrams, with significant amounts of additional incorrectly folded and less active materials also produced
DAS	1000 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.	• The amount of toxin that can be found in plant tissue is in the microgram range • 300 µg/ml of toxin in liquid culture or cooked rice cultures • Approximately 50-250 µg/kg of DAS in rotted potato tissue

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Toxin	Regulatory Amount	Natural Environment	Method of Production	Regulatory Point (So long as the quantity is in excess of the regulatory threshold)	Reasonable estimates of toxin in toxin-containing samples
Ricin	100 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.	• Each gram of castor bean contains ~ 9 mg of Ricin • 1/100 of a seed is fatal for a 5 kg baby if delivered intravenously • 10 seeds are fatal for a 5 kg baby if the toxin is delivered orally
Saxitoxin	100 mg	Species of cyanobacteria and marine dinoflagellates and filter feeding shellfish that have concentrated saxitoxin 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 the toxin.	• Tens of mg of crude STX per 500 L culture, under optimized conditions • Concentrations found in shellfish considered unsafe for human consumption range from 80 µg STX equivalents per 100 g edible shellfish to tens of thousands of micrograms of STX equivalents per 100 g shellfish
Staphylococcal enterotoxin A,B,C,D,E subtypes	5 mg	1) *Staphylococcus aureus* strains that produce those SE subtypes, and 2) Original food and clinical samples that contain the toxin for which no additional procedures have been done to collect or extract the toxin.	Toxin can be extracted from food, stool, serum, and liquid cultures of *S. aureus*.	Intentional toxin collection or extraction.	• 200 ng per milk sample (example given from one investigation) • 0.37-0.38 ng/g with an estimate that 20 ng SEA would make a person sick • Risk assessment model estimates the level of SE to be 1-51 ng/serving of pork products
T-2 toxin	1000 mg	1) *Fusarium sporotrichioides* cultures that produce T-2 toxin, or 2) Food (e.g., oats) that was naturally contaminated by the fungi	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.	• The amount of toxin that can be found in plant tissue is in the microgram range • 300 µg/ml of toxin in liquid culture or cooked rice cultures • Approximately 10-400 µg/g of T-2 in oats and barley
Tetrodotoxin	100 mg	Aquatic animals and amphibians and organs that contain the toxin so long as no additional extraction of the toxin occurs.	Several Japanese companies produce TTX commercially from puffer fish liver and/or ovaries. Toxic organs are homogenized with water and a weak	Extraction from aquatic animals and amphibians and organs that contain the toxin in acidified water or organic solvent.	• 1-2 g toxin from 100 kg of ovary • One puffer fish liver or ovary from a large highly toxic Asian species could contain tens of mg of TTX, making possession of a low number of these organs enough to meet the regulatory threshold

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			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.		• Many frog skins, or other toxic non-puffer fish specimens

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