

Policy 120: Select Agents and Toxins

I. PURPOSE

Dartmouth College is not a registered entity with the Federal Select Agent Program; therefore only research involving excluded select agents or toxins or permissible amounts (exempt quantities) of select agent toxins (SAT) is permitted. This Institutional Biosafety Committee (IBC) policy describes the requirements on the use of exempt or excluded select agents and toxins at Dartmouth. These requirements have been established to ensure safe handling and disposal procedures, effective tracking and security, and to be in compliance with federal regulations regarding select agent and toxin use. Any person wishing to begin research involving excluded or permissible amounts of select agents or toxins must receive prior approval from the Dartmouth IBC before possession of the agent/toxin.

II. DEFINITIONS

Select Agents and Toxins: a subset of biological agents and toxins that the Departments of Health and Human Services (HHS) and Agriculture (USDA) have determined to have the potential to pose a severe threat to public health and safety, to animal or plant health, or to animal or plant products.

- The current list of select agents and toxins is updated every two years and can be found at: <http://www.selectagents.gov/SelectAgentsandToxinsList.html>.

NOTE: Dartmouth is not part of the Federal Select Agent Program, so only excluded select agents or exempt quantities of select agent toxins are permitted.

Select Agent and Toxin Nucleic Acids: nucleic acids that are capable of producing infectious forms of select agents and functional forms of select toxins, including complete genomes as well as recombinant and/or synthetic nucleic acids. The following are regulated as select agents:

- Nucleic acids that can produce infectious forms of any of the select agent viruses.
- Recombinant and/or synthetic nucleic acids that encode for the functional form(s) of select toxins if the nucleic acids:
 - 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*.
- Select agents and toxins that have been genetically modified.

NOTE: Dartmouth is not part of the Federal Select Agent Program, so select agent and toxin nucleic acids, as defined above, are not permitted.

Select Agent Regulations: Select agents are governed by several regulations based on whether they affect humans, animals, or plants. They can be found at <https://www.selectagents.gov/regulations/index.htm>.

- 42 CFR Part 73 = covers human pathogens/toxins (HHS/CDC select agents/toxins) and those that can also affect animals (called the “overlap” select agents/toxins)



- 9 CFR Part 121 = covers animal pathogens/toxins (USDA/APHIS) and those that can also affect humans (called the “overlap” select agents/toxins)
- 7 CFR Part 331 = covers plant pathogens/toxins (USDA/APHIS Plant Protection and Quarantine)

Permissible Toxin Amounts (Exempt Quantities): Certain toxins are not regulated if the amount under the control of a principal investigator does not exceed, at any time, the amounts indicated in Section VI.ii below. Please refer to the list provided on the Select Agent website for the most current information: <http://www.selectagents.gov/PermissibleToxinAmounts.html>.

Excluded Select Agent or Toxin: An attenuated strain of a select biological agent or toxin that does not pose a severe threat to public health and safety, animal health, or animal products may be excluded from the requirements of the select agent regulations. Please refer to the Excluded Select Agents List for the most up to date information: <http://www.selectagents.gov/SelectAgentsandToxinsExclusions.html>.

Tier 1 Agent: A subset of select agents and toxins have been designated as Tier 1 (per Executive Order 13546) because these biological agents and toxins present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence, and pose a severe threat to public health and safety.

NOTE: NO Tier 1 agents are permitted at Dartmouth. Please refer to the Select Agents and Toxins list for the current Tier 1 agents: <http://www.selectagents.gov/SelectAgentsandToxinsList.html>.

III. REGULATORY BACKGROUND

The Federal Select Agent Program (FSAP) is jointly comprised of HHS/CDC Division of Select Agents and Toxins (DSAT) and the USDA/APHIS Agriculture Select Agent Services (AgSAS). The FSAP oversees the possession, use, and transfer of biological select agents and toxins that have the potential to pose a severe threat to public, animal or plant health or to animal or plant products. The select agent regulations implement the *Bioterrorism Preparedness and Response Act* of 2002 (42 USC 262a), *Agricultural Bioterrorism Protection Act* of 2002 (7 USC 8401) and Executive Orders 13486 and 13546 (<http://www.selectagents.gov/faq-legislature.html>). Any individual or entity wishing to work with select agents or toxins must register with the FSAP to comply with these regulations.

IV. REPERUSSIONS OF NON-COMPLIANCE (pursuant to the *USA Patriot Act*)

A. Administrative

- i. The Federal Select Agent Program has the authority to deny, suspend, or revoke registration to use, possess, or transfer select agents and toxins.
- ii. The Federal Select Agent Program has the authority to deny an individual access to select agents and toxins to protect public health and safety.



B. Civil

- i. In addition to any other penalties that may apply, any person who violates any provision of select agent regulations shall be subject to a civil money penalty in an amount <\$250,000 (individual) or <\$500,000 (institution).

C. Criminal: Violations of 18 USC 175b

- i. A "restricted person" that possesses a select agent or toxin, or transfers select agent or toxin in interstate or foreign commerce, (and is not excluded or exempted under select agent regulations) is subject to a criminal fine and/or imprisonment for up to 10 years.
- ii. Whoever transfers a select agent or toxin to a person who the transferor knows or suspects is not operating in accordance with the select agent regulations is subject to a criminal fine and/or imprisonment for up to 5 years.
- iii. Whoever knowingly possesses a select agent or toxin and is not operating in accordance with the select agent regulations is subject to a criminal fine and/or imprisonment for up to 5 years.

V. RESPONSIBILITIES

A. Principal Investigators (PIs) are responsible for

- iv. Compliance with the Federal Select Agent Regulations.
- v. Restricting use of select agents and toxins to those that are on the exclusions list or are of permissible levels (*see above, Definitions*).
- vi. Obtaining approval for any select agent and toxin work with the Dartmouth Institutional Biosafety Committee prior to possession.
- vii. Never exceeding the permissible quantity at any time of any select agent toxin on the permissible level list (*see Section VI.iii below*).
- viii. Maintaining an active inventory of any select agent or toxin. This includes tracking the use, transfers, and destruction/disposal.
- ix. Keep any permissible amounts of select agent toxins locked and secured.
- x. Training (and documenting training) of all lab personnel in the safe handling, PPE, and decontamination and segregation procedures of select agents/toxins.
- xi. The proper deactivation/decontamination and disposal of any select agent or toxin or material that has been in contact with a select agent or toxin. See decontamination procedures below.
- xii. Notifying the IBC of any loss of containment, non-compliance, or exposures.



B. Dartmouth College is responsible for

- i. Ensuring compliance with the Federal Select Agent Regulations.
- ii. Institutional Biosafety Committee (IBC) oversight of select agent and toxin use.
- iii. Reporting any instances of non-compliance.
- iv. Ensuring compliance with this institutional policy.
- v. Registering as an entity with the Federal Select Agent Program if the need arises.

VI. PROCEDURES

A. Authorization

Any person wishing to begin research involving excluded or permissible amounts of select agents or toxins must receive prior approval from the Dartmouth Institutional Biosafety Committee (IBC) before possession of the agent/toxin. A lab-specific standard operating procedure (SOP) must be submitted to the IBC as part of the review. Contact the Biosafety Officer for assistance.

B. Lab Specific Standard Operating Procedures (SOPs)

Lab specific SOPs for safe use of excluded or permissible amounts of select agents or toxins must be based upon the recommendations found in Biosafety in Microbiological and Biomedical Laboratories (BMBL). Modifications or additional requirements may be made by the IBC. SOPs should contain information that includes the name of the agent or toxin, laboratory procedures using the agent or toxin, a risk assessment and how potential exposures will be avoided, appropriate personal protective equipment and facility/engineering containment measures, required training, deactivation and disposal procedures, signage, and any occupational health considerations (signs/symptoms of exposure, how to respond to an exposure, available treatments, etc.) See Appendix A for an example.

C. Permissible Levels

The following toxins are not regulated if the amount under the control of a principal investigator does not exceed, at any time, the amounts indicated in the FSAP Permissible Amounts List (Table 1).

Table 1. Permissible Amounts of Select Agent Toxins (as of 2/16/17*)

HHS Toxins [§73.3(d)(3)]	Amount
Abrin	100 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	100 mg
Diacetoxyscirpenol (DAS)	10000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal Enterotoxins (Subtypes A, B, C, D, and E)	100 mg
T-2 toxin	10000 mg
Tetrodotoxin	500 mg

*Consult current list here: <http://www.selectagents.gov/PermissibleToxinAmounts.html>



In the event that a PI mistakenly acquires a quantity of select agent toxin greater than the permissible amount, they should contact the Biosafety Officer immediately.

D. Training

All personnel possessing, using, transferring, or receiving select agents or toxins must have agent-specific training as required by the IBC and Environmental Health & Safety (EHS). Training may be included as part of the lab-specific SOP. Retraining is required annually.

At a minimum, agent specific training should include:

- Review SDS and physical properties and hazards of toxin
- Discuss routes, signs, and symptoms of exposure
- Discuss potential risks of experimental procedures that may result in an exposure
- Plan emergency actions and what to do in case of exposure
- Review available vaccinations or post-exposure (anti-toxin) management procedures
- Outline control measures for working with toxin (PPE, fume hood, transporting, never working alone, etc.)
- Identify appropriate methods for decontamination and disposal
- Discuss inventory control, lock box, and restricted access
- Review BMBL Appendix I “Guidelines for work with toxins of biological origin”

This training is specific to each lab and is the responsibility of the PI (assistance is available from EHS). Documentation of training including dates, material covered and signatures of attendees must be kept on file in the lab. These records should be maintained indefinitely.

E. Lab Inspections

Laboratories and campus facilities possessing, using, transferring or receiving exempt quantities or excluded select agents and toxins will be inspected periodically by EHS. If a facility is found to be in non-compliance, approval to work with the materials may be revoked by the IBC. See Appendix B for an example inspection checklist.

F. Security

All laboratories using, transferring, or receiving excluded or permissible amounts of select agents or toxins must comply with all of the security, inventory, and containment requirements as follows:

- Select agents/toxins must be secured against unauthorized access
- Select agents/toxins must be under lock and key security when not in use, including freezers, refrigerators, cabinets and other storage containers.
- An accurate inventory must be maintained (see Appendix C).
- Only authorized personnel are allowed in laboratory use areas during select agents/toxins use or transfer. Door signage must be in place when SAT are in use.
- Key control programs must be in place to track lab door and storage cabinet keys, including dates issued and returned. Locks must be changed if keys are lost or unaccounted.



G. Decontamination/Deactivation of Select Agents and Toxins

- i. Excluded Select Agents: Decontamination of excluded select agents should be conducted in accordance with the Dartmouth College [Biohazardous Waste Disposal Guide](#) and the [Emergency Response and Biohazard Exposure Control Plan](#).
- ii. Select Agent Toxins (permissible levels): General guidelines for laboratory decontamination of selected toxins are summarized Biosafety in Microbiological and Biomedical Laboratories, Appendix I, Guidelines for Work with Toxins of Biological Origin.
- iii. General guidelines for laboratory decontamination of selected select agent toxins are summarized in Table 2. Many toxins are susceptible to inactivation with dilute sodium hydroxide (NaOH) at concentrations of 0.1-0.25N, and/or sodium hypochlorite (NaOCl) bleach solutions at concentrations of 0.1-0.5% (w/v). Use freshly prepared bleach solutions for decontamination; undiluted, commercially available bleach solutions typically contain 3-6% (w/v) NaOCl. Consult the BMBL Appendix I, Guidelines for Work with Toxins of Biological Origin for more specific information.

Depending upon the toxin, contaminated materials and toxin waste solutions can be inactivated by incineration, autoclaving, or by soaking in suitable decontamination solutions. All disposable material used for toxin work should be placed in secondary containers, decontaminated in accordance with the appropriate method used to inactivate the toxin, autoclaved, and disposed of as hazardous waste. The initial decontamination step (ex: bleach soak) should occur immediately following use of the SAT. Contaminated waste should be autoclaved promptly. Contact EHS for assistance.

Contaminated or potentially contaminated protective clothing and equipment should be decontaminated using suitable chemical methods or autoclaving before removal from the laboratory for disposal, cleaning or repair. If decontamination is impracticable, materials should be disposed of as hazardous waste. Contact EHS for assistance.

In the event of a spill, avoid splashes or generating aerosols during cleanup by covering the spill with paper towels or other disposable, absorbent material. Apply an appropriate decontamination solution to the spill, beginning at the perimeter and working towards the center.

Inactivation procedures should not be assumed to be 100% effective without validation using specific toxin bioassays. Please review the table below for appropriate measures for biological toxins. If you are working with a biological toxin not listed here, consult the EHS office for further assistance.



Table 2. Inactivation Methods for a Few Select Agent Toxins

Toxin	Chemical Inactivation Minimum 30 min contact time, unless otherwise noted below				Steam Autoclave	Dry Heat (°F) 10min exposure			
	2.5% NaOCl + 0.25N NaOH	2.5% NaOCl	1.0% NaOCl	0.1% NaOCl		121°C for >1 hr, liquid cycle, slow exhaust	200	500	1000
Abrin	YES	YES	YES	YES	YES	YES	YES	YES	YES
Botulinum neurotoxins	YES	YES	YES*	YES	YES	YES	YES	YES	YES
Diacetoxyscirpenol (DAS)	YES	NO	NO	NO	NO	NO	NO	NO	YES
Ricin (2 protein chain, multimeric)	YES	YES	YES*	YES	YES	YES	YES	YES	YES
Saxitoxin	YES	YES	YES*	YES	NO	NO	YES	YES	YES
Staphylococcal enterotoxins (A-E)	YES	YES	YES*	YES	YES?	YES?	YES?	YES?	YES?
T-2 Toxin	YES*	NO	NO	NO	NO	NO	NO	NO	YES
Tetrodotoxin	YES	YES	YES*	NO	NO	NO	YES	YES	YES

NaOCl = Sodium hypochlorite; NaOH = Sodium hydroxide; YES = complete inactivation; YES? = assumed inactivation; NO = no inactivation; * see recommendations below

Adapted from *BMBL*, Appendix I; *Biological Safety Principles and Practices*; and UC Denver/Anschutz EHS

*Recommendations

- i. For complete inactivation of T-2 mycotoxins, all liquid samples, spills, and non-burnable waste shall be soaked in 2.5% NaOCl with 0.25N NaOH for 4 hours. All solid wastes will be segregated in a closed container for incineration in the chemical hazardous waste stream. It is further recommended that cages and bedding from animals exposed to T-2 mycotoxin be exposed to 0.25% NaOCl and 0.025 N NaOH for 4 hours, prior to further cage-cleaning/cage-wash operations.
- ii. Exposure to 1% NaOCl for 30 minutes is an effective inactivation method for saxitoxin, tetrodotoxin, ricin, botulinum toxin, or staphylococcal enterotoxins (SEB) for working solutions, equipment, working area and spills.
- iii. Autoclaving can be used for protein toxins, i.e. ricin, botulinum toxin, and SEB, but should not be used with any of the low molecular weight toxins.
- iv. Conotoxin inactivation depends on the presence or absence of disulfide bonds.
 - For conotoxins with disulfide bonds: incubate with an excess of dithiothreitol (DTT) (10-20 mM buffered solution, pH 8.7) for at least 1 hour at room temperature or 30 minutes at 50°C. Add an equal volume of 50-100mM solution of iodoacetamide (buffered solution, pH 8.7) and incubate for 1 hour at room temperature.
 - For conotoxins without disulfide bonds, incubate in a fume hood in a closed glass vial with 6N HCl for 24 hours at 90-110°C.



H. Transfers - Due Diligence Provision

The Federal Select Agent Program has a provision to address the concern that someone might stockpile toxins by receiving multiple orders below the excluded amount. The "Due Diligence Provision" requires a person (registered or unregistered) transferring toxins in amounts that would otherwise be excluded from the provisions to:

- i. use due diligence to assure that the recipient has a legitimate need to handle or use such toxins; and
- ii. report to FSAP if they detect a known or suspected violation or become aware of suspicious activity related to the toxin. The "due diligence" provision required under Section 16 applies to anyone (registered or unregistered individuals or entities) that transfers toxin listed under Section 73.3 in amounts that otherwise would be excluded from the regulations.

To document those individuals who have a legitimate purpose to handle and use such toxins, the transferor can either require the recipient to complete documentation stating their intended use of the toxins or the transferor can document their knowledge of the recipient's legitimate need for the toxins. Information pertinent to the person requesting and using the toxins includes the individual's name, institution name, address, telephone number, and e-mail address.

I. Reporting Suspected Violation of Federal Law Suspicious Activity

The FSAP has established a confidential means for reporting safety and security issues associated with the possession, use and transfer of select agents and toxins. The HHS, Office of Inspector General (OIG) maintains a hotline that allows individuals to anonymously report fraud, waste and abuse in all departmental programs. This hotline is now available to anonymously report safety or security issues related to select agents and toxins. When reporting these issues to OIG, please ensure that you indicate it is a "Select Agent Program" issue.

OIG Hotline Info:

Phone: 1-800-447-8477

Fax: 1-800-223-8164

Web: <http://oig.hhs.gov/fraud/hotline>

Mail: Office of Inspector General
Dept of Health & Human Services
Attn: Hotline
PO Box 23489
Washington, DC 20026



VII. RESOURCES

- Biohazardous Waste Disposal Guide. Dartmouth IBC 2022
http://www.dartmouth.edu/~ehs/biological/policies_sops.html
- *Biosafety in Microbiological and Biomedical Laboratories*, 6th ed. Centers for Disease Control and Prevention and National Institutes of Health, 2020 June
<https://www.cdc.gov/labs/BMBL.html>
- Emergency Response and Biohazard Exposure Control Plan. Dartmouth IBC 2022.
http://www.dartmouth.edu/~ehs/biological/policies_sops.html
- Evers DL, Allen KP, Fowler CB, Mason JT, Blacksell SD. *Laboratory Decontamination of HHS-listed and HHS/USDA Overlap Select Agents and Toxins*. *Applied Biosafety* 2013; 18(2); 59-71
- Federal Select Agent Program: <http://www.selectagents.gov/>
- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. National Institutes of Health Office of Science Policy, 84 FR 17858, 2019 April
<https://osp.od.nih.gov/biotechnology/nih-guidelines/>
- Wooley DP and Byers KB, eds. *Biological Safety Principles and Practices*, 5th ed. ASM Press, 2006.



Appendix A. Example Select Agent or Toxin Lab SOP

Select Agent/Toxin Lab Standard Operating Procedure (SOP)

Date:	Building/Room:
Principal Investigator:	Alternate Contact:
PI Phone:	Alt Phone:
Revision Date:	IBC Approval Date:

Agent/Toxin Name: _____

Risk Assessment:

- Max amount stored: _____
- Amount used in experiments: _____
- LD50: _____
- Describe risks inherent to procedures (*use of powders, aerosol formation, potential routes of exposures, etc.*): _____
- Engineering Controls (*fume hood, biosafety cabinet, etc.*): _____
- Proper PPE (*lab coat, gloves, safety goggles are a minimum; respiratory protection may be required*): _____
- Signage (*on lab door, on fume hood, etc.*): _____

Inactivation and Disposal Procedures (*refer to BMBL Appendix I; IBC Select Agents and Toxins Policy*):

Personnel Training Requirements (*list lab specific training required to work with material*):



Spill/Accident Response Procedures (*describe what to do in the case of a spill or exposure; refer to the Dartmouth College [Biohazardous Waste Disposal Guide](#) and the [Emergency Response and Biohazard Exposure Control Plan](#)*):

Occupational Health Info (*describe available and/or required vaccines, prophylactic treatments etc.*):

Storage/Security (*describe how the material will be securely stored*):

Inventory Maintenance (*describe how the inventory will be maintained*):

List of PI-Approved Users

Name	Signature	Date



Appendix B: Lab Inspection Checklist

Lab Inspection Checklist for Select Agents and Toxins

PI name: _____

Date of inspection: _____

Inspected by: _____

YES <input type="checkbox"/>	NO <input type="checkbox"/>	Area where toxin is used/stored is clean and organized (free from clutter) Location of toxin (building, room): _____
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Lab has a written SOP specific to the toxin(s) used in the lab. (Plan should (1) identify the hazards encountered during normal use of the toxin and those that could be encountered during a spill or other accident; and (2) specify the policies and practices to be used to minimize risks (e.g. containment and PPE, management of spills, management of accidental exposures, decontamination, etc.)
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Everyone in the lab who has potential exposure to the toxin has had documented (EHS) training for that specific toxin.
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Inventory control system is in place
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Inventory log is current and up to date (Current amount of toxin _____)
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Access to toxins is restricted to authorized personnel
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Toxin is locked up
YES <input type="checkbox"/>	NO <input type="checkbox"/>	All manipulations with dry forms of the toxin is done in appropriate containment
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Rooms where toxins are used are appropriately posted
YES <input type="checkbox"/>	NO <input type="checkbox"/>	High risk operations are done with at least two knowledgeable persons present.
		How often are high risk operations done? (_____)
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Work surfaces and storage containers are decontaminated regularly (including interior of any hood/bsc)
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Waste associated with the toxin is being properly decontaminated and disposed of
YES <input type="checkbox"/>	NO <input type="checkbox"/>	Appropriate PPE is available and used during all manipulations and potential exposure conditions
YES <input type="checkbox"/>	NO <input type="checkbox"/>	SDS is available for the toxin

NOTES: _____
