Toxin Standard Operating Procedure

1. Purpose

The purpose of this Standard Operating Procedure (SOP) is to provide guidance and instructions for working with biological toxins and Select Toxins at UAB. This SOP and the Safety Data Sheets (SDSs) for toxin must be included in the laboratory safety manual. Principal investigator (PI) and laboratory personnel must document that they have read and understood these materials.

2. Scope

This SOP applies to all personnel who handle, store, use, transport or dispose of toxins on UAB campus. Procedures not covered in this SOP require either a supplemental document describing the deviation or an alternate SOP. All supplemental documents and alternate SOPs must be approved by the Biosafety Office.

Toxin work must be registered with IBC by sending documents to projects@uab.edu. Institutional Care and Use Committee (IACUC) approval is required for animal studies involving toxins. See **Appendix A** for LD50 (ug/kg) of select toxins and some of commonly used biological toxins in research.

3. Introduction

Biological toxins are non-infectious, non-replicating substances produced by microorganisms, plants, or animals that can cause significant harm at low exposure levels and must be managed with the same care as infectious biological agents. Appropriate controls, training, and protective measures must be in place to prevent exposure. Certain biological toxins are designated as Select Toxins by the Federal Select Agent Program (FSAP) due to their potential risk to human, animal, or plant health. Their possession, use, transfer, and disposal are strictly regulated. Select Toxins possessed below regulatory threshold amounts may qualify for exemption (see **Appendix B**); however, the PI must submit an exemption checklist for approval by the Responsible Official (RO) before use. Select Toxin possession above the exempt threshold requires registration with the FSAP. Select Toxin inventory records documenting acquisition, use, storage, and inactivation must be maintained and retained for at least three years.

4. Roles and Responsibilities

4.1 Principal Investigator (PI)

- Ensure toxin work is reviewed and approved before initiation.
- Maintain toxin SOP/SDS on file.
- Get approval for laboratory-specific toxin SOPs when procedures deviate from this SOP.
- Ensure personnel receive required training before handling toxins.
- Ensure appropriate PPE, engineering controls, and supplies are available.
- Coordinate toxin work with the IBC/biosafety office.

4.2 Laboratory Personnel

- Read and follow approved toxin SOP and SDS.
- Complete required training before handling toxins.
- Use required PPE and containment practices.
- Report spills, exposures, and incidents immediately.
- Maintain accurate records of toxin acquisition, use, storage, and disposal.
- Clearly label any aliquots of toxins outside their original containers.
- Access to toxins must be restricted to trained and approved personnel only.

4.3 Biosafety Office/IBC

- Review and approve toxin SOPs.
- Provide guidance regarding containment, training, disposal, and regulatory compliance.
- Assist with exposure response and incident investigations.
- Conduct audits to ensure compliance with institutional and regulatory requirements.

4.4 Responsible Official (RO)

- Review and approve Select Toxin exemption requests and SOP.
- Oversee compliance with FSAP requirements.
- Maintain required records related to Select Toxin exemptions and approvals.

5. PPE and Workplace Practices

5.1 Personal protective equipment (PPE)

1. Double gloving is recommended and change gloves immediately if contaminated, torn, or punctured and dispose of them immediately after removal.
2. Wear a laboratory coat with long sleeves, disposable gown, apron, or coveralls. Disposable PPE is recommended.
3. Wear closed-toe shoes that fully cover the feet.
4. Wear an N95 respirator if required by the risk assessment.
5. For toxin administration to animals, wear gloves, disposable gown, and a face mask in addition to animal facility requirements.

5.2 Invitro work

1. Biosafety Level 2 (BSL-2/ABSL-2) practices are appropriate for most toxin work. However, some toxins or procedures may require BSL-3/ABSL-3 facilities or practices (e.g., aerosolization studies, or use of non-exempt quantities of select toxins).
2. Post sign on room door when toxins are in use stating, “Toxins in Use - Authorized Personnel Only.”
3. Store toxins in a designated, secure location. Select toxins must be maintained in a locked location.
4. Use the toxin in liquid form whenever possible. If you must use a solid or powder, purchase the smallest amount needed, preferably already pre-measured, and add the diluent using syringe needle through the sealed top without opening the container.
5. Perform all procedures that may generate aerosols, droplets, or splashes in a certified biosafety cabinet (BSC) or other approved containment device. Use plastic-backed absorbent pads while

working with toxins. After completion of tasks, neutralize or dispose of pads in waste streams effective for the toxin used.

6. Utilize safe sharps procedures (i.e. sharps container in the immediate vicinity). Luer lock syringes or disposable syringe needle units are recommended and should be properly disposed of promptly after use.
7. Decontaminate the exterior of toxin containers before removal from primary containment devices. Closed primary containers should be transferred in a clean, labeled secondary container to prevent contamination during transport.
8. Decontaminate the BSC or approved containment and all surfaces used upon completion of tasks with appropriate inactivating agent and contact time.
9. All potentially contaminated disposable items (such as gloves used in toxin preparation) must be decontaminated and placed in an ASTM-D certified biohazardous waste bag and autoclaved or incinerated, depending on the toxin used.

   Refer Appendix C: Chemical and physical inactivation of toxins table at the end of this SOP
10. Transport toxins only in labeled, leak/spill-proof, non-breakable secondary containers.
11. Wash hands thoroughly upon completion of tasks.

5.3 Animal work

1. Administration of toxins to animals must be done inside a certified BSC.
2. Animals should be anesthetized or placed into a restraining apparatus before procedures with toxin are performed. Once the animal has been properly fitted into the restraining apparatus, the syringe will be loaded just prior to injection.
3. Animal Use Safety Information (AUSI) must be posted at the entrance to the animal room. Cages containing animals that have received the toxin must be labeled. Cages must remain in place for a minimum of 14 days following the toxin administration.
4. Dirty animal cage bedding must be autoclaved prior to disposal.
5. If the toxin is not sensitive to autoclave, spray NaOCl or NaOH (Refer Appendix C) on cage bedding and allow for 30 min followed by incineration through stericycle. It is the responsibility of investigator to inform ARP staff and perform chemical inactivation.

6. Inactivation Procedures

According to the CDC, inactivation of a biotoxin means to render the toxin non-functional so that it is no longer capable of exerting its toxic effect. This is different from inactivation of biological agents, which renders the agent non-viable, or no longer capable of growing, replicating, infecting, or causing disease. Inactivation methods used for biotoxins must be specific for the toxin, published and validated, or developed and validated with thorough testing. Note that disinfection solutions and products may not inactivate biotoxins. Similarly, autoclaving does not inactivate all toxins and should only be used when known to inactivate a specific toxin.

All toxin waste must be chemically or physically inactivated prior to disposal.

When using bleach solution for inactivation of toxins and decontamination of surfaces, prepare working solution daily, as diluted bleach is effective for only 24 hours. Undiluted, commercially available bleach solutions typically contain 3-6% (w/v) NaOCl (sodium hypochlorite). (Refer Appendix C).

7. Waste Disposal Procedures

1. For toxins inactivated by autoclaving: Place contaminated PPE, disposable materials, and spill cleanup debris in a biohazardous waste bag. Autoclave if the toxin is susceptible, then dispose via Stericycle for incineration. Contact medwaste@uab.edu for the appropriate account to use for incineration.
2. For mixed waste (i.e., toxin waste mixed with radioactive waste) consult Biosafety at biosafety@uab.edu for disposal instructions.
3. For chemically inactivated toxins: Manage materials as hazardous chemical waste based on the inactivation agent and submit through UAB's Support Facility using EHSA. If inactivating using non-hazardous chemicals, dispose down UAB's sanitary sewer. Contact medwaste@uab.edu for clarifications regarding disposal.

8. Spills or Releases

Toxin spills must be cleaned up immediately by trained personnel wearing the appropriate PPE. For questions on spill cleanup, contact EH&S at biosafety@uab.edu or (205) 917-4766 or call UAB Police at (205) 934-3535 for emergency assistance.

8.1 Liquid Spills

1. Don the appropriate PPE including at least a lab coat or disposable gown, goggles, and two pairs of nitrile gloves.
2. Restrict access to the affected area until cleanup is complete.
3. Determine the appropriate inactivation method and waste stream for the toxin and any potentially contaminated surfaces or materials.
4. For chemical inactivation, cover the spill with absorbent paper towels and inactivate by applying the appropriate chemical agent, starting at the perimeter and working toward the center. Allow the appropriate contact time before cleaning up. Return to the spill site and clean the area with more of the inactivating agent, allowing prescribed contact time, then clean with soap and water. The inactivated spill waste can be double bagged and disposed of in accordance with the toxin-specific SOP.
5. For physical inactivation use absorbent paper towels to wipe up liquid. Place waste in a biohazardous bag and autoclave/incinerate, as appropriate.

8.2 Powder Spills inside a BSC or containment

1. The required PPE for cleaning up contained spills includes a lab coat or disposable gown, goggles, and two pairs of nitrile gloves.
2. Determine the appropriate inactivation method and waste stream for the toxin and any potentially contaminated surfaces or materials.
3. Gently cover powder spill with dampened absorbent paper towels to avoid raising dust.
4. For chemical inactivation, apply the appropriate chemical inactivating agent, starting at the perimeter and working toward the center. Allow the appropriate contact time before cleanup. Return and clean

the spill area with inactivating agent (See Appendix C), allowing prescribed contact time, then clean with soap and water. Inactivated spill waste is disposed of in accordance with the toxin-specific SOP.

5. For physical inactivation, use dampened paper towels to wipe up the powder spill. Place waste in a biohazardous bag and autoclave/incinerate, as appropriate, according to the toxin-specific SOP.

8.3 Powder spills outside of primary containment- (BSC, fume hood, glove box, or other containment)

1. Remove all personnel from the laboratory, exit, and restrict access; do not attempt to clean up the spill.
2. Immediately call EH&S at (205) 917-4766

9. Emergency Procedures

In the event of an exposure, follow your laboratory's toxin-specific Exposure Response Plan. The steps described below are generally applicable to biological toxin exposures:

9.1 Provide First Aid

1. Sharps injury (needlestick and subcutaneous exposure): Scrub exposed area thoroughly for 15 minutes using warm water and soap. Do not squeeze the wound.
2. Skin exposure: Use the nearest sink (localized exposure) or safety shower (gross exposure) for 15 minutes. If using a sink, continue rinsing for 15 minutes. If using a shower, continue for 15 minutes, while removing clothing. Use a clean lab coat or spare clothing for cover-up.
3. Eye exposure: Use the eye wash for 15 minutes while holding eyelids open.
4. Inhalation: Move to an area physically separated from the contaminated space.

9.2 Obtain Medical Guidance

1. Report the exposure to personnel in the area.
2. Call **(205) 934-3411** and ask for "Needlestick and Exposure Team" and provide details of the exposure (i.e., agent, dose, route of exposure, time since exposure). Take SDS and SOP of the specific biotoxin to the UAB clinic or Emergency Department (in case of emergency).
3. Notify your supervisor as soon as possible for assistance.
4. Ensure the area is secure before leaving.

9.3 Report the Incident

1. All incidents should be reported to Human Resources by following instructions on the On-The-Job-Injury/Illness Program website. Employees should report incidents within 48 hours to ensure eligibility for workers' compensation benefits.
2. After medical evaluation, notify biosafety office at biosafety@uab.edu.

Appendix A: LD50 (ug/kg) of biological/select toxins

TOXIN	LD50 (ug/kg body weight of vertebrate)
Abrin*	**0.7**
Aerolysin	7
Anthrax Lethal toxin	1000
B-bungarotoxin	14
Botulinum toxins*	**0.0004 to 0.0025**
Caeruleotoxin	53
Cereolysin	40-80
Cholera toxin	250
Clostridium difficile enterotoxin A	0.5
Clostridium difficile cytotoxin B	220
Clostridium perfringens kappa toxin	1500
Clostridium perfringens perfringolysin O	13-16
Clostridium perfringens enterotoxin	81
Clostridium perfringens beta toxin	0.4
Clostridium perfringens delta toxin	5
Clostridium perfringens epsilon toxin	0.1
Conotoxins*	**12-30**
Crotoxin	82
Diacetoxyscirpenol (DAS)*	**1100**
Diphtheria toxin	0.1
Listeriolysin	3-12
Leucocidin	50
Modeccin	1-10
Microcystin	25-150
Nematocyst toxins	33-70
Notexin	25
Palytoxin	0.041-1.81
Pertussis toxin	15
Pneumolysin	1.5
Pseudomonas aeruginosa toxin A	3
Ricin*	**2.7**
Saxitoxin*	**8**
Shiga Toxin	0.25
Shigella dysenteriae neurotoxin	1.3
Staphylococcal enterotoxin B*	**25**
Staphylococcal enterotoxin F*	**2-10**
Streptolysin O	8
Streptolysin S	25
Taipoxin	2
Tetanus toxin	0.001
T-2 toxin*	**5-10**
Tetrodotoxin*	**8**
Viscumin	2.4-80
Volkensin	1.4
Yersinia pestis murine toxin	10

*** Select Toxins (see Appendix B for exempt quantities)**

Appendix B: Select Toxins and their Permissible Toxin Amounts (exempt quantities/ Inventory limits)

Select Toxin	Exempt Quantity
Abrin	1000 mg
Botulinum neurotoxins	1 mg
Short, paralytic alpha conotoxins	200 mg
Diacetoxyscirpenol (DAS)	10,000 mg
Ricin	1000 mg
Saxitoxin	500 mg
Staphylococcal enterotoxins (Subtypes A, B, C, D, and E)	100 mg of all subtypes combined
T-2 toxin	10,000 mg
Tetrodotoxin	500 mg

Note: For working with select toxins more than permissible toxin amounts, approval permit must be obtained from the Federal Select Agent Program through Responsible Official (RO) before acquiring the toxin.

Appendix C: Chemical and physical inactivation of toxins

TOXIN	AUTOCLAVE 1hr @ 121°C	NaOCl (30 min)	NaOCl + NaOH or NaOH only (30 min)
Abrin [1]	Yes	≥0.7% [a]	ND
Alpha Conotoxin [2, 3]	ND	≥0.5%	10 N NaOH
Anthrax Lethal Toxin (PA, LE) [1]	Yes	≥0.5%	ND
Botulinum neurotoxin A-G [1]	Yes [b]	≥0.1%	ND
Brevetoxin (PbTx-2) [2,	No[d]	≥2.5% [c, e]	0.25%+0.25 N [c, e]
Cholera toxin [1]	Yes	0.5%	ND
Clostridium perfringens epsilon toxin [1]	Yes	0.5%	ND
Conotoxin [2]	ND [d]	0.5%	ND
Diacetoxyscirpenol (DAS), Deoxinalenol (DON), Zearalenone (ZEA) [2]	No [d]	≥2.5%	0.25%+0.25 N
Diphtheria toxin [1]	Yes	0.5%	ND
Microcystin [2]	No [d]	≥0.5% [c]	0.25%+0.25 N [c]
Palytoxin [2]	No [d]	≥0.1% [c]	0.25%+0.25 N [c]
Pertussis toxin [1]	Yes	0.5%	ND
Ricin [1]	Yes	≥1.0% [c]	>0.1%+0.25 N [c]
Saxitoxin [2]	No [d]	≥0.1% [c, f]	0.25%+0.25 N [c, f]

Shigatoxin and Shiga-like ribosome inactivating proteins [1]	Yes	≥0.5%	0.25%+0.25 N
Staphylococcal Enterotoxins [1]	Yes [b]	≥0.5%	ND
T-2 mycotoxin [2]	No [d]	≥2.5% [c, e]	0.25%+0.25 N [c, e]
Tetanus toxin [1]	Yes	0.5%	ND
Tetrodotoxin [2]	No [d]	≥0.5% [c, f]	0.25%+0.25N [c, f]

Foot notes:

1. *Autoclave liquid and powder stocks, waste, and disposable materials and dispose of through Stericycle as medical waste.*
2. *Inactivate liquid and powder stocks, as described in table, and dispose of the inactive, non-hazardous solutions down sanitary sewer. Decontaminate and deface stock vials prior to disposal. Dispose of all potentially contaminated solid waste through Stericycle as medical waste (labeled as "Incinerate Only").*
3. *Conotoxins can also be inactivated using reducing agents such as dithiothreitol β- mercaptoethanol or tris (2-carboxyethyl) phosphine (100 mM) at 65–100° C for 15 min.*

a. *Exposure of crude abrin solution and dried abrin to 0.67% NaOCl eliminate over 90% of cytotoxicity within 5 min.*
b. *Steam autoclaving should be at ≥121°C for 1 h. For volumes larger than 1 liter, especially those containing Clostridium botulinum spores, autoclave at ≥121°C for 2 h to ensure that sufficient heat has penetrated to kill all spores.*
c. *The minimal effective concentration of NaOCl was dependent on toxin and contact time; all LMW toxins tested were inactivated at least 99% by treatment with 2.5% NaOCl, or with a combination of 0.25% NaOCl and 0.25 N NaOH.*
d. *non-burnable (non-autoclavable) waste should be chemically inactivated.*
e. *For T-2 mycotoxin and brevetoxin, non-burnable waste should be soaked in 2.5% NaOCl with 0.25% N NaOH for 4 h., whereas cages and bedding should be treated with 2.5% NaOCl + 0.25 N NaOH for 4 h.*
f. *Exposure for 30 min to 1% NaOCl is an effective procedure for the laboratory (working solutions, equipment, animal cages, working area and spills) for the inactivation of saxitoxin or tetrodotoxin.*
N. *Normality of NaOH solution.*
ND. *Indicates "not determined" from available decontamination literature.*

General Notes:

- *Commercially available bleach contains 5% NaOCl.*
- *Prepare working solution daily as diluted bleach is effective for only 24 hours.*
- *Decontaminate work surfaces and spills with solutions appropriate for the specific toxin for 30 min.*
- *The UAB BARB Core provides toxin inactivation services listed in Table 5.2 (standard fees apply).*
- *If you have any questions about inactivation of toxins, contact Biosafety Office at biosafety@uab.edu*