RUTGERS
Rutgers Environmental Health and Safety

Standard Operating Procedures: Conotoxin

Principal Investigator (print):	
Principal Investigator Signature:	
Date Reviewed:	
Location:	Rutgers University
Campus:	
Building:	
Designated Use Area / Room(s):	
Designated Storage Area/Room	
IBC Approval Number:	
IACUC Approval Number:	

Physical Characteristics:

Conotoxins are neurotoxins derived from marine cone snails of the genus *Conus*. The snails use their venom to immobilize and kill fish, shellfish, and marine worms.

The extreme toxicity results from several different classes of conotoxins acting synergistically by different mechanisms. Some of the toxins by themselves are not lethal but produce tremors or deaden pain. Some alpha-conotoxins by themselves are lethal by injection at 0.025 mg/kg or even 0.01 mg/kg of body weight, from mouse injection tests.

On a molecular scale, conotoxins differ from other biotoxins in that they are relatively small, compact peptides made up of 12 to 40 amino acids held tightly together by disulfide bonds. The disulfide bonding network as well as the order of the specific amino acids and how they are configured determine the specifically of conotoxins.

There are probably over 50,000 different conotoxins in existence from perhaps 500 different species of cone snails. Any cone snail species can inject a mix of many different conotoxins.

Health Hazard Summary:

Conotoxin can be fatal if swallowed, inhaled or if exposed by the percutaneous route. Death can occur in 1-5 hours after exposure due to paralysis of the respiratory system.

The main lab risks for this toxin are exposure by direct contamination of mouth, eyes, or other mucous membranes and by accidental inoculation or other accident that may compromise the normal barrier of the skin.

Signs and symptoms of conotoxin exposure include: faintness, ptosis (dropping eyelids), poor coordination, absent gag reflex, abnormal sensations such as burning or tingling, blurred vision, speech difficulties, double vision, difficulty swallowing, weakness, nausea, generalized numbness and respiratory distress.

No antidotes are available.

Safety Data Sheet (SDS): (Attach manufacturer-specific SDS to this SOP)
Read the manufacturer's SDS, formerly called the material safety data sheet (MSDS), and maintain a copy in your safety binder along with this SOP. For safety questions, contact Rutgers Environmental Health & Safety (REHS) at 848-445-2550.

Personnel Requirements:
All laboratory members working with Conotoxin must review the manufacturer specific SDS and be up to date on all required health and safety trainings.

Exposure Control:
- Purchase Conotoxin in quantities that can be dissolved at one time, within the original vial.
- Handle Conotoxin inside the chemical fume hood/certified biosafety cabinet (consult with REHS).
- Wear double nitrile gloves, eye protection and lab coat when handling Conotoxin.
- Conotoxin must be kept in a secured location.
- Use syringe with integral safety feature, as applicable.
- Keep a solution of 10% bleach solution readily accessible (made fresh daily).
- Avoid inhalation and physical contact with Conotoxin.
- Ensure that a safety shower and eyewash station are nearby.

First Aid Procedures:
- *Call for medical advice immediately:*
  - Occupational Medicine Services (Newark) 973-972-2900
  - Hurtado Health Center (New Brunswick) 848-932-8254
  - Emergencies & After Hours – Call the Rutgers University Police Department (RUPD) or visit nearest hospital Emergency Room
    - 732-932-7211 (Piscataway & New Brunswick)

- 973-972-4490 (RBHS - Newark / Scotch Plains)
- 973-353-5111 (Rutgers-Newark)

- Additional first aid based on route of exposure:
  - *Ingestion/oral exposures* – rinse mouth with water.
  - *Inhalation exposure* – move person to fresh air and call for an ambulance if breathing becomes difficult.
  - *Contact exposure (eyes, nose, skin)* – flush the affected area with copious amounts of water for at least 15 minutes.
  - *Accidental Injection / Percutaneous* – call RUPD and request an ambulance or go to the nearest hospital emergency room.

Injury / Exposure Reporting:

Any exposure incidents must be reported in the REHS Accident Database located online at http://myrehs.rutgers.edu. The injured/exposed person's direct supervisor (e.g., PI or lab manager) needs to submit the incident report by the end of the work shift.

Spill Clean-up:

For small quantities (less than 5ml or 100 ug).

- If you don't feel comfortable cleaning up the spill, follow the instructions for large spills (below).
- Wear double nitrile gloves, lab coat, and safety glasses/goggles.
- Any broken glass fragments should be picked up with tongs, forceps or a small scoop (never use your fingers). Place the broken glass in a wide-mouthed plastic container. Tightly seal the container and contact REHS (http://rehs.rutgers.edu) for disposal.
- Liquids should be absorbed with paper towels and saturated with 10% bleach solution – 20 minute contact time!
- Solids should be wiped up with wetted paper towels saturated with a 10% bleach solution – 20 minute contact time!. Contaminated surfaces should then be cleaned three times using a detergent solution and paper towels followed by clean water.
- Inside a ducted hood, contaminated re-usable items (e.g., glassware and scoops) should be disinfected with a 10% bleach solution, washed three times with detergent by a trained employee wearing two pairs of nitrile gloves, eye protection and fully fastened lab coat or gown.
- Contaminated disposable items & spill clean-up waste (gloves, paper towels, absorbent pads, spill pads/pillows) must be bagged and autoclaved at 121°C and 15 psi for 60 minutes on liquid cycle (slow exhaust). The materials must then be disposed as biomedical waste.
- If your building does not have an autoclave, collect all spill clean-up

materials in tightly sealed containers, and contact REHS (http://rehs.rutgers.edu) for disposal.

For large spills (greater than 5ml or 100 ug) or possible airborne Picrotoxin:

- Evacuate the area.
- Report the spill to Rutgers University Police Department (RUPD)
  - 732-932-7211 (Piscataway & New Brunswick)
  - 973-972-4490 (RBHS - Newark / Scotch Plains)
  - 973-353-5111 (Rutgers-Newark)
- The police dispatcher will contact on-call REHS personnel.
- REHS staff will clean-up the spill.

General Safety Precautions for Dissolving and Aliquoting:

1. Post signs such as "Caution – Conotoxin" in the designated area(s) when working with the toxin until the Conotoxin has been returned to storage and the work area has been decontaminated.
2. Handle lyophilized Conotoxin powders and concentrated Conotoxin solutions in a fume hood, while wearing personal protective equipment (PPE): lab coat, safety glasses, and two pairs of nitrile gloves.
3. Place absorbent pad in the bottom of the hood/cabinet to contain potential spills.
4. Aliquot 5ml of a 10% bleach solution into a conical tube. Place the open tube in a tube rack to serve as waste receptacle for contaminated filtered pipette tips – minimum of 30 minute contact time!
5. Open vials carefully. If your gloved finger slips from the screw-top lid and touches the rim of the vial, change your outer gloves immediately to avoid spreading α-Conotoxin contamination to other items.
6. Aliquot suspension into plastic tubes labeled: 'Conotoxin, concentration, your name and date'.
7. Place tubes in leak-proof secondary plastic container. Label container: ''Conotoxin, concentration, your name, date, TOXIN – DO NOT HANDLE'.
8. When ready for use, remove aliquot from freezer and let thaw to room temperature.
9. Use a pipette with filtered tip to transfer contents. Treat tips as described in #4 above.
10. Use extreme caution when preparing/handling needles of Conotoxin. Use needles with integral safety feature (e.g., BD Safety Glide™). Dispose of contaminated needles immediately in sharps container.
11. Animal Administration: restrain or anesthetize animal during the injection, label the cage card with a chemical hazard label and Conotoxin information, wear PPE when handling the animals, and collect carcasses of Conotoxin -administered animals in a separate bag with a chemical hazard warning label and Conotoxin information when returning carcasses to the research animal facility for disposal.

12. Animal Housing: use static or microisolator cages. Never use ventilated cage racks without first consulting with REHS.
13. Cage/bedding changing: First bedding change (minimum 72-hour post-dosing):
    - Performed by laboratory personnel in a biosafety cabinet.
    - Use 10% bleach solution to thoroughly wet the inside of cage and soiled bedding.
    - Allow 30 minutes contact time prior to cage wash.
    - Dispose disinfected bedding into regulated medical waste (RMW) container.
    - Subsequent changes performed by LAS staff. No special handling precautions...
14. Carcass Disposal: place in red biohazard bag and then into Vivarium biohazard freezer for incineration.
15. Inactivate Conotoxin stocks and Conotoxin -contaminated items by chemical inactivation with 10% bleach solution (30 minutes of contact time) prior to disposal. Surfaces must be decontaminated with 10% bleach solution.

Lab-Specific Procedures & Safety Precautions (to be completed by Principal Investigator). You may attach separate pages if more space is required.

Materials: List manufacturer, catalog number, quantity to be ordered and form of material – e.g., lyophilized powder.

Preparation: List specific steps for preparing aliquots, specify containment controls, PPE to be worn, disinfection steps for surfaces and equipment used, storage information.

Procedure for Use in Mice (if applicable): Include description of containment controls, injection dilution, method of injection, dosing and cage marking information, procedures for bedding changes, carcass disposal, etc.

Procedure for Use In-Vitro (if applicable): Specify containment controls used, describe preparation of cell culture and how cell culture is treated and disposed.

Signatures:
By signing below, I certify that I have read this SOP and attached material, that I understand the procedures for working with Conotoxin toxin, that I understand the hazards associated with using Conotoxin toxin, and that I will use the procedures described in this SOP to safely handle and use Conotoxin toxin.

Name (typed)	Job Title	Signature

References:

CDC, 'The Emergency Response Safety and Health Database'
http://www.cdc.gov/niosh/ershdb/EmergencyResponseCard_29750019.html

Anderson, P.D. and G. Bokor. 2012. Conotoxins: Potential Weapons from the Sea. Bioterrorism & Biodefense. 3:3. http://dx.doi.org/10.4172/2157-2526.1000120