

Conotoxins | standard operation procedure

Work with conotoxins at MT9

The purpose of this document is to acquaint lab members and guest researchers with local rules, regulations as well as security and safety procedures for working with conotoxins.

Conotoxins/Conopeptides

Conotoxins are molecules found in the venom of the ~800 living species of marine cone snails. They can either be extracted from venom or predicted based on coding DNA (cDNA) sequenced from venom gland tissue or DNA sequenced from any cone snail tissue. However, the term “conotoxins” derives from “cono” for cone snail and “toxin”. The latter describes that cone snails use these compounds to capture prey. The term “toxin” does not imply that these compounds are toxic to non-prey species, including human. Because the majority of conotoxins are peptides, conotoxins are also often referred to as conopeptides. These two terms are interchangeable.

Conotoxin Research

Research on conotoxins at BMI focuses on functional characterization of conotoxins as novel probes for ion channels and receptors, and as potential drugs. Conotoxins are well established as valuable tools in brain research, and therapeutically for various human disorders, including pain, epilepsy, and diabetes.

Conotoxin Regulation

Work with and storage of conotoxins is subject to Danish rules and regulations administered by the Center for Biosecurity and Biopreparedness (CBB) (<https://www.biosikring.dk/home/>), which refers to the Ministry of Health.

Personnel working with conotoxins in the lab must contact Dr. Helena Safavi or Prof. Nicole Schmitt for training prior to commencing any work with conotoxins. Conotoxins cannot be transferred or taken outside of the lab without prior approval from Dr. Safavi or Prof. Schmitt.

Storage of conotoxins

Conotoxins are stored in freezers in rooms 7-09-118 and 7-09-154 (freezer #1).

Conotoxin risk assessment

Conotoxins are peptides and as such they do not replicate, are not infectious, and are difficult to transmit mechanically or manually from person to person. Moreover, they have very low volatility. A very small fraction of conopeptides (e.g., the α -conotoxins) are paralytic in higher vertebrates, including humans, when injected at doses exceeding the amount used by the majority of researchers today. The LD₅₀ of α -conotoxins is 10-100 μ g/kg. We do not work with α -conotoxins. The vast majority of conotoxins either display very mild phenotypes when injected into vertebrates, or have no phenotypic activity at all. Moreover, since conotoxins are peptides, they are unlikely to be absorbed after oral ingestion or through dermal absorption.

Based on this, the main laboratory risk posed by conotoxins is accidental injection. Other risks include accidental exposure by direct contamination of mouth, eyes or other mucous membranes and by inadvertent aerosol generation.

Security and safety measures and procedures when working with conotoxins

In your work, always wear a lab coat, disposal gloves (if required do double gloving) and if necessary safety glasses.

In addition, you must adhere to the following rules:

- Avoid the use of glassware (e.g. do not use glass Pasteur pipettes, but only plastic pipettes). Be especially careful if your work involves pressure or vacuum.
- For concentrated solutions ($> 100 \mu\text{M}$) use nitrile gloves, pipette tips with filters, and transport in tubes with tight-fitting, leak-proof caps.
- When dealing with freeze-dried conotoxin, in quantities higher than 100 nmol, use mouth and nose protection, goggles, gloves and a lab coat.
- When working with concentrated solutions ($> 1 \text{ mM}$) as well as freeze-dried conopeptide, decontaminate your work areas and apparatus with a solution of 6% sodium hypochlorite. This solution is stored together with the general spill kit in room 7.09.142A.
- Before discharging conotoxins, treat the conotoxin with 6% sodium hypochlorite for 20-30 min. For larger amounts ($> 100 \text{ nmol}$), additionally treat conotoxin with a solution of 50 mM DTT for 1 h at 37°C prior to hypochlorite treatment.
- New personnel (e.g. new students and guest researchers) must receive thorough instruction in the work with conotoxins from an authorized biosecurity-educated person (at the moment this is Dr. Helena Safavi and Dr. Walden Bjørn-Yoshimoto).

To decontaminate a larger spill, follow this procedure:

1. Put on gloves, lab coat, and eye and face protection (face shield or mask and safety glasses)
2. Prepare a solution of 6% sodium hypochlorite.
3. Clean up any broken glass with a mechanical device such as forceps, and place the glass in a yellow sharps GMO container.
4. Place damaged tubes of toxin in a solid chemical waste container.
5. Pour 6% sodium hypochlorite in the center conotoxin spill. Allow 20-30 min for the bleach to interact with the spilled conotoxin.
6. Place paper towels on top of your spill working from outside in.
7. Scoop up the paper towels working from outside in, into a disposable bag and place it in a solid chemical waste container.
8. Dispose of all personal protective equipment, including lab coat, in the disposable bag before closing it. Then place the bag in the solid chemical waste container, and close the lid.

In case of any accident always report details to Dr. Helena Safavi (+45 6143 6096) and work environment responsible Paul Meyer Mai (+45 2875 6005) - and seek medical assistance if there was a risk of exposure!