41. Conotoxins

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41.1 Introduction

Cone snails are a group of carnivorous marine gastropods, having perfected venom needed for rapid prey immobilization. This neurotoxic cocktail is skillfully delivered using a hollow, lone, disposable radula harpoon, which is also designed to impale and tether its prey (Figure 41.1A). Armed with this combination, certain cone snail species have been responsible for human fatalities [1-3]. To understand their toxic nature, science has taken these highly prized snails, to discover a wealth of biological materials that separate them from most other venomous organisms.

(Insert Figure 41.1 here)

The genus of ~600 species, carrying >100,000 peptides (termed 'conotoxins' or 'conopeptides'), provides clear indication to immense chemical evolution and diversity. What variation these snails have achieved potentially parallels the diversity of bioactive constituents observed in the microbial world. This evaluation has been recently reiterated examining milked venoms of the piscivorous/fish-eating members of *Conus* [4-7]. Coinciding with their characterization [8-10], synthesis [11-13] and bioengineering [14-16], these peptides have operated as pharmacological probes to dissect ion channel and receptor functions since the 1970's [17-19] and have now transitioned into analgesic pharmaceuticals [20,21].

On a regulatory basis conotoxins/conopeptides represent a grey area in their formal classification listing. Exemption status as 'Select Agents' has been provided by U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) and Centers for Disease Control and Prevention (CDC; see Section 41.8), but they remain as security sensitive materials, with specific biosafety concerns. However, combining the many thousand conotoxins/conopeptides into one general regulatory classification brings about a complex

situation, as the various described peptide families demonstrate diversity in their pharmacology, offering differential phyla-selectivity in higher organisms (Section 41.2). Synthetic techniques are used in the production of *Conus* peptides to compensate for their limited natural availability [22-24]. To a lesser extent recombinant protein expression technology may be used [25-26]; this is limited due to the abundance of post-translational modifications (PTMs). Some of these modifications are required for biological function (Section 41.6). Such synthetic or biotechnological approaches potentially present their own unique regulatory concerns.

Only a few laboratories in the world have access to these native venom extracts; thus far from those efforts <2% of materials have been described. Yet of the described compounds, some have demonstrated unparalleled scientific and medical worth [27,28]. As correctly gauged by the scientific community and federal regulatory agencies, placing restrictive access and practices on these 'agents' may actually hinder potential drug development for major human afflictions such as pain, as seen with the analgesic properties of ω-conotoxin MVIIA/Prialt® [29-31]. Thus, establishing a basic understanding of the biology of these venomous organisms (Section 41.2), the chemical and pharmacological diversity of their toxins (Section 41.5), as too the practices employed in safely procuring materials and handling (native and synthetic materials) within the confidences of a research laboratory environment becomes essential. To achieve this, we have provided Standard Operating Procedures (SOPs; Section 41.9), detailed First Aid protocols (Textbox 41.1), and Notes to Physicians (Textbox 41.2) to ensure a centralized document to assist forming individual requirements and protocols while dealing with conotoxins and conopeptides as security sensitive agents.

(Insert Textbox 41.1 and Textbox 41.2 here)

41.2 The Biology of *Conus*

The genus *Conus* owes much of its 60 million years of evolutionary success as marine predators to its distinct venom apparatus. The main structure, a highly convoluted, longitudinal secretory duct, can range from <2 to 80 cm in length [32]. Crude venom extracts can yield a few micrograms to 10's of grams of venom, depending on the species. Histological examination of the venom duct epithelial cells provides clear evidence of their secretory nature [1,33,34]. Different sections of the secretory venom duct possess varying degrees of toxicity, yet these observations remain unexplained [35,36].

The radula harpoon plays a significant role in the process of prey capture and venom delivery, and this tooth completes the terminal interaction between predator and prey [1,37]. The hollow, chitinous harpoon is a single projectile that is morphologically particular to each *Conus* species [38-40] (Figure 41.1A). Structural features can be clearly correlated to specific feeding types observed in *Conus* [41,42].

Cone snails are either: (a) piscivorous/ fish-eaters, (b) molluscivorous/ mollusk-eaters, (c) vermivorous/ worm-eaters, or (d) omnivorous. Although representing a small number of species, the piscivorous cone snails' venoms are considered life threatening to humans, while molluscivorous feeders are considered dangerous, via their aggressive nature – unconfirmed fatalities have been attributed to some members (see below). The vermivorous class represents the largest group within the genus; most are considered timid and non-threatening, unlike their previous counterparts. Lastly, omnivorous species show no preferential feeding behavior. These four feeding classes have a few 'general' morphological distinctions that include aperture size, weight, and general pigmentation patterns.

Vermivore shells are generally heavy in comparison to other classes of feeders and possess a narrow parallel aperture. Molluscivores typically tend to have multiple white-tented marks on a brown background medium-weighted shell, with a graduated aperture. While piscivore shells are ornate, typically light weight in comparison, and have the widest apertures to accommodate vertebrate prey (Figure 41.1A). Despite these delineations, snail classification is an onerous undertaking due to the morphological differences between the species.

Cone snails are nocturnal but are mainly active around sunset and sunrise. Vermivores impale polychaetes/worms with the radula affixed to the extended, freely mobile proboscis, as prey is drawn into the rostrum [43]. In a second strategy, the foot locates and orientates the prey for consumption. The foot moves the worm to the rostrum and using a ‘spaghetti-sucking’ approach, with or without active envenomation, engulf the prey [44].

Molluscivores are aggressive hunters, feeding on mitres, trochus, cowries, including other cone snails [40,45-47]. Stimulated snails use their proboscis to probe for the prey’s shell and orifice. Once the prey is found, it is impaled with multiple radulae. At times, a 'puff' of surplus venom is observed, and this action may be repeated during subduing strikes [45]. Once paralyzed, the cone snail’s rostrum enters the prey’s shell cavity so partial digestion and extrapolation can occur. Indigestible material and used radulae are regurgitated after digestion [40,48].

Piscivores also use two feeding approaches [4,40,43]. The first is via 'tag and reel' harpooning, in which the extended proboscis and internalized radula impale, inject, and draw the paralyzed fish into the fully expanded rostrum [49,50] (see Figure 41.1B). After digestion, a mass of fish scales, bones and used radula(e) are regurgitated [51,52]. *Conus geographus* and

Conus tulipa have exhibited an alternative 'passive' strategy. The unsuspecting fish is ‘netted’ by the extended rostrum, and envenomation occurs after engulfment [40,51,53], but variations of this behavior have been observed [54].

The milked venom, resulting from piscivore envenomation, possesses a pharmacological cocktail to block pre- and post- synaptic ion channels at neuromuscular junction. Injected venom containing various α- and ω-conotoxins (i.e. by *C. geographus*) [54] provides dual assurance in accomplishing rapid prey immobilization. Terminating action potential propagation is achieved by simultaneously inhibiting the pre-synaptic Ca^{2+} influx through voltage gated calcium channels (VGCCs; ω-conotoxins) and post-synaptic muscle type acetylcholine receptors (AChR; α-conotoxins). Other synergistic strategies exist, in which *Conus* peptides combine targeting Sodium (Na_V) and Potassium (K_V) voltage gated channels to induce an excitotoxic state in prey [55-57]. These combined mechanisms intensify the venom’s effectiveness in rapid ‘lightning-strike cabal’ immobilization; Na_V channels are opened simultaneously (via μ-conotoxins) alongside blockage of K_V channels (via κ-conotoxins) [58]. Further discussion on conopeptide pharmacology is presented in Section 41.5.

41.3 The venom of *Conus*

Venom Appearance. Dissected venom gland extracts can be opaque, milky white to sulfur yellow in color. The milked venom is clear, unless hydrophobic peptides, such as the δ-conotoxins, are present. Milked venom consists to a lesser extent of proteins and low molecular mass organic compounds, with peptides being the dominant constituent; the majority of the milked venom volume is equivalent to seawater. Freeze-dried or lyophilized materials, both native (desalted) and synthetic, can be fluffy/velutinous, electrostatic and hydroscopic in nature.

Venom Solubility. Synthetic and extracted conotoxins/conopeptides are soluble in water, producing a slightly translucent solution that may foam if agitated. Native venoms contain small insoluble particles or granules [59-61], these being heavily pronounced in dissected whole duct venom extracts, along with other cellular debris. Whole venom extracts require centrifugation and secondary extraction. This process results in a translucent peptide containing supernatant. To achieve maximal solubility, small amounts of immiscible organic solvent, such as acetonitrile (5% v/v), are added to aqueous solvents; sonication may assist in dissolving peptidic materials.

Venom Stability. When stored correctly under laboratory conditions, conotoxins/conopeptides are highly stable identities. The analysis of dissected *C. geographus* duct venom obtained from the late Dr. Robert Endean (University of Queensland, Australia), these being collected, extracted and freeze dried in 1962, demonstrated to contain many recognizable peptide molecular masses that correlated to known conotoxins/conopeptides [62]. Due to disulfide bonding, a high level of intrinsic structural stability is present within the conotoxins. Yet, as peptides, they are not resistant to enzymatic digestions, microbial breakdown, disulfide reduction and/or chemical oxidation. Any chemical modification to the native peptide(s) will typically lead to a decrease or removal of biological activity. Although heating leads to their degradation, prolonged exposure to heat, such as autoclaving alone, is regarded as an ineffective measure to completely remove biological functionality. An effective combined laboratory approach for peptide neutralization is discussed in Section 41.11.

41.4 Epidemiology of *Conus*

Cone snail related deaths have been recorded dating back to 1705; first from a molluscivore, *Conus textile* [63]. Since then there have been around 30 recorded fatalities,

principally, by one of the most studies species, *C. geographus* [34,40,64]. In humans, the fatality rate from this species alone is approximately 70% [3].

Shell collectors have the highest risk of cone snail envenomation, whether being a tourist or hobby malacologist. Increased exposure and handling increases the likelihood of envenomation (see Textbox 41.1). This includes researchers that handle cone snails in the same category. Collecting live cone snails is dangerous! Specimen handling, containment and separation in the field requires due diligence, behavioral knowledge and positive identification of species. Removal of cone snails from their natural environment induces a defensive response. When stressed, they can eject 5-30 times more venom than during a predatory response, a contribution to their lethal nature. Radula harpoons are fired at a velocity of 200 m/sec [64,65] and can penetrate 5 mm neoprene wet suite material [66]. The proboscis has been observed to extend 2-3 shell lengths from the snail, probing for the source of antagonism; careful handling is essential.

Moreover, even cone snails have become 'taboo'; certain cultures of the South Pacific Islands are long accustomed and wary of the dangers from cone snail envenomation. If an islander has been stung they resort to 'on-the-spot' bloodletting, inflicting numerous deep lacerations on the afflicted limb [67]. Although not recommended, such actions would likely decrease circulatory distribution. Table 41.1 lists reported symptoms observed after a cone snail envenomation, representing details from numerous species [68-71]. Also compiled are First Aid measures that should be immediately implemented. These expectations too have equal relevance in the laboratory setting, particularly when involving injectable materials. As there is misinformation on how to handle cone snail envenomation, a list of 'interventions to avoid' is also presented; these having no effect in lessening the affect or venom distribution and may

cause more harm. An advance note to physicians is also provided (Textbox 41.2), as a reference if such a case is encountered.

(Insert Table 41.1 here)

41.5 Conotoxin and Conopeptide Classification

Bioactive constituents within *Conus* venom demonstrate inter- and intra-species diversity [72]. An individual duct venom extract can contain ~200 different pharmacological components [18,73,74], while only ~20% of these elements appear in milked venom. The milked venom may also demonstrate differential peptide toxin expression leading to intra-specimen variation [57,75]. This unique ability, to vary venom constituents, potentially makes production of an effective antivenom/antivenin impossible.

There are ~5500 individual sequences derived from *Conus*, with ~80% originating from genomic origin [76-78]. A majority of these peptides have been organized into seven main superfamilies (A-, M-, O-, P-, S- T- and I-Superfamilies; Table 41.2). These groupings are based on a combination of their pharmacological selectivity, their cysteine frameworks, together with pre-propeptide genetic sequence homology. The naming convention of *Conus* first assigns a Greek letter to indicate the pharmacological targeting; the main families of interest are the α-, ω-, μ-, δ-, κ-'conotoxins' (see below), while other bioactive peptides such as conantokins, contulakins, contryphans, and others *Conu*s peptides are assigned as 'conopeptides'. Distinguishing between conotoxin/conopeptide sequences, one or two letters are used to indicate the source species, which the first (capitalized) and second letter (typically a sequential non-vowel character) derived from the species name i.e. Tx = *Conus* ***t****e****x****tile*. Use of a single letter assignment is typically reserved for piscivorous species i.e. M = *Conus* ***m****agus*.

(Insert Table 41.2 here)

Conus nomenclature is partly influenced by disulfide bridges, these are essential in forming and maintaining the necessary three-dimensional structures that convey biological activity. Assigning of a Roman numeral indicates the disulfide framework category, as illustrated in Table 41.2. Lastly, a sequential upper case letter is used to signify the order of discovery. Usually the initially described peptide, within the individual species, often lacks this designation. A discussion outlining the major described superfamilies is provided below:

41.5.1 A-Superfamily

The A-superfamily is separated into four toxin families, α-, αA-, κA- and ρ-conotoxins, these consisting of ~200 individual sequences (excluding the precursor sequences). The α- and αA-conotoxins are nicotinic acetylcholine receptor (nAChR) antagonists, ρ-conotoxins have structurally similar cysteine arrangement to the α-conotoxins but are $_{1B}$-adrenoceptor antagonists [Table 41.2], while κA-conotoxins target potassium (K^+) ion channels and are typically glycosylated and larger in size [79,80]. The classical α-conotoxins contain ~40 individually isolated venom peptides and have been discovered throughout the genus. They range from 12 to 22 amino acids (αα) and contain four cysteine moieties that form two disulfide bonds. Postsynaptic inhibition at the neuromuscular junction by α-conotoxins results in paralysis and death from respiratory failure [3,68,70]. Other α-conotoxins have the ability to distinguish between different AChR subtypes, as observed with α-conotoxin ImI (Table 41.2) that targets neuronal isoforms.

41.5.2 M-Superfamily

The M-Superfamily is comprised of three families, ψ-, μ- and κM-conotoxins [81-83]. About 300 peptide sequences (excluding the precursor sequences) from *Conus* fall into this class, a large majority being described from genetic sources. These peptides structurally contain 14-28 αα residues, six cysteines - forming three disulfide bonds in a CC-C-C-CC framework. Some members contain high concentrations of *4-trans*-hydroxyproline, a PTM αα (Section 41.6.4). ψ-Conotoxins target nAChRs, non-competitively, while the μ-conotoxins block Na_V channels in excitable cells of muscle, heart, skeletal and nerve tissue [81]. Na_V channels modulate rapid electrical signaling in both neuronal and muscle cell types [84]. μ-Conotoxins GIIIA, GIIIB, and GIIIC are examples of conotoxins from *C. geographus* that act upon Na_V channels in muscle [85,86; Table 41.2]. These peptides work in unison with other milked venom constituents to induce rapid paralysis in fish [54]. At a molecular level, they selectively bind to the ion channel pore, blocking the electrochemical flux of Na^+ ions into the cell. This feature makes them extremely useful for electrophysiological research.

41.5.3 O-Superfamily

The O-Superfamily is the largest group, with ~500 peptide sequences (excluding precursor sequences), in five distinct families, μO-, δ-, ω-, κ-, and γ-conotoxins [87-89]. Structural similarities exist between pharmacologically unrelated conotoxins (Table 41.2). This superfamily is primarily dictated by a highly conserved disulfide framework of C-C-CC-C-C or commonly referred as a '6/4-Cys loop' pattern, which is observed in the mature peptide sequence [90]. The inter-relation of this superfamily is then compounded by peptide precursor sequence homology [91]. Pharmacologically, μO-, and δ-conotoxin families target Na_V channels but will not compete with μ-conotoxins for the pore binding site [92,93]. While ω-, κ- and γ-conotoxins

target VGCCs, K^+ channels, and Pacemaker channels, respectively [94]. The δ-conotoxins typically consists of about 30 ααs, retaining three disulfide bridges and demonstrates a characteristic hydrophobic nature. The ω-conotoxins following these same biochemical properties, but they lack the hydrophobicity. Known as the 'shaker peptides' these induce persistent tremors when injected into mice and have drawn much pharmacological interest. ω-Conotoxin MVIIA, a N-type selective VGCC isolated from *C. magus,* was the first conotoxin to be clinically approved for the treatment of chronic intractable pain, mostly targeting patients who have high tolerances to opioids [95]. Other ω-conotoxins are under investigation as therapeutic leads.

41.5.4 P-Superfamily

The P-Superfamily, which consists of nine peptide sequences, has been granted the family name, 'spastics,' because their effects on their molecular target manifest a spastic paralysis in the test organism [96]. Presently, these peptides have only been isolated from molluscivorous and vermivorous cone snails, with the majority being derived from genetic sequences. An example of a spastic-conotoxin is Tx9a, a PTM peptide found in the duct venom of molluscivorous *C. textile* [97] (Table 41.2). Pharmacological targeting of this unique peptide family remains to be established.

41.5.5 S-Superfamily

The S-superfamily targets muscle type nAChR or competitively antagonizes the serotonin $5HT_3$ receptor and consists of seven peptides (excluding the precursor sequences). Isolated from all feeding types, these sequences are unique, both in size (<40 αα) and disulfide arrangement; these having the highest content observed in any conotoxin/conopeptide with the formation of

five individual disulfide bonds [98]. Those isolated from the duct venom of piscivores demonstrate extensive PTM, including bromotryptophan, as seen in σ-conotoxin GVIIIA from *C. geographus* (Table 41.2).

41.5.6 T-Superfamily

The T-Superfamily includes both τ- and χ-conotoxin families and consists of ~110 peptide sequences (excluding the precursor sequences); with most (<95%) being derived from genetic sources representing all feeding groups within the genus. The majority of these peptides have four cysteines in a -CC--CC- framework producing two disulfide bonds [99]. τ-Conotoxins function as pre-synaptic Ca^{2+} channel blockers [101], while χ-conotoxins inhibit norepinephrine transporters (NETs). χ-Conotoxins are receiving much interest due to their pharmacological transition into clinical drug trials to combat pain [102].

41.5.7 I-Superfamily

A large number I-superfamily members have been disclosed, ~88 individual sequences (excluding precursor sequences), most representing genetic derived sequences from vermivorous cone snails. Most of these sequences remain pharmacologically undefined. These peptides possess eight cysteine moieties, with a pattern of -C-C-CC-CC-C-C-, and from those few isolated native peptides demonstrate to contain multiple PTM ααs. Examples include κ-conotoxin BtX (BeTx), isolated from *Conus betulinus*. Pharmacologically, BtX demonstrated to target the calcium activated potassium channel [103], without affecting other voltage-gated channels [104]. The pharmacological selectivity and differentiation within the I-superfamily towards K_V channels is illustrated with conotoxin ViTx (*Conus virgo*), which demonstrated specific selectivity inhibiting the K^+ ion channel subtypes $K_V1.1$ and $K_V1.3$ subtypes, but not

$K_V1.2$ [105]. However, the role of PTMs in this class is basically unknown.

41.5.8 Single disulfide bonding and linear conopeptides

A number of additional classes of conopeptides are gaining momentum together. Most are not classified within the conotoxin superfamily structure, presently. These contain either a single disulfide bridge, or as with the linear conopeptides, possess no cysteine moieties at all. Conopressins and contryphans contain one disulfide bond, which are vasopressin receptor antagonists and target unknown receptor types, respectively [106,107]. Being absent of disulfide bonds, the Conantokins are structurally stabilized by the chelating ability γ-carboxy glutamate. These peptides selectively target the *N*-Methyl-D-Aspartate subtype of glutamate receptors, ligand-gated Ca^{2+} channels, involved in seizures of intractable epilepsy [108]. The Contulakins target the neurotensin (NT) receptors antagonistically [109]. It is a given that the pharmacological repertoire of ion channel and receptor targeting capabilities within *Conus* is not fully complete. Proposed extensions to these superfamilies is now being undertaken to accommodate the discovery of new conotoxins and conopeptides.

41.6 Post Translation Modification in Conotoxins and Conopeptides

Native post-translational modifications (PTMs) in conotoxins and conopeptides play an important biochemical and pharmacological role, as seen with their incorporation and dominance throughout the peptide families [110]. Many PTMs have multiple implications in peptide folding and structural stabilization [111], providing resistance to degradation [112] and importantly increasing pharmacological potency [113]. These will be briefly discussed below, but do not represent all PTMs observed in *Conus.*

41.6.1 Disulfide bond formation

Biological activity is reflective on native three-dimensional structure. In conotoxins, and some conopeptides, this is conveyed by specific, directed formation of disulfide bonds between individual cysteine moieties. Each class of native peptides has a specific connectivity pattern. Modification of the resulting structure by changing their connectivity can lead, in most, to biological inactivity [114]. This PTM represents the most commonly observed in *Conus*.

41.6.2 *C*-terminal amidation

C-terminal amidation is common to most conotoxins. Its presence can be genetically predicted, with a level of certainty, by establishing the rear flanking sequence region of the predicted mature peptide toxin [115]. *C*-terminal amidation has a role in the disulfide coupled folding of conotoxins, which can structurally influence the biological activity of the peptide [116], but also provides a level of enzymatic resistance to carboxypeptidase degradation. Its removal from native-like peptides has demonstrated minor decreases in potency [117]. This represents the second most common PTM seen in *Conus*.

41.6.3 *N*-terminal cyclization

A few conotoxins contain an *N*-terminal glutamic acid; these residues can undergo *N*-terminal cyclization via Glutaminyl cyclase to form pyroglutamic acid [79]. This results in changing the *N*-terminal charge state that can effect target affinity as well as provide resistance to endo-peptidase degradation. This alteration represents one of the least observed αα modifications in *Conus*.

41.6.4 Hydroxylation of proline

Hydroxylation of proline to *4-trans*-hydroxyproline is a common PTM undertaken by proline hydroxylase. Observed in a various conotoxins and conopeptide classes, it has been implicated in assisting in three-dimensional folding [118]. For the μ-conotoxins, incorporating multiple moieties is important for interacting with the Na_V channel. This represents the third most commonly observed PTM in *Conus*.

41.6.5 Carboxylation of glutamic acid

About 10% of conotoxins/conopeptides contain the vitamin K-dependent carboxylation of glutamic acid, which results in γ-carboxyglutamic acid. Structurally it promotes the formation of helices and Ca^{2+} ion binding [111]. γ-Carboxylation is predominant in the conantokins (Table 41.2). Its removal or exclusion often causes inactivity [111]. It is also seen as a mechanism to increase the chemical/charge state diversity within conotoxins and conopeptides. The presence of γ-carboxyglutamic acid represents the forth most common PTM seen in *Conus*.

41.6.6 Isomerization of amino acids

L-amino acids are the biologically active and prevalent chemical precursors of proteins, but some ααs undergo epimerization. Although uncommon, a number of D-amino acids have been observed in *Conus*, most being restricted to D-tryptophan, phenylalanine, valine, and leucine [119]. Epimerase enzymes cause their occurrence, and the biological value of such modifications in these peptides remains elusive.

41.6.7 Bromination of tryptophan

Halogenation of amino acids in *Conus* is presently restricted to the bromination of L-tryptophan [120]. Produced by bromo-peroxidase, forming the analog 6-L-bromotryptophan, this

unique PTM is present in the I- and S-superfamilies. There is no specific functionality assigned to peptides that contain 6-L-bromotryptophan. This represents one of the more unusually PTMs observed in *Conus*.

41.6.8 *O*-glycosylation

Glycosyltransferase enzymes post-translationally modify serine and threonine residues by *O*-glycosylation. These modifications can have different sugar interlinkages within different conformations. Contalukin-G contains one of the most common glycan linkages, β-D-Gal(1→3)α-D-GalNAc, which is found attached to threonine. Glycosylation can increase the efficacy of certain conotoxins. Without the glycosylation, the bioactivity is lower or abolished; this is evident with the κ-conotoxins and their actions upon K_V channels [79]. Glycosylation is expected to have a higher occurrence in conotoxins than presently discussed in current literature. Glycosylation events can be found predominantly in the milked venoms of *Conus* (Bingham unpublished results).

41.6.9 Sulfation of tyrosine

Tyrosyl sulfotransferase results in the sulfation of the hydroxyl group of tyrosine, producing sulfotyrosine. Identified originally in the α-conotoxin EpI, its non-PTM form, EpI[Tyr]^{15}, demonstrated to have similar potency as a competitive nicotinic antagonist on bovine adrenal chromaffin cells [121]. Thus, the biological consequences of this PTM remain undetermined. Although an uncommon PTM found it *Conus*, it reveals a different chemical feature from its hydroxylated counter-part.

41.7 Bioengineered Conotoxins

Conotoxins/conopeptides have been subjected to extensive chemical manipulation and bioengineering. These endeavors outweigh the combined efforts undertaken with other peptide toxins derived from scorpion, spider, ant and bee venoms. The resulting bioactive, non-native conotoxin/conopeptide hybrids and peptidomimetic analogues have advanced our present understanding of structural biology [122] and drug design [123]. Development of the 'cono-polytides' hybrids represents a significant step forward in conotoxin/conopeptide bioengineering [124]. This approach incorporates replacements of the peptide backbone and side functionalities to streamline molecular size and make 'redundant' or minimize 'non-essential' interactions that may deter pharmacological selectivity or directed targeting [124,125].

Other mechanisms in conotoxin/conopeptide bioengineering revolve around the all-essential disulfide bridges, replacing them with organic non-reducing structures. These include cytsathionine thioether and dicarba linkages, as seen applied to α-conotoxin ImI [126,127]. Here mimicking structural constraints has pharmaceutical benefit, providing potentially great stability *in vivo*. This is also observed with the more recent approach in undertaking peptide backbone cyclization of the *N*- to *C*- termini by incorporating both a space linker with Native Chemical Ligation for the desired cyclic backbone structure. The bioengineering approaches as applied to Vc1.1 and α-conotoxins AuIB [128] RgIA [129] and MII [130] increase oral bioavailability and systemic stability. This offers advancement in seeing conotoxins/conopeptide leads increasing their therapeutic potential.

Synthetic bioengineered conotoxins/conopeptides potentially represent a perplexing situation in 'Select Agent' regulation. Here, more resilient procedures may be required in neutralization and decontamination (see Section 41.9 – Standard Operating Procedures).

Granted, the complications regarding the bioengineering of orally active ‘full’ or ‘partial’ peptidometics, represent unique circumstances that need to be evaluated to ensure personal and work place safety. These measures may include: (i) handling procedures; (ii) decontamination and neutralization procedures; (iii) animal disposal procedures; (iv) security measures; (v) possible re-classification and regulatory delineation between bioengineered and native materials. Present recommended Standard Operating Procedures for conotoxins/conopeptides are detailed below.

41.8 Select Agent Classification of Conotoxins and Conopeptides

Preamble. The words conotoxin and conopeptide are typically used interchangeably, however the term ‘conotoxin’ is usually reserved for their pharmacological-hierarchical classification (see Section 41.5). These peptides vary in molecular mass, solubility, phylogenetic (dependent) bioactivity and toxicity. General safety precautions in handling and storing these materials must be undertaken in the laboratory, as with any potentially toxic venom constituent(s). Here we describe the United States National Select Agent Registry’s[1] regulatory classification regarding conotoxins/conopeptides and provide recommended Standard Operating Procedures (SOPs) for their laboratory use.

41.8.1 Select Agents exclusion - Effect 4-29-2003

For research purposes, the United States Departments of Health and Human Services (HHS) and Agriculture (USDA) excludes specifically:

[1]Maintained jointly by the Animal and Plant Health Inspection Service (APHIS) and the Centers for Disease Control and Prevention (CDC) Select Agent Programs

> "the class of sodium channel antagonist μ-conotoxins, including GIIIA; the class of calcium channel antagonist ω-conotoxins, including GVIA, GVIB, MVIIA, MVIIC, and their analogs or synthetic derivatives; the class of NMDA-antagonist conantokins, including con-G, con-R, con-T and their analogs or synthetic derivatives; and the putative neurotensin agonist, contulakin-G and its synthetic derivatives"…. as select agents[2].

41.8.2 Permissible amounts

Also, excluded by the National Select Agent Registry, are permissible amounts <100 mg of any conotoxin/conopeptide under the control of a principal investigator[3] (PI). These present regulatory exclusions that meet normal research demands are founded on current experimental evidence and scientific literature, which indicates that these conotoxins/conopeptides do not possess sufficient or acute toxicity to pose a significant threat to public health or safety.

41.8.3 'Nonfunctional' conotoxins and conopeptides

'Nonfunctional' conotoxins/conopeptides are also excluded from these regulations[4] – this includes synthetic peptides in either their on- or off-resin state, which are not folded/oxidized and remain in an inactive/'non-functional' state.

41.9 Standard Operating Procedures for Laboratory Use of Conotoxins and Conopeptides

41.9.1 Purpose

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[2] http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20Exclusions.html
[3] http://www.selectagents.gov/Permissible%20Toxin%20Amounts.html
[4] http://www.selectagents.gov/Guidance_for_the_Inactivation_of_Select_Agents_and_Toxins.html

This Standard Operating Procedure (SOP) describes the techniques and procedures for safe and proper handling, storage and preparation for experimental use, and disposal of conotoxins/conopeptides as ‘select agents’.

Note: Not all conotoxins/conopeptides demonstrate toxicity, but the user must regard all materials, independent of source or species, as having the ability to be toxic.

41.9.2 Minimum Personal Protective Equipment (PPE)

At minimum, personal protective equipment for handling of conopeptides should include the following:

(i) Eye protection: Safety glasses or chemical-splash goggles must be worn at all times when handling conotoxins/conopeptides. Adequate safety glasses must meet the requirements of the “Practice of Occupational and Educational Eye and Face Protection” (ANSI Z.87.1 2003).

(ii) Face shield: An optional face shield may be worn in addition to safety glasses or chemical-splash goggles if the potential for splashing exists.

(iii) Gloves: (a) Appropriate gloves shall be worn when handling solutions contain conotoxins/conopeptides; (b) Due to strength and durability, nitrile gloves are recommended when handling conotoxins/conopeptides. (c) Gloves that protect against the generation of static charges are also preferred, especially when handling dry or powdered forms of conotoxins/conopeptides. This is important when weighing out materials as static charge may cause conotoxins/conopeptides to become motile and airborne.

(iv) Respirator: During the handling of bioengineered, aerosol-derived conopeptides, a respirator should be utilized (e.g. half-mask respirator equipped with HEPA cartridges[5]). A respirator should be used during events of spill-cleanup or decontamination. Potential users should have obtained prior medical evaluation, training and fit testing before the usage of a respirator.

(v) Protective clothing: A traditional cotton-polyester lab coat may not provide adequate protection against chemicals. Wear impervious protective wear such as a polyvinylchloride (PVC) or polyethylene (PE) apron, lab coat, or at minimum a smock with elastic cuffs and high neck collar. This may be required in possible events of decontamination.

41.9.3 Hazardous Warning Signs

Any room associated with the usage of conotoxins/conopeptides should be posted with a sign "Caution! Toxins in Use" (User Name/Date). Visitors to the laboratory should be notified to take precaution by the researchers present.

41.9.4 Delivery of Conotoxins and Conopeptides

(i) When a shipment of conotoxins/conopeptides is received, the specification sheet is dated and goes in the Select Agents Notebook. Log in the agent's name and quantity shipped on the select agent inventory sheet. Notify the sender within 24 hours of receipt of the toxin.

(ii) Inspect external package for damage during shipment. If damaged, immediately contain and isolate, notify Principle Scientist/PI, Environmental Health and Safety (EH&S), and sender; undertake decontamination and disposal of the material upon instruction.

[5]High-Efficiency Particulate Air

(iii) All primary vials containing select agents should be handled in a glove box or Class III biosafety cabinet – when practical.

(iv) For storage, the primary vial is placed in a secondary sealed container, upright, within a lockable freezer. The primary vial remains factory sealed until 'Working Solution' stocks are required.

41.9.5 Handling Procedures for Conotoxins and Conopeptides

(i) A 'buddy-system' should be practiced during any high-risk operations. Each must be familiar with the applicable procedures, maintain visual contact with the other and be ready to assist in the event of an accident.

(ii) If the conopeptide is received as a powder, place a pad on the inside of a glove box or Class III biosafety cabinet in order to minimize the spread of contamination during the solvation process. Ensure the ducted biosafety cabinet is working properly with an inward airflow prior to initiating work. All work should be performed within the operationally effective zone of the biosafety cabinet.

(iii) To avoid static charge buildup on glass or plastic-ware, use static discharger i.e. Zerostat® 3 Antistatic Gun; use caution to avoid flammable or combustible solvents/materials during its use.

(iv) Avoid unnecessary handling or/and transfer of materials by making a stock solution using manufacturers vial and weight specifications; adjust volume addition to achieve desired stock concentration. Carefully suspend the select agent by slow titration, preventing possible foaming or aerosolization. Buffer such as 100 mM NaCl, pH 7.0 (±100 mM Glycine) is

recommended; addition of 1 mg/mL BSA can be used to minimize non-specific binding, if this is suspected.

(v) Aliquot stock solution into separate labeled vials (agent name; concentration; buffer type; date) for storage and later concentration re-adjustments to obtain desired experimental concentration ranges – this being referred to as ‘Working Solution’. Dispose of used pipette tips into a beaker containing a 10% v/v bleach or alternatively 1% v/v solution of glutaraldehyde.

(vi) Decontaminate/neutralize the exterior surfaces of all materials leaving the glove box and biosafety cabinet, including the closed aliquot vial(s), with a 10% v/v bleach solution. Decontaminate all work surfaces with a 10% v/v bleach solution. Until the glove box or ducted biosafety cabinet is decontaminated, the equipment should be posted to indicate that select agents are in use, and access to the equipment and apparatus is restricted to authorized personnel.

(vii) Select agents should be transported in a leak-proof closed secondary container, kept upright, and placed into a lockable freezer, best kept at -80°C for longer-term storage, while -20°C is good for short term (1-6 weeks).

(viii) If primary stock material is in a powder form and requires weighting, transfer material from primary stock vial into pre-weighted capped vial, operating within the inside of a glove box or Class III biosafety cabinet. Wipe outside of closed vial with 10% v/v bleach solution and air dry. Reweigh closed vial and calculate the required buffer addition to achieve working stock volume, and then continue as from (iii) above, with modification. This becomes a ‘Working Solution’.

41.9.6 ‘Working Solutions’ Storage

While not in use, keep select agents within a lockable storage freezer. Make sure to secure any vial with parafilm in order to prevent any possible spill or leakage. Ensure permanent labeling – with date/amount/concentration etc. An inventory control system should be in place – detailing inventory, stocks and physical state (i.e. in-solution vs. lyophilized form) and dates received/used/accessed.

41.9.7 'Working Solutions' Usage

Remove required 'Working Solutions' adjusting inventory lists when required. Thaw the vial at 4°C in lockable refrigerator and once in liquid form, mix and centrifuge before opening. Open the vial with caution as material may have pressurized. It is advisable to plan the use of 'Working Solutions' with care, avoiding prolonged and repeated vial openings, minimizing vial handling and movement. Also of consideration is stability of materials with repeated usage. Avoid repeated freeze-thawing if possible. However, some conotoxins/conopeptides have demonstrated extreme stability if handled and stored correctly. Yet, more conservative considerations are required when using native crude venoms of unknown composition, as degradation does occur, which is seen as a decrease in biological activity or chromatographic component abundance.

41.10 Animal Handling

Note - Practice Standard: Sharps must not be used with conotoxins/conopeptides unless specifically approved by Environmental Health and Safety (EH&S) and Principle Scientist/PI. If it is absolutely necessary to use sharps with conotoxins/conopeptides, sharps with engineering controls must be used (see below).

41.10.1 Use of Syringes and Needles

Always wear gloves prior to usage of needles or syringes. Locate a sharps container and bring it to the area of usage. Do not attempt to recap the syringe after usage, instead dispose of it immediately, sharp end first, into the container. Remain stationary; do not walk with any needles. Keep sharp ends away from you at all times.

41.10.2 Pre injection

Any animal used as a candidate for conotoxin/conopeptide analysis should be properly obtained and humanely taken care of, complying with approved and current governing animal protocols. It is important to ensure careful experimental planning and organization to decrease stressors that may affect the health of the organism, and thus observations and data. Follow guidelines provided in regards to protective wear and safety. Extra precautions should always be taken during the handling of needles and syringes. Engineered controls include a Luer lock with a simple tubular needle guard that can assist leak avoidance and in reproducible needle depth penetration.

41.10.3 Injection

If hypodermic needles are used, the injection site should be targeted to an area of high vascular activity. This will ensure maximum efficiency of the toxin, and prevent unnecessary pain directed towards the organism. It is recommended to obtain help from a second lab-mate during this procedure. While one person handles the organism, the other records observations and data. Use of a basic platform to immobilize animals is recommended; with use of fish ensure dose is administered in a quick fashion, returning the animal to its observation habitat.

41.10.4 Post injection

The use of conotoxins/conopeptides may cause paralysis, which might induce the false appearance of death. Obtain knowledge about each organism used, in regards to the most pain-free and efficient way to euthanize, and make sure that each organism is deceased prior to processing/disposal. All small animals used are considered pathological waste and must be incinerated.

41.11 Waste Disposal and Decontamination/neutralization of Conotoxins and Conopeptides

All biological liquid waste must be decontaminated/neutralized using a 10% v/v sodium hypochlorite (Clorox®) or solution of sodium hypochlorite and sodium hydroxide (10% w/v) for a 30-minute contact time and may be drain disposed with plenty of water. Alternatively, use of reactive disinfectants such as glutaraldehyde and formaldehyde (1% v/v solution) for 30-minute contact time; however resulting material requires biohazards waste disposal.

The majority of native conotoxins/conopeptides are susceptible to (i) reducing agents followed by thiol alkylation [Reducing agents: dithiothreitol, (DTT); β-mercaptoethanol (BME); tris(2-carboxyethyl)phosphine (TCEP), 50-100 mM, 65-100°C, 15 min; alkylation: 50-100 mM maleimide or substituted maleimides in isopropanol, 65°C, 15 min [131]] and (ii) hydrolytic activity (acid or base; 10 M, 100°C, 60 min) leading to their degradation and 'non-functionality'. This approach is usually reserved for destruction of concentrated materials or stock solutions. Dry waste must be placed in double red biohazard bags autoclaved at 121°C for 60 minutes at 18 psi. Contaminated and potentially contaminated protective clothing and equipment should be decontaminated using methods known to be effective against the conotoxins/conopeptides before removal from the laboratory for disposal, cleaning or repair. If decontamination is not possible/practical, materials (e.g. used gloves) should be disposed of as hazardous waste.

Note: 'Nonfunctional' peptides after disposal/neutralization must represent a zero risk of acquiring toxicity – thus, simple disulfide reduction alone is not an advised process for conotoxins/conopeptide neutralization, but combining thiol reduction with thiol alkylation is satisfactory.

41.12 Emergency Procedures

Follow these steps when an emergency involving toxins occurs. Circumstances may include spills, fire and evacuation, personal exposure or injury, and power and ventilation failure.

41.12.1 Spill

If a spill occurs outside of the fume hood or ducted biosafety cabinet, notify lab personnel and evacuate immediately. Close the doors as you exit to allow the aerosols to settle. Notify EH&S immediately! Do not re-enter the facility. Personnel decontaminating the spill must wear the minimum PPE of safety goggles, disposable lab coat, double gloves and shoe coverings. Respiratory protection may be necessary based on a risk assessment of the Select Agent.

Cover the spill area with absorbent material and cover with 10% bleach solution for a 30-minute contact time. Avoid raising dust when cleaning up. Ventilate the area and wash spill site after material pickup is complete. Wash contaminated clothing before reuse. Refer to an MSDS for proper cleanup measures.

Clean up the material and place in double red biohazard bags. Ventilate the area and wash the spill site again after decontamination is complete.

Note: Notify the Principle Scientist/PI and EH&S of the spill immediately.

41.12.2 Fire & Evacuation

Refer to standard procedures and responsibilities to the emergency, following an established procedure for laboratory/building evacuation – account for all personnel.

41.12.3. Personal Injury/Exposure, First Aid, & Medical Emergency

Undertake all standard first aid procedures, paying particular attention to respiratory support.

A note to physicians: There are no known antidotes/antivenoms for conotoxins/conopeptides. Therapy is supportive. Supplemental oxygen, airway management and mechanical ventilation may be required (see Textbox 41.2).

41.12.4 Power/Ventilation Failure

Undertake the following procedures: (i) Stop the work; (ii) Secure and cover the toxin; (iii) Whenever possible, store the toxin(s) in a secure storage location or chemical fume hood; (iv) Lower the hood's sash completely; (v) Post an alert or warning on the sash, and; (vi) Alert all personnel.

41.13 Control, Security and Training

41.13.1 Equipment Control

Engineering controls such as fume hoods and biological safety cabinets must be used as primary containment in order to limit personnel exposure to conotoxins/conopeptides. Engineering controls must be inspected in order to ensure efficient removal of hazard, must have a visual indication of airflow, and alarms to indicate that airflow has fallen below acceptable standards. Notification of laboratory personnel must be made prior to any maintenance that will impact the capture velocity of ventilation systems. Local exhaust ventilation systems including

laboratory-type chemical fume hoods and biosafety cabinets must be certified upon installation, after maintenance and annually thereafter.

41.13.2 Administrative Control

Written Standard Operating Procedures should be developed for all laboratories possessing, using, transferring or receiving conopeptides in order to ensure consistently safe work practices are being used. Standard Operating Procedures must be reviewed annually or as necessary to reflect changes to procedures. Standard Laboratory Practices for Guidelines for Work with Toxins of Biological Origin are addressed in the BMBL Appendix I (http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf).

41.13.3 Security

Laboratory areas using conotoxins/conopeptides, including those housing cone snails, must be locked at all times. All entries (including entries by visitors, maintenance workers, and others needing one-time or occasional entry) should be recorded by signature in a logbook. Only workers required to perform a job should be allowed in laboratory/holding areas, and workers should be allowed only in areas and at hours required to perform their particular job. Access to areas containing conotoxins/conopeptides should be restricted to those whose work assignments require access. All unknown persons must be politely confronted and questioned to their presence, then escorted to a safe hazard-free area and await consultation. Access for students, visiting scientists, etc., should be limited to hours when regular employees are present. Access for routine cleaning, maintenance, and repairs should be limited to hours when regular employees are present. The laboratory must be cleared of hazardous materials by EH&S prior to maintenance work or cleaning.

41.13.4 Training

All research personnel possessing, using, transferring, or receiving conotoxins/conopeptides must have appropriate training as to (i) the symptoms of conopeptide exposure, (ii) Post-Exposure Management Protocol, (iii) spill cleanup and conotoxin/conopeptide decontamination/neutralization and disposal. Research personnel also require training in the proper use of (i) engineering controls, (ii) administrative and work practice controls, (iii) personal protective equipment, and (iv) security requirements for conotoxin/conopeptide possession and use. Research personnel must be trained on the (i) Chemical Hygiene Plan and the (ii) conotoxin/conopeptide specific Standard Operating Procedures. Training is required before initiation of research involving conotoxins/conopeptides and annually thereafter. Principal Investigators are responsible for ensuring that all laboratory workers and visitors understand security requirements and are trained appropriately and equipped to follow established procedures.

41.14 Conclusions and Future Perspectives

The study of venomous organisms or the utilization of their potentially toxic extracts in research comes with inherent risks. Yet, these risks can be minimized with a basic understanding of the organisms' biology, an awareness of their toxicity and a healthy respect for their pharmacology complexity. Conotoxins/conopeptides are well recognized for advancing the current developments in analgesic medicines and have continued to increase our understanding of receptor-ligand interactions. Cone snails will undoubtedly continue to be a source of novel phyla selective agents, demonstrating unique isoform pharmacological targeting abilities, but as

conotoxins/conopeptides develop and now transition, more so in the synthetic world, examination and reconsideration of these risks becomes an evolving issue. Various regulatory bodies have recognized the scientific communities need for access to these ‘agents’. In doing so, the agencies have made classification exemptions, providing specific measures and guidelines in their research use. Such access will continue the present momentum in conotoxin and conopeptide drug development.

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Disclaimer

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Figure 41.1 (A) Representatives of piscvorous cone snail *Cone striatus* – illustration of morphological differences and representative geographic distribution: (i) and (ii) Oahu Hawai'i, (ii) Pago Pago, American Samoa (vi) Great Barrier Reef, Australia, (v) Cebu, Philippines. (vi) The radula harpoon from *C. striatus* – each individual snail species has a different radula structure. **(B)** The Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)/Ultraviolet detection profile of the duct and milked venom extracts from *C. striatus*, Oahu Hawai'i – Note dramatic differences in venom composition, some peaks (conotoxins) are common in both extracts. **(C)** Live *C. striatus* showing siphon (S) and proboscis (P) – the latter holds the radula for venom delivery.

Table 41.1 Symptoms of Cone snail envenomation and First Aid

Table 41.2 Representative conotoxins and conopeptides from *Conus*

Textbox 41.1 First hand account

Textbox 41.2 Notes to Physicians

Table 41.1 Symptoms of Cone snail envenomation and First Aid

<table>
<tr><th colspan="2">Cone snail envenomation</th></tr>
<tr><th>Symptoms</th><th>First Aid</th></tr>
<tr><td>

• Mild to sharp burning sensation at the site of the sting
• Sensation of tingling, burning, pricking, or numbness (Paresthesia) especially about lips and mouth
• Pruritus - desire to scratch at site of penetration
• Edema at site of penetration; actual puncture wound may not be evident; possible localized discoloration; edema may show effects within entire limb
• Fatigue and malaise
• Faintness or altered mentation
• Nausea, prolonged stomach cramps
• Facial muscle paralysis
• Ptyalism – drooling/hypersalivation
• Slurred speech and potentially aphonia (total loss of speech)
• Blurred vision or diplopia (double vision)
• Ptosis - abnormal drooping of the upper eyelid(s)
• Progressive muscle paralysis and numbness
• Absence of reflexes in lower legs/limbs and arms
• Dyspnea - difficulty in breathing
• Unconsciousness
• Respiratory arrest (40 min to 5 hours after sting)
• Cardiac impairment, leading to cardiac arrest
____
• **DO NOT** cut or excise the bitten or stung area
• **DO NOT** attempt to suck out the venom
• **DO NOT** unnecessarily move the victim
• **DO NOT** submerge limb in hot water; or pour hot water, vinegar, denatured alcohol or ethanol etc. on sting area
• **DO NOT** apply an arterial tourniquet
• **DO NOT** elevate sting site
• **DO NOT** operate vehicle if envenomated

</td><td>

As there is no antivenom/antivenin for cone snail venom; death is typically due to respiratory failure then cardiac arrest. Initial first responder measures:
• General **DRABC** - (**D**anger, **R**esponse, **A**irway, **B**reathing, **C**irculation)
• Activate emergency medical services
• Seek assisted medical evacuation
• Go directly to an Emergency Room – escorted
• Keep victim calm
• Prolonged artificial respiration, even mechanical ventilation, may be required. Be prepared.
• Cardiopulmonary resuscitation (CPR), maybe required.
• If possible, and with great caution, retain specimen(s) for identification process, which may aid in prognosis.
Bandage Application:
• Apply a broad pressure bandage directly over the sting area about as tight as an elastic wrap to a sprained ankle.
• Bind bandage distally, away from the heart – this avoids venous congestion and discomfort.
• Ensure arterial circulation is not cut off; ensure fingers or toes stay pink and warm.
• Immobilize limb-using splint; use a sling if the stung area is on the arm or hand.
• In cases that involve swelling of the affected area, the compression bandage may need to be more proximally positioned to wrap ahead of the swollen area.
• In route, hold the stung site below the rest of the body.
• The bandage pressure should be released within 8 hours or as soon as medical care is reached.

</td></tr>
</table>

Superfamily	Disulfide framework	Family	Target (receptor/channel)	Example	Amino acid sequence	Pharmacological Affinity IC$_{50}$ [nM] (organism)	*Conus* species	Feeding type	References
	CC-C-C	α	nAChR	α-ImI	GCCSDPRCAWRC*	250-500 (frog)	*C. imperialis*	V	[132]
A		ρ	nAChR	ρ-TIA	FNWRCCLIPACRRNHKKFC*	150 (rat)	*C. tulipa*	P	[133]
	CC-C-C-C-C	αA	nAChR	αA-PIVA	GCCGSYONAACHOCSCKDROSYCGQ*	350 (fish)	*C. purpurascens*	P	[134]
		κA	K$_v$	κA-SIVA	ZKSLVPSVITTCCGYDOGIMCOOCRCTNSC*	0.050-5 (fish) [LD$_{50}$]	*C. striatus*	P	[135]
		μ	Na$_v$	μ-GIIIA	RDCCTOOKKCKDRQCKOQRCCA*	100 (rat skeletal muscle) [K$_D$]	*C. geographus*	P	[84,85]
M	CC-C-C-CC	ψ	nAChR	ψ-PIIIE	HOCCLYGKCRRYOGCSSASCCQR*	127 (eel skeletal muscle)	*C. purpurascens*	P	[8]
		κM	K$_v$	κM-RIIIK	LOSCCSLNLRLCOVOACKRNOCCT*	20 (fish)	*C. radiatus*	P	[104]
		μO	Na$_v$	μO-MrVIA	ACRKKWEYCIVPIIGFIYCCPGLICGPFVCV	500 (chromaffin cell)	*C. marmoreus*	M	[136]
		δ	Na$_v$	δ-TxVIA	WCKQSGEMCNLLDQNCCDGYCIVLVCT	0.0025 (rat CNS) [K$_D$]	*C. textile*	M	[47,137]
O	C-C-CC-C-C	ω	Ca$_v$	ω-MVIIA	CKGKGAKCSRLMYDCCTGSCRSGKC*	0.03 (rat CNS)	*C. magus*	P	[138]
		κ	K$_v$	κ-PVIIA	CRIONQHCFQHLDDCCSRKCNRFNKCV	360±70 (frog) [K$_D$]	*C. purpurascens*	P	[139,9]
		γ	Voltage-gated pacemaker	γ-PnVIIA	DCTSWFGCTVNSγCCSNSCDQTYCγLYAFOS	0.0632 nM/100 mg body weight (mussel) [ED$_{50}$]	*C. pennaceus*	M	[140]
P	C-C-C-C-C-C	Spastics	Undetermined	Tx9a	GCNNSCQγHSDCγSHCICTFRGCGAVN*	>0.250 nM/g body weight (mouse brain) [LD$_{50}$]	*C. textile*	M	[97,141]
S	C-C-C-C-C-C-C-C-C-C	σ	5-HT$_3$	σ-GVIIIA	GCTRTCGGOKCTGTCTCTNSSKCGCRYNVHPSGwGCGCACS*	53±3 (HEK 293)	*C. geographus*	P	[98,18]
T	CC-CC	τ	Ca^{2+}	τ-TxIX	γCCγDGWCCT†AAO	0.20±0.029 (fish) [ED$_{min}$]	*C. textile*	M	[18,99]
		χ	Norepinephrine transporter	χ-MrIA	NGVCCGYKLCHOC*	500 (rat); 1700 (human)	*C. marmoreus*	M	[100]
I	C-C-CC-CC-C-C	Excitatory	Undetermined	Sr11a	CRTEGMSCγγNQQCCWRSCCRGECEAPCRFGP*	640 (frog)	*C. spurius*	V	[142,143]
No assignment	C-C	Conopressins	Vasopressin	Lys-conopressin-G	CFIRNCPKG*	0.010 (mouse) [ED$_{min}$]	*C. geographus*	P	[98]
		Contryphans	L-type Ca^{2+} channel	Am975	GCDOWDPWC*	<100 (rat PNS)	*C. amadis*	M	[144]
	No cysteines	Conantokins	Glutamate (NMDA)	Pr1	GEDγYAγGIRγYQLIHGKI	200 (mouse NR2B)	*C. parius*	P	[145]
Linear		Conorfamide	RF amide	CNF-Sr2	GPMγDPLγIIRI*	active range of ±0.25-1.00 (mouse)	*C. spurius*	V	[146]
	O-linked	Contulakins	Neurotensin	Contulakin-G	ZSEEGGSNATKKPYIL*	960 (human); 250 (mouse)	*C. geographus*	P	[79]

*, amidated *C*-terminus; O, 4-*trans*-Hydroxyproline; Z, pyroglutamate; S, *O*-glycosylated serine; γ, gamma-caboxyglutamic acid; w, L-6-Bromotryptohan; W, D-tryptophan; T†,*O*-glycosylated threonine; T, threonine + β−D-Gal(1→3)α−D-GalNAc; P, piscivorous; M, molluscivorous; V, vermivorous; ED, effective dose.

Table 41.2 Representative conotoxins and conopeptides from *Conus*

First hand account of treatment of a cone snail sting

In 1974, I was a Government Medical Officer at Kavieng, in New Ireland, which at that time was still a part of the Commonwealth of Australia. Spear-fishing on the reefs at night was a popular pastime with the local inhabitants, using a home-made spear gun powered by strong elastic bands, and a waterproof flashlight. They also used to keep an eye open for specimens of *Conus gloriamaris,* which at that time were selling for several hundred dollars each. Although papers had been published many years earlier both in Australia [64], Papua & New Guinea [147] and elsewhere [1] reporting the potential risk to humans from cone snails, both expatriates and indigenous people alike were generally unaware of the danger. Although an amateur shell collector myself, I was equally ignorant of the potential hazards.

The victim in this case was a 35 year old male, and he was brought to hospital unconscious at first light. The history was obtained in part from family members, and later from the patient himself after recovery. While spear-fishing on a reef in the dark, he had seen and picked up a cone snail, which he had placed in the pocket of his shorts. Soon after he felt a sharp prick in his thigh, but thought nothing of it. While cycling home from his fishing expedition, he began to feel weak, and went straight to bed. After a while, his wife with exceptional vigilance observed that his chest wall did not move when he breathed and on attempting to rouse him, found that he was unresponsive and paralysed below the neck. They urgently brought him to hospital and with commendable diligence also brought the cone snail, which was still very much alive. Examination revealed a flaccid paralysis to the level of T1, with diaphragmatic respiration. No lesion was identified on the thigh where the victim had felt the sting. Rapid consultation of a textbook, and identification of the shell as *Conus geographus* made the diagnosis all too clear.

There was nothing to be done but to prepare for intubation and mechanical ventilation, which in a small hospital on a Pacific island was a somewhat daunting prospect for a young doctor of only three years experience. Fortunately, the paralysis progressed no further, and soon began to resolve. Consciousness gradually returned, and after 24 hours the patient could move his legs. The next day he was out of bed and two days later left hospital, apparently none the worse for his experience.

The cone shell spent a short time in the freezer, and was then added to my collection. I developed a healthy respect for cone snails, and, although I never saw another case of envenomation in my subsequent 38 years of medical practice, I have always treated them with great respect and encouraged others to do the same [148]. Prevention as always is better than cure, particularly if there is not one.

Col. Robert Likeman MA BM BCh (Oxon), FRCOG, FRANZCOG, FACTM, FACRRM

Textbox 41.1 First hand account

NOTES TO PHYSICIAN

■ All cases, or suspected cases, should be treated as potentially life-threatening, and moved as quickly as possible to a medical facility, which can provide mechanical respiration for 24 hours.

■ The only serious consequence of a sting is paralysis of respiratory muscles; however, this paralysis is not permanent, and in most cases resolves in less than 24 hours.

■ Treatment should be directed at maintaining respiration until the paralysis resolves by itself. Mechanical ventilation is the treatment of choice.

BASIC TREATMENT: Establish a patent airway with suction where necessary. Watch for signs of respiratory insufficiency, and assist ventilation as necessary. Administer oxygen by non-rebreather mask at 10 to 15 L/min. Monitor and treat, where necessary, for pulmonary edema. Monitor and treat, where necessary, for shock. Anticipate seizures. DO NOT use emetics.

NO recurrent or persistent coagulopathy has been reported in humans.

Direct cardiac toxicity by a cone snail envenomation has not been demonstrated. If cardiac arrest occurs, it is likely secondary to respiratory arrest and secondary cardiac ischemia. Management should focus on airway protection and ventilation, with cardiac interventions (such as chest compressions, anti-arrhythmic medications, and cardioconversion) given only as indicated once the respiratory status is stabilized.

ADVANCED TREATMENT: Consider orotracheal or nasotracheal intubation for airway control in unconscious patient or where respiratory arrest has occurred. Positive-pressure ventilation using a bag-valve mask or mechanical ventilator might be of use. Monitor and treat, where necessary, for arrhythmias.

Start an IV and provide intravenous fluid resuscitation as needed, but avoid volume overload. Drug therapy should be considered for pulmonary edema. Hypotension with signs of hypovolemia requires the cautious administration of fluids. Fluid overload might create complications.

Treat seizures with midazolam.

LABORATORY SAFETY: Proparacaine hydrochloride should be used to assist eye irrigation. Peptides from cone snails are also poorly absorbed dermally or via oral route. Treat needle stabs as above.

Textbox 41.2 Notes to Physicians

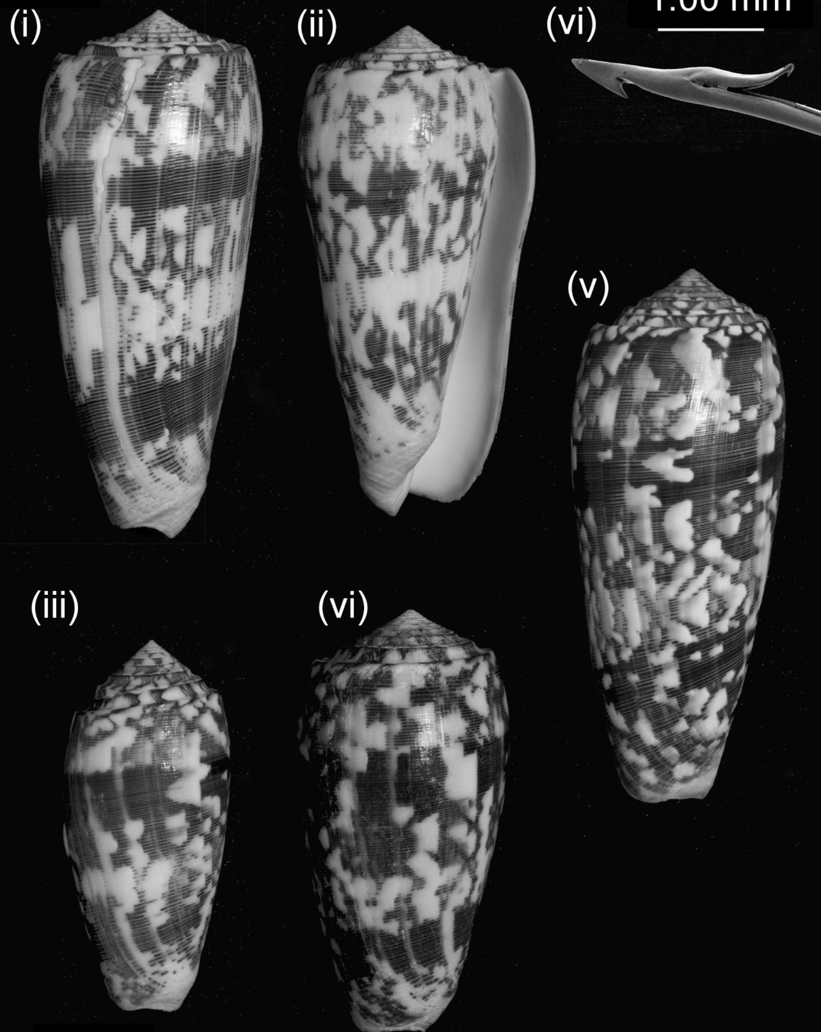

Fig.1A
1.00 mm
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2 cm

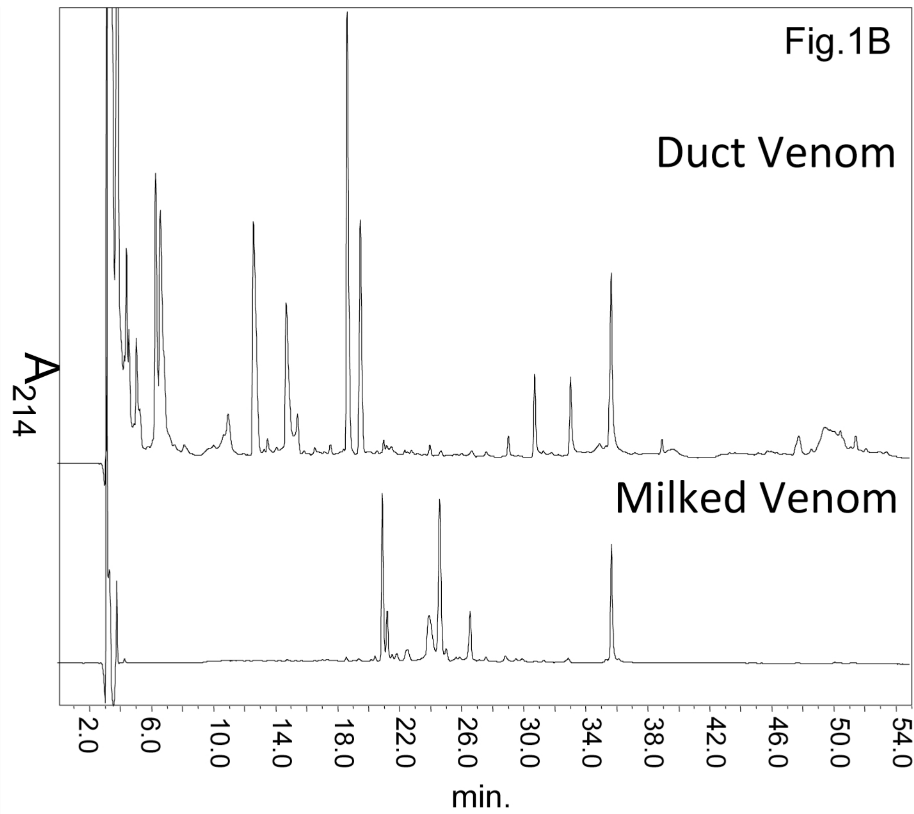

Fig.1B
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Milked Venom
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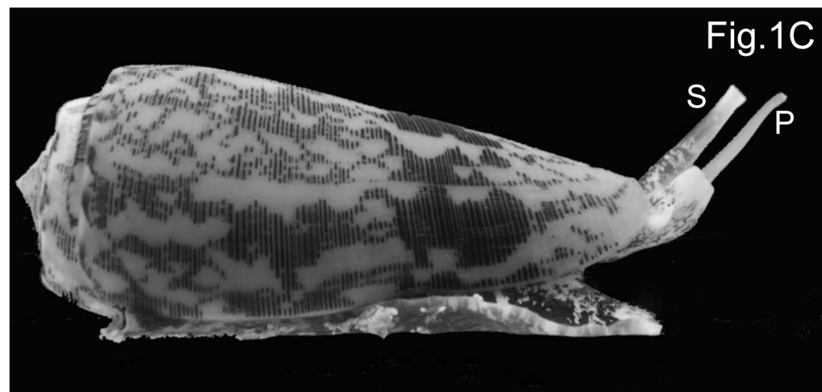

Fig.1C
S
P