

# Cone shell envenomation: epidemiology, pharmacology and medical care

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## Abstract

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The marine environment presents much danger, specifically in regards to the numerous venomous inhabitants within tropical and subtropical waters. The toxins from one such group of venomous marine snails, commonly referred to as 'cone snails', have been well documented in causing human fatalities. Yet information regarding medical treatment for cone snail envenomation is limited and poorly accessible. To correct this, medical and scientific expertise and literary review on *Conus* provide a basic and comprehensive directive focused on the medical treatment and post-mortem investigative analysis of cone snail envenomation. We emphasize what we expect to be the most lethal feeding group of *Conus* and provide a brief background to the epidemiology of their stings. We describe the venom apparatus of *Conus* and its utility of rapid venom delivery. We have compiled the documented incidences of *Conus* envenomation to offer thorough reference of known signs and symptoms – this too drawing on personal experiences in the field. We have also made available a brief background to the biochemistry and pharmacology of *Conus* venoms to highlight their complex nature.

## Key words

First aid; envenomation; toxins; pharmacology – marine; deaths; symptoms; treatment; review article

## Introduction

In a recent paper, we illustrated the molecular composition of the milked venom obtained from *Conus geographus* (Table 1).<sup>1</sup> We believe this particular species, given the documented human fatalities, provides a 'worst case scenario' to use for revising medical treatment protocols in treating cone snail envenomation. The potential need to access this information is warranted by public interactions with cone snails and more so with increased activities in field collection and venom milking for scientific and medical research.

The photo of *Conus geographus* in Table 1 is of the actual specimen, collected on 27 June 1935 at Hayman Island, Northern Queensland, Australia, that caused a well-documented human fatality in 1935.<sup>3</sup> Accessioned on 19 July 1935 (Albert H Longman, Director of the Queensland Museum, Reg. # QMMO 1689), its length is 84 mm and the dried animal is inside shell (Photo: J Healy, Queensland Museum)

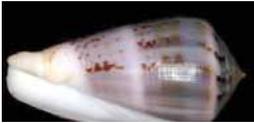
Here, we summarise the epidemiology of *Conus* envenomation, review the symptoms and signs of envenomation and provide revised recommendations for first aid and medical treatment. These details are based on personal, medical, laboratory and field experiences with cone snails and their toxins, together with literary research. A concise background into venom biochemistry and pharmacology is provided to deepen clinical awareness and assist in emergency treatment.

## Epidemiology of *Conus* envenomation

Cone snails, representing the genus *Conus*, have been a source of interest and injury, with cases cited as early as 1706.<sup>2</sup> Fifty-five reported stings from the Indo-West Pacific, the Atlantic shores of Brazil, and the islands of the Indian and Pacific Oceans have been reviewed and reported.<sup>3-7</sup> Shell collectors, scuba divers and beachcombers are the typical cohort of cone snail victims as the collecting and handling of live specimens risk envenomation.

The most potentially dangerous cone shell species belong to piscivores (fish eaters; ~10% of genus; Table 1). Envenomation by *C. geographus* has the greatest mortality rate at 67% and has been responsible for nearly 85% of all lethal cases reported.<sup>24</sup> The principal conotoxins discussed that contribute to the mortality rate in humans, have been mathematically modelled to estimate a human lethal dose, based on the correlations found between dry weight per volume of injected venom and the size of the shell; the human lethal dose (0.029–0.038 mg·kg<sup>-1</sup>),<sup>25</sup> was extrapolated based on the historic fatal case of *C. geographus*.<sup>3</sup> Revised estimates indicate the mortality rate due to piscivorous envenomations to be 15–25%.<sup>4,26</sup> Molluscivore species (mollusk eaters; approx. 25% of the genus) have been inadvertently implicated in several fatal cases. This may be attributed to specimen misidentification, as the likelihood of this occurring is high considering there are 600 species in the genus. Moreover, molluscivores are known to be aggressive when removed from their natural environment. The remaining *Conus* species, the vermivores (worm eaters, approx. 65% of the genus), have no reported human deaths

**Table 1**  
Known and suspected highly venomous cone snails; \* the information was not explicitly indicated in the article  
GBR – Great Barrier Reef

<i>Conus species</i>	Place of collection	Foraging behaviour
 <i>C. geographus</i>	Marinduque, Philippines  Boult Reef, GBR, Australia	Only feed on specific species including a puffer fish and small eels; also feed on frozen and thawed anchovies after spending some time in aquarium; length 70–153mm. <sup>8</sup> Feed guppies and milked for venom. <sup>1</sup>
 <i>C. californicus</i>	Western coast of Baja, California  Monterey Bay, California	Fed on variety of fresh and saltwater fish and/or Canadian night crawlers; attack lasted for ~30 min; display organized/cooperative attacks upon shrimps and snails. <sup>9</sup> Starved for a week before feeding with live juvenile specimens of three species of prickleback; length 15–35 mm. <sup>10</sup> Fed as above, with milked venom collected by arousing with squid skin (internal layer) stretched over a 0.5 ml microfuge tube and enticing a venom injection into the vial. <sup>11</sup>
 <i>C. striatus</i>	American Samoa Hawaii* Various locations; GBR, Australia; Oahu, Hawaii	Fed on commercially procured goldfish; <sup>12</sup> Fed on several fish species; <sup>13</sup> Fed on goldfish and milked for venom; <sup>14</sup> Fed on swordtail once a week after milking; <sup>15</sup> length 60–130 mm.
 <i>C. catus</i>	Hawaii Kauai, Hawaii  Hawaii*	Feed on small marine fish, generally sculpins; <sup>12</sup> Fed on freshly killed killifish and arrow goby; uses a high-speed hydraulic prey capture mechanism similar to the fish-hunting <i>C. pennaceus</i> . <sup>13</sup> Feed on goby; <sup>16</sup> length 25–50 mm.
 <i>C. purpurascens</i>	Gulf of California; Near Smithsonian Tropical Research Institute, Panama	Fed twice weekly on goldfish after milking. <sup>17</sup> Fed weekly on goldfish and milked for venom; length 30–84 mm. <sup>18</sup>
 <i>C. ermineus</i>	Palm Beach County, FL, USA	Fed on goldfish; locally acquired for bait/feeding and milking purposes; length 30–70 mm. <sup>19</sup>
 <i>C. consors</i>	Chesterfield Islands, New Caledonia	Most fed on live fish (not specified) for milking purposes; one snail displayed scavenging feeding behavior on dead fish; length 50–80 mm. <sup>20,21</sup>
 <i>C. obscurus</i>	Oahu, Hawaii	Fed on goldfish and milked for venom; length 20–41 mm. <sup>22</sup>
 <i>C. magus</i>	Night Island, GBR	Specimens were fed weekly on juvenile goldfish and milked for venom for 12 months. <sup>23</sup>

**Table 2**

Symptoms and signs of cone snail envenomation

**Local manifestations**

- Mild to sharp burning sensation at the site of the sting;
- Sensations of tingling, burning, pricking (paraesthesiae) or numbness;
- Pruritus at site of penetration;
- Oedema at site of penetration; actual puncture wound may not be evident; possible localized discoloration;
- Oedema may show effects within the entire limb.

**Systemic manifestations**

- Spreading paraesthesiae and numbness, especially about lips and mouth;
- Blurred vision or diplopia;
- Fatigue and malaise;
- Faintness or altered mentation;
- Nausea, prolonged stomach cramps;
- Facial muscle paralysis;
- Ptyalism (drooling/hypersalivation);
- Slurred speech and potentially aphonia;
- Ptosis;
- Progressive muscle paralysis and numbness;
- Absence of limb reflexes;
- Dyspnoea;
- Unconsciousness;
- Respiratory arrest 40 min to 5 h after sting;
- Cardiac impairment, leading to cardiac arrest;
- Death (typically from acute respiratory failure).

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associated with stings, mostly owing to their timid nature and phyla-selective toxins.

Human cone snail envenomations are uncommon due to the animal's nocturnal nature and the lack of public knowledge regarding their habitats, although chance discoveries can lead to their handling and removal for their attractive shell patterns. This dangerous practice is frequently dismissed with the delusion that a snail cannot kill. South Pacific islanders are aware of the dangers associated with these marine gastropods, and typically group all cone snails, called "*intrag*" or "*nunus*", as dangerous and revered. Undocumented envenomations have occurred, with local inhabitants stating they were told or know of such occurrences. In these locations, a potential indicator of a [surviving] cone snail victim can be the presence of multiple laceration scars to the affected limb caused by bloodletting; a treatment islanders wrongly believe limits the circulation of injected venom.<sup>7</sup>

Scientific researchers use cone snail toxins to dissect ion channel functions.<sup>27</sup> Due to their toxic nature, the US Centers for Disease Control and Prevention classify these as 'select agents'.<sup>28</sup> Thus, governmental regulated safety measures are employed within the research laboratory. Regardless of toxin

**Table 3**

First aid and advanced care for cone shell envenomation; there is no antivenom for cone shell toxin

- General **DRSABC** (**D**anger, **R**esponse, **S**end for help), **A**irway, **B**reathing and **C**irculation);
- Administer Basic Life Support (BSL) as indicated;
- Activate emergency medical services;
- Seek medical evacuation;
- Advanced Life Support (ASL) as indicated;
- Pressure immobilization (see below);
- If possible, with caution, retain specimen(s) for identification;
- If first aid measures of BSL and ASL are effective, remember that the victim may be paralyzed but fully conscious. Thus, reassurance and talking to the victim is important.
- Prolonged cardio-respiratory support may be required, including mechanical ventilation, iv fluids and inotropes;

**Pressure immobilization**

- Apply a broad pressure bandage directly over the sting area about as tight as elastic wrap to a sprained ankle.
- Ensure that arterial circulation is not cut off and fingers or toes stay pink and warm.
- 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.
- Bind splint or any rigid object to support limb/affected area; focus on immobilization to limit toxin circulation.
- Reassure patient and prevented patient from walking or physically moving.

**DO NOT:**

- cut or excise the stung area;
- attempt to suck out the venom;
- submerge limb in hot water or pour hot water, vinegar, denatured alcohol, ethanol or other home remedies on sting area;
- apply an arterial tourniquet;
- elevate sting site;
- operate vehicle if envenomed.

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source (synthetic or native), incident locations (laboratory or field sites) or post-exposure symptoms and signs, the principles and practices for medical treatment are identical.

**Venom delivery**

The route of venom delivery is unique to this family (toxicoglossa, meaning 'poisonous tongue,' includes cone snails, turrids, and terebra). These predatory snails are armed with a quiver of single-use, hollow, barbed and serrated hypodermic-like harpoons or radula. The structures are commonly used as a taxonomical tool in species identification, since each species has a distinct form and

structure.<sup>29</sup> Typically in piscivorous *Conus*, these eyelash-sized chitinous impalers can penetrate woven layers and even 5 mm neoprene wetsuit materials at high velocity, indicating that a wetsuit provides little protection (Gilly, personal communication, 2014).<sup>30–32</sup> The volume of venom delivered ranges from 1 to 50 µL. The effective harpoon trajectory range is increased by the proboscis, an extendable and flexible tongue-like structure that can rapidly extend one-to-two shell body lengths. Thus, holding a snail at the rear (the broadest end of its shell) offers little protection. In rare occurrences with antagonism, such as scraping the shell with a knife or dropping it, cone snails have been known to ‘shoot’ harpoons.<sup>6</sup> Therefore, avoiding handling these live shells represents the best prevention.

### Envenomation

Reported envenomations<sup>30,32,33</sup> have provided various symptoms and signs, summarised in Table 2. These reports concur with personal experience and observations.<sup>32</sup> The puncture and envenomation sensations vary. Most victims feel an immediate stinging sensation and later local numbness. Localized swelling may occur, accompanied by redness or discoloration from ischaemia/cyanosis. Local numbness and paraesthesia spread quickly from the affected area about 10–30 min after the sting. Many victims report these sensations intensely around the lips and mouth. Less common manifestations are nausea, muscle cramping, headache, and itching. The venom is a neurotoxin, therefore, after about 30 min, systemic abnormalities such as muscle weakness (including respiratory), diplopia, dysarthria, the inability to swallow, and an absent gag reflex start to develop.<sup>4</sup> Within an hour, generalized paralysis and respiratory failure can occur and, without medical support, coma and death may follow.<sup>3,4,6,33</sup>

### Medical treatment

#### FIRST AID

Because envenomation may cause paralysis, coma and death, it is essential to first remove the victim from water in order to prevent drowning and subsequently transport the victim to a medical facility as quickly as possible. Owing to the chemical complexity, rarity of stings, and geographic diversity of cone snail venom, no attempts to produce cone snail antivenom have been successful.<sup>21,23</sup> To slow venom distribution, a pressure immobilization technique, as recommended for snake envenomations, should be used (Table 2). There have been documented cases in which medical attention was not possible, and the course of action was vigorous rubbing and squeezing to expel contaminated serum. More drastically, some cases have involved wound lancing/bloodletting and sucking fluids from the site.<sup>33</sup> There is no evidence to support these harmful measures and so they are not recommended. Prioritize the patient’s airway, watch for signs of respiratory insufficiency, and administer

oxygen as indicated (10–15 L·min<sup>-1</sup>). Gag reflex may be absent and suction may be required. Management should focus on airway protection and ventilation with Basic and Advanced Life Support (ALS) as indicated. The victim may be paralyzed but remain conscious, thus reassurance and talking to the victim are important.

#### ADVANCED CARE

Provide on-going ALS, including mechanical ventilation, anti-arrhythmic agents, inotropes and cardioversion as indicated. No coagulopathy has been observed in cone snail envenomation.<sup>35</sup> The onset of respiratory paralysis can require mechanical ventilation for more than 24 hours. Paralysis is not permanent and typically resolves within 12–36 hours. Intravenous fluid and inotropes may be required for hypotension. Administer fluids cautiously since some patients have exhibited pulmonary oedema. Continue monitoring respiratory and cardiac function until autonomic and motor function are fully regained. Animal studies suggest that seizures are unlikely as, owing to the peripheral peptidic neurotoxic nature of the venom, it does not penetrate the blood brain barrier. If seizures occur, these are likely secondary to hypoxia.

#### POST-ENVENOMATION CARE

The wound should be regarded as potentially contaminated, and treatment may be required for secondary bacterial infection.<sup>4,33,36</sup> Treatment for secondary soft tissue infections should be directed at usual flora and additionally, those unique to the marine environment. For this circumstance, consideration of empiric therapy with ceftriaxone and doxycycline is recommended.<sup>37</sup>

Ulceration may occur and may require long-term wound care with the potential of recurring infection (Jackson, personal communication, 2014).<sup>26</sup> In such cases, it is likely that foreign material is still present within the dermis. Thus, to avoid infection, the wound must be examined and debrided. Nerve damage at or around the sight of envenomation is possible, leading to temperature perception reversal – a similar permanent localized effect to that seen with ciguatera poisoning (East, personal communication, 2005). Most recovering patients seem no worse for wear, with some having been medically discharged within 48 hours of envenomation.

#### Post-mortem examination

In the event of post-mortem examination, external inspection of the extremities is paramount. It is possible to locate the area of radula harpoon penetration, indicated by a small area of dermal pigment differentiation and/or the presence of the imbedded radula harpoon(s). It is possible to see < 50% of the exposed harpoon length. The imbedded harpoon(s) may still retain a posterior thread-like ligament, which can be

equal in length to the chitinous harpoon itself. The naturally barbed structure of the harpoon may be damaged if forcibly removed from the dermis. Typical points of entry are hands, fingers and waist region if the animal was collected and then stowed or 'pocketed'.

Animal studies of the toxic effects of cone snail venom have demonstrated decreased red blood cell count, increased immunoglobulins, and elevated serum enzyme levels, including glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, lactate dehydrogenase, and both alkaline and acid phosphatases.<sup>35</sup> Unlike snake-bite envenomation, no forensic use of immunoassay approaches have been conducted for cone snail venoms, and very limited data are available in regards to attributed blood changes in humans.

Respiratory failure is typically stated as the cause of death, often with minimal pathological findings. In the rare instance of post-mortem examination of a human *C. geographus* victim, the victim "showed that all the organs, heart, lungs, et cetera were quite healthy".<sup>3</sup> Such lack of findings fits with the known pharmacological diversity of toxic peptides that primarily illustrate neuromuscular targeting (Table 4).<sup>1</sup> The speed of lethality, venom volume, and toxin concentration are elements for consideration, as these may cause different findings at autopsy.

### Biochemistry of venom

Potential for death from a piscivorous cone snail envenomation comes from the mixture of high-affinity peptide toxins that target different ion channels and receptors.<sup>1,14,38</sup> There are an estimated half-million bioactive peptides, commonly referred to as conotoxins or conopeptides within the genus (see Table 4 for examples). Recent evidence indicates that *Conus* has the ability to deploy different venom profiles for prey capture and for defense, resulting in changes within the injected venom.<sup>39</sup> These features highlight the unparalleled biochemical nature of these venomous marine snails.

Biochemically these small peptide neurotoxins (5–100 different peptides per milked venom; 10–40 amino acids in length) commonly contain stabilizing chemical modifications such as disulfide bonds.<sup>40–42</sup> Venom extracts collected in 1962 by the late Dr. Robert Endean exemplify venom constituent stability; even today, these extracts still demonstrate potency and high molecular mass composition compared to freshly dissected venom from the same species.<sup>14</sup> This stability is reflected in the inability to minimize venom toxicity by heat and renders hot water submersion as an ineffective first-aid procedure.<sup>43</sup>

Proteinaceous material (> 6,000 Da) is also expressed within the milked venom of *Conus*. These proteins potentially include phospholipases and proteases.<sup>44,45</sup> The expression of phospholipases within *Conus* is neither as predominant nor as lytic as those found in some snake venoms.

### Pharmacological properties

Isolated conotoxins from *C. geographus* venom, our hallmark for human lethality, have been pharmacologically characterized, and their elicited and complex symptoms have been individually observed (Table 4). The pharmacological predation/defensive strategy for all piscivorous *Conus* species is the same – to rapidly paralyze. In laboratory animals, a wide range of behaviours of head swinging, kicking on back, scratching, uncoordinated jumping, trembling, back leg dragging, depressed activity, sleeping, convulsing and bleeding, and finally paralysis and coma are revealed upon intracranial injection.<sup>54</sup> In nature, the venom's pharmacological effectiveness is maximized by a synergistic binding strategy that achieves paresis more rapidly than other observed venomous groups (snakes, scorpions, spiders, anemones).<sup>42,55</sup>

As in *C. geographus* venom, a single venom may contain both pre- and post-synaptic inhibitors that specifically target voltage-gated calcium channels and acetylcholine receptors, respectively. The pre-synaptic inhibitors act upon the neuronal calcium channels located in the axonal terminal; when these receptors are blocked, incoming action potentials that would allow for the influx of Ca<sup>2+</sup> ions through the external vestibule are obstructed. Otherwise, the influx of Ca<sup>2+</sup> would have triggered the release of neurotransmitter (acetylcholine) into the synaptic cleft to stimulate the propagation of another neuronal action potential or a muscular contraction event. Thus, the post-synaptic neuronal inhibitors which have high binding affinity for neuronal acetylcholine receptors block the downstream propagation of the action potential by inhibiting the ability of the acetylcholine receptors to respond to increased concentrations of their associated ligand, acetylcholine. This synergistic blocking prevents synaptic nerve action potential propagation completely, a common trend in underlining its neurotoxicity. Those peptide toxins responsible are identified as the  $\omega$ -conotoxins:  $\omega$ -GVIA and  $\omega$ -GVIIA or 'shaker peptides' and members of the  $\omega$ -conotoxins:  $\omega$ -GI and  $\omega$ -GIIA (Table 4).<sup>42,56</sup> This represents a highly conserved strategic pharmacological targeting process within all piscivorous *Conus*.<sup>57</sup>

The venom may also contain  $\mu$ -conotoxins: GIIIA, GIIIB and GIIIC, which act by blocking the voltage-activated sodium channels in muscle membranes (Table 4). These conotoxins are also lethal in mice whether by intracranial or interparietal injections and potentiate the mechanism for rapid onset of paralysis.<sup>42</sup> Other  $\omega$ - and  $\mu$ -conotoxin variants have been reported in other piscivores. While within the genus and encompassing all feeding groups, many phyla-selective  $\omega$ -conotoxins are reported which demonstrate a preferential molecular selectivity.<sup>58</sup>

Conantokin-G represents a non-paralytic toxin in *C. geographus* venom that subdues young mice to a sleeping state upon intracranial injections (Table 4).<sup>42</sup> This peptide

**Table 4**

The dominant and consequential conotoxins within milked venom of *Conus geographus*, tested for activity in mice; LD50 = lethal dose; ND = Structure not determined; \* C-terminal amidation; O = 4 trans-hydroxyproline;  $\gamma$  = gamma-carboxyglutamic acid; W = bromotryptophan

Conotoxin	Amino acid sequence	Target	Intracerebral injection	Intraperitoneal injection	Symptoms	Reference
$\alpha$ -GI	ECCNPACGRHYSC*	Neuronal nAChR	-	LD <sub>50</sub> 12 $\mu$ g/kg BW	Paralysis, death	8,46
$\omega$ -GVIA (SNX-124)	CKSGSSCSOTSYNCCRSCNOYTKRCY*	Ca <sub>v</sub> 2.2	3 $\mu$ g·kg <sup>-1</sup> BW	-	Persistent tremors	47-49
$\mu$ -GIIIA	RDCCTOOKKCKDROCKOQRCCA*	Na <sub>v</sub> 1.4	-	LD <sub>50</sub> 340 $\mu$ g/kg BW	Paralysis, death	50,51
Conantokin G (CGX-1007)	GEYWLQYNOQLIRYKSN*	NMDA receptor	11 $\mu$ g·kg <sup>-1</sup> BW	-	Drowsiness/hyperactive (age dependent)	52,53
$\omega$ -GVIA (SNX-178)	CKSGTQCSRGMRDCCCTSCLLYSNKRRY	Ca <sub>v</sub>	<2 nmol per mouse	-	Persistent tremors	42

targets the NMDA receptor. It is not consequently deadly in mice, but its presence may act on the peripheral circuits of fish.<sup>54</sup> To date, the numerous conantokins that have been isolated and their expression appears to be exclusive to the piscivores of *Conus*.<sup>59</sup> With the potential of hundreds of conotoxins in a single envenomation and many still being unknown, the pharmacological correlation to specific symptoms and signs in humans can be complex and variable. Therefore, *Conus* deserves both respect and caution as a highly venomous marine invertebrate.

**Conclusions**

Although considered rare, cone snail envenomation can be lethal in humans. Given the diversity, potency and specific neurotoxicity of conotoxins and the lack of antivenom, the emergency care of an envenomation must focus on maintaining airway protection and ventilation. The best prevention is education. All cone snails should be considered venomous and thus should be avoided.

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