

Geographic cone breeding project for the production of mu-conotoxin

By Benjamin LISAN, september, the 2 th 2016 (Table of Contents, see [Plan](#)).

1 Introduction

Cone snails are carnivorous marine gastropods. They are found in all tropical waters, especially in reef habitats. They are best known by collectors for their beautiful shell.

These predatory mollusks use a sophisticated venomous device to kill their prey or deter predators.

Fish-eating cone snails, including *Conus geographus*, are by far the most dangerous to humans¹.

The number of species of conical marine snails of the *Conus* genus is estimated to be up to 700. The venom of the cone snails has provided a rich source of novel neuroactive peptides or conotoxins. Prialt® or ziconotid, the first drug, from the marine world, approved by the FDA, is an omega-conopeptid isolated from the venom of *Conus magus*. This new drug selectively targets N-type calcium channels, and its analgesic effect is 1000 times more potent than morphine. At least 10 other cone peptides purified from cone snail venom have entered human preclinical and clinical trials to treat pain, ischemic stroke, epileptic seizure or myocardial infarction. Each of the 700 known *Conus* species produces a rich, unique and highly complex mixture of peptides and natural compounds. Less than 1% of the biodiversity of cone snail venoms is pharmacologically characterized, **and therefore there is a huge potential for discoveries².**

2 The price of some venoms

The **GIIB mu-conotoxin³** du of the **geography cone** snail is trading at almost **500 euros per milligram** at Alomone Labs.

The **PIIIA variant** of the geography cone costs **800 euros per milligram** at Smartox Biotech ... or **800 million euros per kilo**. Some **variants** of the **jingzhao toxin**, derived from the venom of the **Chinese tarantula** (*Chilobrachys jingzhao* venom), are close to **1,000 euros per milligram**, or one **billion per kilo**.

The **hainantoxin**, extracted from the **Chinese spider *Ornithoctonus hainana***, is trading at **950 euros per milligram**.

In comparison, the **sea anemone** (*Anthopleura elegantissima*) would almost seem like a poor relation with its **APETx2 toxin** at **600 euros per milligram**.

As for the **toxic peptide Tx2-6** from the very aggressive **Phoneutria spider**, studied in the treatment of erectile disorders, its cost of synthesis is not yet available.

List of venoms from cone "milking"

Species	Price (Euros) / 500 µg (1/2 gr)
<i>Conus textile</i>	75
<i>Conus victoriae</i>	150
<i>Conus imperialis</i>	95
<i>Conus striatus</i>	150
<i>Conus ammiralis</i>	
<i>Conus catus</i>	
<i>Conus geographus</i>	
Kit BioConus includes 500 µg of each species (4 x 500 µg). Ideal for screening tests, proteomics and discovery purposes.	450 euros

¹ Source : <http://www.bioconus.com/conesnails.htm>

² Source : *High-quality venoms extracted from live marine cone snails*, <http://www.bioconus.com/>

³ µ-Conotoxin GIIB. Synonyme : **Arg-Asp-Cys-Cys-Thr-Hyp-Hyp-Arg-Lys-Cys-Lys-Asp-Arg-Arg-Cys-Lys-Hyp-Met-Lys-Cys-Cys-Ala-NH₂** [ponts Disulfide indéfinis], GTXII, Geographutoxin II

These cone snail venoms are sent lyophilized (freeze-dried directly from pure liquid venom, without dilution) in micro-tubes and sold by dry weight. The minimum order is 500 µg per species.

Source : <http://www.bioconus.com/conesnailvenompricelist.htm>

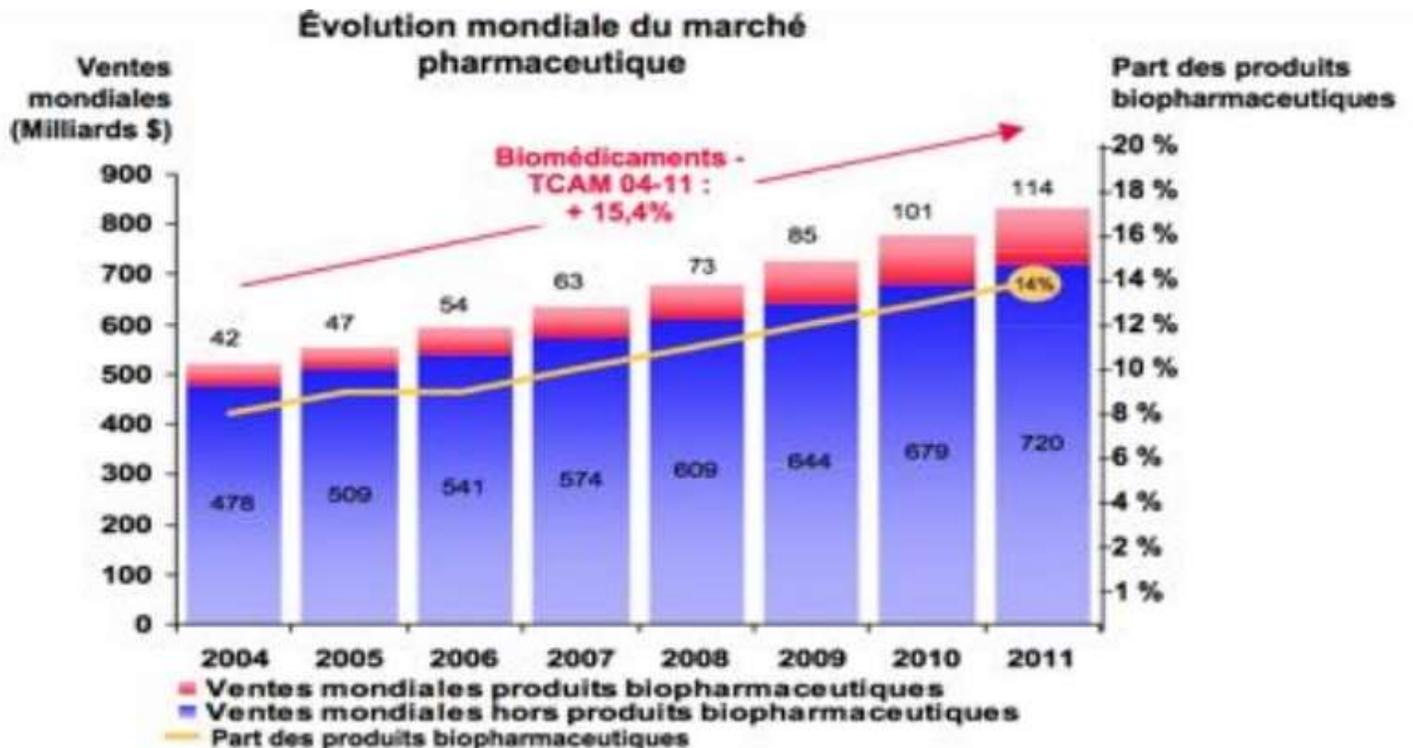
3 Why such prices ?

At the origin of such a surge of venoms, the rise of neurotechnologies, new biotechnologies, which boost the global demand for peptides that we do not know how to synthesize easily, if at all, and which must then be collected on the planet. stupid. Conotoxins, jingzhaotoxins, huwentoxins and hainantoxins affect various sodium channels, including those that neuroscientists inhibit to reveal their action potential and which are also necessary for neurons to communicate with each other. We obviously use the venoms in pharmaceuticals, various **scorpion toxins** treating rheumatoid arthritis or multiple sclerosis. The venom of the **king cobra** can be used as a pain reliever. As for **epibatidin**, an alkaloid accumulated by the *Epipedobates tricolor* frog from the insects it feeds on, it is 200 times more powerful than morphine!

Note: The µ-conotoxin from *Conus geographus* venom is very effective in blocking sodium channels in vertebrate skeletal muscles, thus preventing the spread of muscle action potentials⁴.

4 A potential market

There are more than 200 protein-drugs marketed and / or used in hospitals and several hundred are in clinical trials ...



Source image : *Diversité des toxines d'origine animale et stratégies d'ingénierie de peptides et de protéines* [Diversity of toxins of animal origin and peptide and protein engineering strategies], Dr. Frédéric DUCANCEL (CEA), Service d'ImmunoVirologie, iMETI/DSV/CEA, Fontenay-aux-Roses, 2014, http://www.genie-bio.ac-versailles.fr/IMG/pdf/conference-f-ducancel_12-02-14.pdf

⁴ Source : a) <https://www.scisale.com/VWRH-9015.0500BA.html>, b) <https://www.scisale.com/VWRH-9015.1000BA.html>

5 Authorization to breed dangerous animals

In France, **poisonous animals must be declared to the Directorate of Veterinary Services** and owners must hold a **specific certificate of competence**.

Among the poisonous NACs, the most dangerous are the exotic poisonous snakes.

A Certificate of Capacity is required to open a reptile breeding establishment.

This certificate of competence allows us to know about the occupational risks and the regulations specific to reptiles.

5.1 How to request authorization for prior detention?

Pour faire la demande d'autorisation de détention d'**animaux d'espèces non domestiques** vous devez télécharger et remplir le formulaire [Cerfa n°12447*01](#) et l'envoyer au préfet de département du lieu de détention de l'animal, par lettre recommandée avec avis de réception. Le non-respect de la réglementation vous expose à de lourdes sanctions.

5.2 How to obtain the certificate of competence?

In France, the owners of certain species must hold a certificate of capacity for the maintenance of animals of non-domestic species ([arrêté du 12 décembre 2000 fixant les diplômes et les conditions d'expérience professionnelle requis par l'article R. 213-4 du code rural](#)) [(decree of 12 December 2000 setting the diplomas and the professional experience conditions required by article R. 213 -4 of the rural code)].

The future holder of a specimen of a rare species of NAC will have to follow a training course at the end of which an individual certificate, justifying a minimum duration of experience in contact with the animal, will be issued.

CONTACTS

- Departmental Directorate of Veterinary Services (DDSV)
- Departmental service of the National Office for Hunting and Wildlife (ONCFS)
- Regional Directorate for the Environment (DIREN)
- Veterinarians
- Pet shops: these animal professionals benefit from official authorization for the exercise of their profession
- Breeder associations;

ON THE INTERNET

www.ecologie.gouv.fr

Ministère de l'Écologie et du Développement durable, 20 avenue de Ségur, 75302 Paris 07 SP

A prefectural decree is probably needed to open such a medical biology laboratory (LBM) (?) (With accreditation by COFRAC and declaration by LBM to ARS (?))⁵.



This is the harpoon tip of the radula of a cone snail for hunting fish (*Conus striatus*).

⁵ Cf. Instruction DGOS/R 2 no 2010-333 du 9 septembre 2010 relative aux demandes d'ouverture de laboratoires de biologie médicale, http://social-sante.gouv.fr/fichiers/bo/2010/10-09/ste_20100009_0100_0052.pdf

6 Precautions

Precautions in handling cones:

All **laboratory operators** handling or feeding these live cones **should do so with forceps (pincers, pliers)**.

And they must always grip the cone, with the 2 fingers of the pliers, by the rear part (the wide, flat and spiral part) of the cone.

Even when handling the venom with pipettes, thick diving gloves should be kept on both hands.

A checklist of procedures to be followed for each employee should be set up.



Danger: The cone's proboscis, containing the radula, a hollow tooth, is very mobile and can project its radula (hollow tooth), used as a hypodermic syringe, perfectly well behind the cone and therefore pierce the operator's rubber glove. So this picture shows you that you can still get stung no matter how you pick up the cone.

Source image : Okinawa Churaumi Aquarium,
<https://www.youtube.com/watch?v=8aprazOOvBA>



Feeding the cone, not giving it a dead fish, held by a clamp. As soon as it has palpated its prey, the cone projects its "net" to encompass its prey.

Source image : Cone snail feeding session at the *Institute for Molecular Bioscience, University of Queensland*,
<https://www.youtube.com/watch?v=Vqb8Dc-m7NE>



The cone attack. The prey (the fish) is held between the claws. The cone snail projects its trunk (proboscis) on its prey.

Source image : *Conus feeding*,
<https://www.youtube.com/watch?v=oqu-xt116P8>



"Milking" the cone. The cone is excited by shaking a fish on it. When it projects its proboscis onto its prey, the prey is removed and placed in a tube or pipette (containing the aroma of the fish) and the tube is pushed onto the proboscis while shaking it. He ejects his venom and removes the tube with his venom. Then we give him back the fish.

Source image : *Conus purpurascens* milking, Alex Rodri,
<https://www.youtube.com/watch?v=85J94PtO1Ok>

Precautions against theft of venoms and toxins:

The laboratory must be secure (armored), security level 2 (armored doors with code (recognition by iris and / or fingerprints), triplex (or Kevlar) windows that do not open, alarm system. Fridge closing key).

Those hired must have a clean criminal record.

A checklist of procedures to be followed for each employee should be set up.

Conservation :

Storage temperature: - 20°C.

7 How to set up the breeding of the geographic cone

One would have to imagine building a lot of small individual tanks (or buying aquariums), filled with seawater, for each cone (so that they do not devour each other, because they may be cannibals).

Basins or aquarium reproducing the night life conditions of the cone (same temperature: 25 ° to 30 ° C, same sandy, coral, and rocky soil).

We would regularly feed the cones, with dead fish serving as prey, held by a clamp.

These fish would be bought or bred, accordingly.

To constitute the starting stock of living cones:

You have to pay the fishermen from the coasts of the Indian Ocean (who normally sell these cones in the form of dead shells to tourists for € 3) to sell us (at € 5) the **live cones**, bringing them to us in the water seafood (in plastic bags), directly to our ponds. These fishermen would be provided with **diving gloves and tongs, to grab the cones and keep them from being poisoned (and killed)**.

Make the cones reproduce with each other:

Like most snails, one can imagine that they are hermaphrodites.

So, put two cones stuffed with food (and therefore full), during the mating season, between April and September when the waters are warm (in the Indian Ocean or in Australia).

8 How to extract the venom?

« In the case of the marine cone, the sacrifice of the animal is almost inevitable in order to recover the venom.

Its poisonous apparatus is made up of a muscle gland where toxins are produced. Itself is prolonged by a long duct ending in a poisonous tooth which plays the role of harpoon head when the cone is hunting. Therefore, the extraction of the venom requires a dissection of the shell in order to recover the entire venomous apparatus »⁶.

Another suggestion to preserve the resource:

By taking it out of the water, we could push the cone to prick, in a defense reflex, the forceps covered with a latex glove, which holds the cone, or we could push the cone to prick the operculum of a bottle _ closed by this plastic or paper cap _ bottle that is constantly shaken in front of the proboscis of the cone.

Or we work with a rubber fish, previously rubbed with the fish to get the smell.

Then when he injected his toxin into the rubber fish, then offer him a real fish.

⁶ *Les venins d'animaux, nouvelle panacée ? [nimal venoms, a new panacea] Université de Liège,*
<http://reflexions.ulg.ac.be/upload/docs/application/pdf/2013-07/ath291maiechterbille.pdf>

Example of collecting cones [in the Persian Gulf] and extracting venom from the Textile Cone⁷ :

Until now, conical snail venoms have been of limited access and always obtained from dissected animals, a method that is unsustainable, and at best, ethically questionable.

Fortunately, a method of milking pure venom allows it to be collected from living cone snails.

In addition, the Traits Conus venom has many beneficial properties over the venom of dissected cones:

Dissected cone venoms	Milked Venoms
May include cellular debris and untreated molecules which unnecessarily complicate purification of the active conopeptide.	99,9% pure Free of contamination, cell debris and containing no or traces of unprocessed toxins and degradation products.
Individuals within a <i>Conus</i> species are well known to produce different venom profiles. Effectively, a particular molecule of interest can be produced by a single cone snail, and therefore, when more material is needed for the complete characterization of a stroke, it is impossible to obtain this additional material since the <i>Conus</i> mollusk was sacrificed.	Very homogeneous The venoms of the cone snails are collected and grouped into batches to minimize individual variations.
High content of insoluble matter.	Greater solubility
Sacrifice hundreds of cone snails to purify and characterize a single peptide.	Sustainable and ethically acceptable

« Specimens of *Conus Textile* were collected at Larak Island, at a depth of 7 m. The collected samples were transferred to the laboratory alive and were stored at -70 ° C.

The venom ducts were separated and homogenized with deionized water. The mixture was centrifuged at 13,000 ipm (rpm) for 15 minutes. The supernatant was taken as the extract of the venom and stored at -20 ° C after lyophilization.

The venom protein profile was determined using SDS-PAGE and by HPLC used to study the extracted venom and assess analgesic activity, **formalin** test was performed. He indicated that several SDS-PAGE bands were between 6 and 250 kDa. The venom chromatogram demonstrated more than 44 large and small fractions. The 10 ng amount of raw *Conus* venom and analgesic peptide showed the best pain relieving activity in the formalin test. No deaths observed up to 100 mg / kg, which is 250,000 times higher than the characteristic dose. The potent Persian Gulf venom *Conus textile* may thus be of medical importance and the potential for novel pharmaceutical DMGS⁸ ».

« Nine samples of *C. purpurascens* were collected near the Smithsonian Tropical Research Institute, Panama. Specimens were transported to the USA, acclimatized and housed in a temperature controlled environment in a single 30 gallon artificial seawater tank equipped with a Fuval™ 402 biological filtration system. Samples were fed. per week, using *Carassius auratus auratus* (goldfish, weight 2-5 grams) and therefore the venom was milked [from the verb to milk], using a modified method previously described in Hopkins et al. (1995) and Bingham et al. (2005).

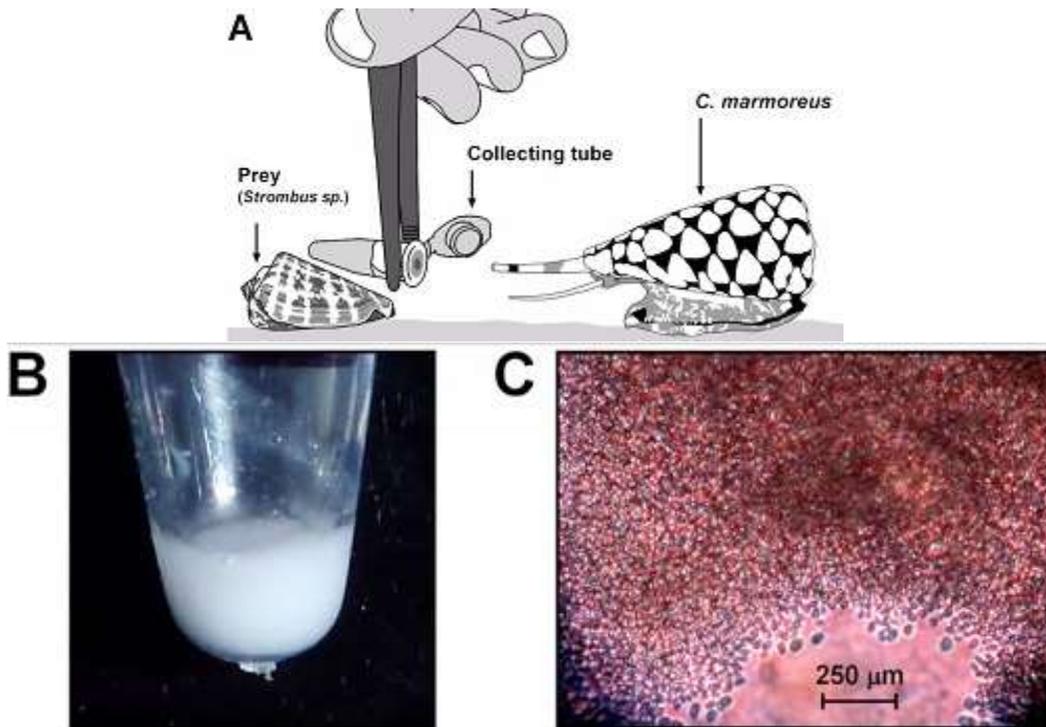
Individual volumes of milked venom were collected, measured, and then lyophilized. The samples were later sexed [whose sex was determined], on dissection, dissected for the venom duct and harpoon [stinger] radula. The venom extracts, together with the harpoons radula were stored at -20 ° C until needed.

⁷ Le *Conus textile*, ou **Toison d'or**, est une espèce de mollusque de la famille des Conidae (proche de l'espèce *Conus geographus*). Son poison, extrêmement puissant, peut tuer un homme en cinq minutes, il n'existe aucun antidote efficace, faisant de cette espèce l'un des cônes les plus venimeux. Sa répartition est de la Mer Rouge et ouest du Pacifique sud jusqu'à l'Australie. Taille : jusqu'à 15 cm. Source : https://fr.wikipedia.org/wiki/Conus_textile

⁸ Source: *Extraction, purification and analysis of conotoxin of Conus textile captured from Persian Gulf and the investigation of analgesic effects of conotoxin in an animal model*, Nasim Tabaraki, Delavar Shahbazzadeh, Ali Mashinchian Moradi, Gholamhossein Vosoghi, Pargol Ghavam Mostafavi, page 90, <http://aquaticcommons.org/19066/1/37856.pdf>

The secretions from the venom duct were dried by Speed-Vac, then homogenized to a fine powder and weighed. Peptide extraction was performed with 95% solvent A (0.1% v / v TFA / aq.) And 5% solvent B (90/10 v / v MeCN / 0.08% v / v of TFA / aq.), with samples typically representing 1 mg ml⁻¹. The samples were treated with ultrasound and then centrifuged [vortexed] for 10 min. The extracts were then centrifuged at 12,000 xg for 10 min. The resulting supernatant was removed, dried by Speed Vac, weighed and then stored at -20 ° C until use. All extracts were resuspended in the above solvent at 1 mg ml⁻¹, sonicated (5 min.) And re-centrifuged (at 12,000 for 10 min.) Prior to chromatographic separation and I MS analysis. "

"MV from *Conus Textile*, not captive, was obtained within 24 hours of collection in the field. The envenomation was stimulated by the presence of live gastropods *Morula marginalba*, *Strombus luhuanus* or *Cypraea caputserpentis*. Upon extension of the proboscis of the cone snail, **a pipette with an L 5000 tip was depressed and placed near the upward facing opening near the prey's foot**. On the subsequent firing of the radula, envenomation was observed by the release of excess venom, typically as a visible "cloud", which was carefully aspirated to avoid dispersal. The collected MV was acidified (1% v / v TFA) and frozen (-20 °C) or lyophilized for further analysis. Aliquots of seawater (white) were collected and treated identically ».



« **Milking the molluscivorous cone snail *Conus marmoreus***. A) The prey (left) is placed at the front end of a *Conus marmoreus* specimen to induce proboscis extension. A centrifuge tube covered with parafilm and a piece of external tissue from the prey's foot is then presented to the tip of the extended proboscis. On contact, a radula is usually drawn and the venom is injected into the collecting tube. Rapid centrifugation is then performed to sediment the venom droplets at the bottom of the tube before storage at -20 ° C. B) the reserve of raw venom injected from several samples of *C. marmoreus*. C) the white color of the injected venom seems to be due to the presence of long oval granules of ~ 25 to 30 μm, as seen under a light microscope (x 40)⁹ ».

« Animals are difficult to keep in captivity. The cone snails from Bingham Farm currently consists of a single reservoir of snails of a single species, *Conus purpurascens*. Although it bears the "Danger - poisonous snails" label, at first glance, the tank appears as if it contains only dirty brown sand. But Bingham calls it "Hilton cone snail". He sprinkles the water in the

⁹ Cf. *Deep Venomics Reveals the Mechanism for Expanded Peptide Diversity in Cone Snail Venom*, <http://www.mcponline.org/content/12/2/312/F4.expansion>

tank with a little fish flavored water. “Where do you expect to walk away?” He asks? The buried mollusks smell the hint of a meal and emerge over the next two minutes, until all nine are visible.

Best of the best

Once the milking time is over, the next task is to place the samples in a high pressure liquid chromatography machine, which separates the toxins from the venom and produces a printed output with peaks that correspond to individual compounds.

For Bingham, these results illustrate the superiority of cone snail farming over the traditional approach of extracting toxins from dead molluscs. Environmentalists, meanwhile, are worried that collecting animals to supply as material for neuroscience research and drug development could have a detrimental effect on wild populations. In October last year, Eric Chivian, director of the Center for Global Health and Environment at Harvard Medical School in Boston, wrote a letter to *Science* ⁸ expressing concern about the overuse of cone snails for the scientific research. “No one really knows the number of cone snails that are killed,” Chivian says¹⁰ ».

9 The extraction of venom conotoxins

Either sell the raw venom (which is the easiest). It is up to large laboratories to extract toxins from the venom (the simplest solution for us). Lower added value.

Either the toxins are extracted from the venom => Very high added value (promising) but large **financial investment** (in **chromatograph** and **mass spectrometer**).

Extractions of key (useful) molecules from the venom:

« To turn a toxin into a drug, you first have to characterize that toxin, that is, find the shape of that key, in order to determine which lock it is capable of activating. To do this, it must be isolated from the venom, made up of hundreds of different keys. The job is arduous. To perform this extraction, the raw venom is injected into an instrument - a **chromatograph** - which will separate the various constituents of the venom according to their physicochemical characteristics such as their electrical charge or their affinity for water. This operation will be repeated the number of times necessary in order to obtain the molecule of interest with the desired purity.

Once the toxin is isolated, characterization of its structure begins. Another measuring instrument, the **mass spectrometer**, will not only make it possible to determine the molecular mass of the compound but also to go back to the sequence with which the amino acids (the beads) are arranged within the molecule (the necklace).

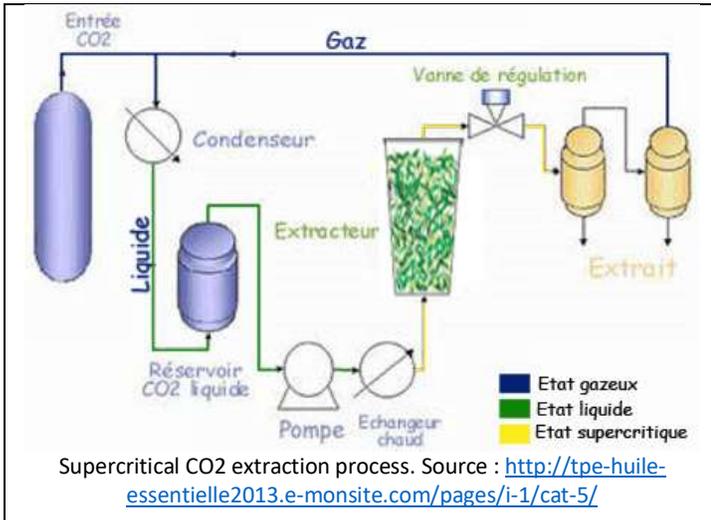
This information is essential because it constitutes the identity card of the molecule (the key) and also gives some indications on the receptor (the lock) which will be targeted by the molecule in question. Once the structure of the toxin has been obtained, the researchers try to determine the biological activity of the toxin, that is, to know which lock will correspond to the key and what the effect of the key will be. activation of this lock on the body. For these tests, the toxin must be reproduced chemically since the amounts extracted from poisonous animals are quite low. For example, venom must be taken from 200 to 300 black widows to obtain 1 mg of dry venom. The work of collecting and maintaining animals as well as the risk they present are also major drawbacks to natural production»¹¹.

To lower the price of extracting key molecules?

Suggestion: either one can imagine the extraction (or entrainment) of conotoxins and conokines, via a fluid such as alcohol (?) Or another solvent (?) Or else the extraction by pressurized fluid, subcritical water supercritical fluid, supercritical CO₂ or ultrasound assisted etc.

¹⁰ *One slip, and you're dead*, Laura Nelson, Nature, <http://www.nature.com/nature/journal/v429/n6994/pdf/429798a.pdf>

¹¹ Les venins d'animaux _ nouvelle panacée ? Université de Liège, <http://reflexions.ulg.ac.be/upload/docs/application/pdf/2013-07/ath291maiechterbille.pdf>



Supercritical CO2 extraction workshop at the Grasse site (Credit: Firmenich). Source : <http://www.processalimentaire.com/Ingredients/Firmenich-mise-sur-l-extraction-au-CO2-supercritique-18700>



A mass spectrometer for proteomic analyzes

Analyzes carried out internally: organoleptic, physico-chemical analyzes.
 Analyzes performed externally: gas chromatography.



Bottles of 1 gr for the packaging of conotoxins (image of Bachem).

10 Who to contact and how to set up the sector

Suggestion : The interested laboratories should be contacted beforehand, which buy, produce and / or resell the conotoxins, to set up the chain together:

Alomone Labs Limited¹² (buy venom, sell conotoxin), <http://www.alomone.com/>
Sigma-Aldrich¹³ (sell conotoxin), <http://www.sigmaaldrich.com/>
Bachem¹⁴ [production of peptides] (sell conotoxin), <http://www.bachem.com/>
Chemos GmbH¹⁵ (sell conotoxin), <http://www.chemos.de/>
Bioconus, SR, Monash University, Melbourne, Australie (sell pure venom), <http://www.bioconus.com/>
((Scientific Sales¹⁶ [a reseller], Inc. Oak Ridge, TN, <https://www.scisale.com/>(((

Bachem Americas sell 1 mg of **Mu-Conotoxin GIIB** at **\$1,134.95**¹⁷. Thus **Bashem** seems to be the concurrent. Idem for **Bioconus**.

The only institutes (to our knowledge) which currently study cone venoms (or even produce them?) are:

- 1) **Bioconus**, SR, Monash University, Melbourne, Australie, info@bioconus.com, <http://www.bioconus.com/>
- 2) **Institute for Molecular Bioscience** (research Institut in Australia) - The University of Queensland (Brisbane).
Addresses: a) St Lucia QLD 4072, Australie, b) St Lucia, Brisbane, Australie
Phone: +61 7 3346 2100, Fax: +61 7 3346 2101, email: imb@imb.uq.edu.au , Site: <http://www.imb.uq.edu.au/>

(For the moment, the Institut Pasteur, <http://www.pasteur.fr/> does not seem to be doing research on conotoxins).

11 The appearance of the venom

« *Appearance of Venom: Dissected venom gland extracts may be opaque, milky white to sulfur yellow. The processed venom is clear unless hydrophobic peptides, such as S-conotoxins, are present. The venom from trafficking consists largely of proteins and low mass organic molecular compounds with peptides being the dominant component; most of the volume of venom from milking is equivalent to seawater. Lyophilized or lyophilized material, both native (desalted) and synthetic, can be fluffy / velvety, electrostatic and hygroscopic in nature.*

Venom Solubility: Synthetic and extracted conotoxins / conopeptides are soluble in water, producing a slightly translucent solution which may foam when stirred. Native venoms contain small, insoluble particles or granules [59-61], these being strongly pronounced in venom from duct dissection as well as other cellular debris. Whole venom extracts require centrifugation and secondary extraction. This process results in a supernatant containing a translucent peptide. To achieve maximum solubility, small amounts of immiscible organic solvent such as acetonitrile (5% v / v), are added to the aqueous solvents; The use of ultrasound [sonication] can help dissolve peptide materials.

Stability of Venom: When stored properly under laboratory conditions, conotoxins / conopeptides are very stable compounds ».

12 Quantity of venom extracted, by cone milking

The venoms from the milking of *Conus purpurascens* (n = 100 milkings) were measured in volume ranges of **6-480µL** (moyenne ± Std Dev .; 56.21µL ± 105.21µL).

Cf. Milked venoms from *C. purpurascens* (n = 100) were measured to have volume ranges of 6–480µL (Mean ± Std Dev.; 56.21µL ± 105.21µL). Source : <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3565753/>

¹² Cf. a) <http://www.absave.com/products/buy-omega-conotoxin-gvia-c-300> , b) <http://www.absave.com/suppliers/405-alomone-labs-limited?page=87>

¹³ Cf. <http://www.sigmaaldrich.com/catalog/product/sigma/c1676?lang=fr®ion=FR>

¹⁴ [http://shop.neobits.com/bachem americas h 9015 1000 mu conotoxin giiib bachem each 1mg 1032629404.php](http://shop.neobits.com/bachem%20americas%20h%209015%201000%20mu%20conotoxin%20giiib%20bachem%20each%201mg%201032629404.php)

¹⁵ Cf. [https://www.buyersguidechem.com/chemical_supplier/a-Conotoxin IMI](https://www.buyersguidechem.com/chemical_supplier/a-Conotoxin_IMI)

¹⁶ Cf. a) <https://www.scisale.com/VWRH-9015.0500BA.html>, b) <https://www.scisale.com/VWRH-9015.1000BA.html>

¹⁷ Source [http://shop.neobits.com/bachem americas h 9015 1000 mu conotoxin giiib bachem each 1mg 1032629404.php](http://shop.neobits.com/bachem%20americas%20h%209015%201000%20mu%20conotoxin%20giiib%20bachem%20each%201mg%201032629404.php)

Annex: The geographic cone, by Miranda Hall



13 Distribution area

The geographic cone snail, *Conus geographus*, is indigenous to the tropical and subtropical Indo-Pacific regions, found specifically along the northern shores of Australia, ranging from the west coast (Brisbane, Queensland), central (Darwin, Northern Territory), and east coast (Exmouth, Western Australia). Rare sightings (and recorded fatalities) have also been reported in New Caledonia. (Chadwick, 2011; Estival, 1981; Walls, 1978).

Biogeographic Regions: native: australian, indian ocean, pacific ocean.

14 Habitat

Geographic cone snails are most commonly found in the sublittoral epipelagic zone. Their surrounding habitat includes living or fragmented coral reefs, and sandy regions within tidal zones. They are less commonly found in deeper waters. (Chadwick, 2011; Estival 1981; Lim and Wee 1992).

Regions: tropical habitat

Aquatic biomes: coastal benthic (average depth of 7 m).

Other habitat characteristics: intertidal or coastal

Depth:

0-200 m

0.00 to 656.17 ft

15 Physical description

A calcareous, smooth shell covers the mollusk's soft body. The shell spire is obconical (having a length of less than or equal to 10% of the entire structure) featuring coronation (small bumps) at and above the shoulder along the edges of the larger whorls. The spire is concave with smooth sutures and a prominent point at the protoconch apex. The body whorl terminates in an elongated aperture that has a width of about 1/3 of the overall shell width. The outer shell's coloration ranges from ground colors of white, cream, or rose pink overlain with brown or red mottled patterns arranged in horizontal spirals along the body whorl. The shell is covered with a thin yellowish layer of protein-based material called the periostracum, forming tufts on the spire, on the spiral rows, and along the body whorl, following the sculpture of the shell. This protein covering gives the cone a roughened appearance.

The most obvious features of the geographic cone snail are the foot, which extends from the aperture; two small eyes borne on eyestalks, and two features associated with their feeding habits: the proboscis, an extendable protrusion in the oral region that expands to swallow its prey, and the siphon, an extension of the mantle tissue, used for chemoreception of its prey. The cone snail uses an elaborately sculpted, hollow radular tooth (housed in the proboscis) as a harpoon to

incapacitate its prey. Venom glands produce deadly toxins and digestive enzymes, and these are injected into the snail's prey through the radular tooth. (Walls, 1978).

Other physical characteristics/features: ectothermic, heterothermic, bilateral symmetry, venomous
Sexual dimorphism: similar sexes (sexes alike).

Range mass	Average mass	Range length
13,3 à 62 g	38,8 g	70 à 150 mm
0,47 à 2,19 oz	1,37 oz	De 2,76 à 5,91 inches

16 Development

Very little is known of the cone shell's natural history from neoteny to adulthood. After the mating ritual, clusters of egg sacs (about 40 eggs per sac) are extruded and attached on a suitable hard surface. The eggs incubate within their capsule for 10 to 15 days before maturing into the larval stage. After twenty days, the transparent shells and bodies are visible, and they break from their capsules and drift in the plankton as meroplanktonic veliger larvae (a temporary zooplanktonic stage of the lifecycle). The larval diet is unknown, but assumed to be smaller plankton. Only a low percentage of cone snail larvae survive to metamorphose into benthic juveniles, and even fewer survive to reach adulthood. Planktonic survival rate is affected by weather and oceanographic factors such as water temperature, salinity, and ocean currents, as well as abundance of secondary consumers in the water column. (Cruz, et al., 1978; Estival, 1981).

Development - Life cycle: metamorphosis, undetermined growth.

17 Reproduction

Published observations on breeding behavior have been made in aquariums and direct observations on love rituals or competition for a mate in the wild is lacking. Some researchers have hypothesized that male cone snails may exhibit territorial behavior to ensure access to potential mates.

During copulation, the male mounts the female using his foot. It inserts about 2/3 of a ribbon-like organ called the point (analogous to a penis) into the female's opening near the anal notch. This position is maintained for at least 15 minutes before the male retracts his point. Two to three days later, the female lays several egg capsules on a hard surface. No information is available as to whether mating occurs separately or repeatedly, during its lifespan (Cruz et al. 1978; Estival 1981).

Mating system: monogamous

Sexual maturity can occur between 6 to 12 months. After mating, the female lays her egg capsules on a hard, smooth surface, where they develop into larvae within twenty days. (Cruz et al., 1978; Estival 1981).

Main characteristics of reproduction: iteroparous; seasonal reproduction; gonochoric / gonochoric / dioecious (separate sexes); sexual; fertilization (internal); oviparous.

- **Breeding interval**
Geographic cone snails breed once a year.
- **Breeding season**
Between April and September when the waters are warm.
- **Range number of offspring**
1000 to 5000
- **Average number of offspring**
2500
- **Range gestation period**
2 to 3 days
- **Average gestation period**
3 days
- **Range time to independence**
15 to 25 days
- **Range age at sexual or reproductive maturity (female)**
6 to 12 months
- **Range age at sexual or reproductive maturity (male)**
6 to 12 months

There is no further investment in parental care after eggs are laid, as is the case for most marine invertebrates. (Cruz, et al., 1978)

Parental Investment: no parental involvement; precocial; pre-fertilization.

18 Lifespan/Longevity

There is no recorded data of any longevity in the wild or captivity¹⁸.

19 Behavior

The geographic cone is inactive during the day, burying itself in the sand to camouflage itself for both protection and hunting. At night the snails are active to hunt food. Some researchers hypothesize that male cone snails may exhibit territoriality in their hunting grounds. (Cruz, et al., 1978; Johnson and Stablum, 1971).

Key Behaviors: nocturnal; motile; solitary.

20 Home range

There is no recorded data of any home range information (Johnson et Stablum 1971).
Coral plateaus¹⁹.

21 Communication and Perception

The three main methods of perception used are visual (eyes to detect light), tactile (using its foot) and chemoreception (detecting dissolved chemicals in the water). It is likely that potential mates are detected using all three of these senses (Cruz et al. , 1978 ; Johnson et Stablum, 1971).

¹⁸ Selon cette vidéo : <https://www.youtube.com/watch?v= SvYnNR9byU>

¹⁹ Ibid.

Communication Channels: visual; tactile; chemical

Perception Channels: visual; tactile; chemical

22 Food habits

Conus geographus is nocturnal, hunting at night when its fish prey are the least active or at rest. They crawl on top of the substrate, or crawl while buried beneath the sand. Diet consists of small (30 to 50 mm) and medium (100 to 130 mm) sized fishes that fit into its rostrum (mouth). Larger snails (80 to 87 mm) are able to capture and ingest larger fishes between 130 and 140 mm in length. (Cruz, et al., 1978; Johnson and Stablum, 1971)

Observations show that snails hunt with two methods used by other *Conus* species: the hook-and-line method and the net-hunting method. In the hook-and-line method, the snail slowly approaches its prey, waving its proboscis like a lure to attract the fish before stinging the fish with its radula. The fish jerks violently for a few moments and is injected with a paralyzing excitotoxin venom that stiffens the fish, allowing the cone snail to swallow it whole. Several hours or days later, the snail regurgitates the fish's bones. Another method is net-hunting, wherein a fish is engulfed in one mouthful before being harpooned with the radular tooth. (Chadwick, 2011; Cruz, et al., 1978; Johnson and Stablum, 1971)

Primary Diet : carnivore (piscivore)

Animal Foods: fish.

23 Predation

Predators during its larval period include nektonic fishes and filter-feeding invertebrates that consume zooplankton. As an adult, the only enemies the geographic cone snail may fear are mollusk-eating vertebrates such as sea turtles and rays, and human shell collectors. The radular tooth also serves as a defense mechanism against potential predators. (Johnson and Stablum, 1971)

Anti-predator Adaptations: cryptic

Known Predators

- large fish
- humans

24 Ecosystem roles

The geographic cone snail is a piscivore, thus it influences the ecosystem dynamics of coral reef populations of small fish species. (Chadwick, 2011; Cruz, et al., 1978; Johnson and Stablum, 1971).

25 Economic Importance for Humans: Positive

Conantokins ("sleeper peptides") from the geographic cone snail are a complex mix of short-chain peptides that affect a number of neural receptors in fish and mammals. The potential therapeutic and economic benefits from conantokins have great potential. Conantokins are antagonists to the nicotinic acetylcholine receptors (the means by which the cone snails paralyze their prey) and N-Methyl-D-aspartic acid (NMDA) receptors, which (in humans) are involved with pain reception, drug and alcohol withdrawal symptoms, memory, and learning. Con-G, one of the conantokins from the geographic cone snail, is a potent analgesic, particularly for nociceptive pain (pain that warns the body of tissue injury or other serious damage). Con-G specifically acts on the NR2B NMDA receptor subtype, which means it is more selective than morphine

for treating chronic neuropathic pain found in patients suffering from cancer, arthritis, shingles, diabetes, and AIDS. Therefore, smaller doses can be used, and Con-G does not seem to be addictive or to have side effects in the therapeutic dose range, unlike morphine. In addition, since NMDA receptors are involved with memory, conantokins can potentially be used in treating Alzheimer's and Parkinson's diseases, and possibly used as anti-convulsants in epilepsy or as a means of alleviating drug-induced withdrawal symptoms. In addition, Con-G has been found to act as a neuroprotective agent in brain ischemia from strokes. (Jimenez, 2009; Livett, et al., 2004; Sprackland, 2005)

Positive Impacts: source of medicine or drug.

26 Economic Importance for Humans: Negative

The conantokins in one sting can kill 15 people. Symptoms include an excruciating pain at the penetrated area, much worse than a bee's sting. As the pain fades, numbness soon sets in, followed by dizziness, slurred speech, and respiratory paralysis. Death can follow within half an hour afterward, but this is rare. Presently, there is no known anti-venom; applied pressure on the wound, immobilization and artificial respiration (mouth-to-mouth resuscitation) are the only recommended treatments for the victim. (Chadwick, 2011)

Negative Impacts : injures humans (bites or stings venomous).

27 Conservation status

This species is not listed as vulnerable, threatened, or endangered.

IUCN Red List
Not Evaluated

US Federal List
No special status

CITES
No special status

State of Michigan List
No special status

28 Contributors

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This material is based upon work supported by the National Science Foundation Grants DRL 0089283, DRL 0628151, DUE 0633095, DRL 0918590, and DUE 1122742. Additional support has come from the Marisla Foundation, UM College of Literature, Science, and the Arts, Museum of Zoology, and Information and Technology Services.

The ADW Team gratefully acknowledges their support.

Source: Hall, M. 2011. "*Conus geographus*" (On-line), Animal Diversity Web. Accessed September 01, 2016 at http://animaldiversity.org/accounts/Conus_geographus/

29 Aquarium with a locking system

Karapas Aqua (habitat for aquatic turtles)

The "aqua" habitats are sold with the following equipment:

- A grid with a locking system: positioned on the top of the terrarium, the grid provides security for both the risk of escape and for external intrusions (cats, children, etc.) or falling objects.
- Four locking corners: locking the grille at all four angles, preventing unintentional removal.
- An internal filter: powerful and adjustable, it keeps the water clean, the turtles producing a lot of waste.
- A platform (or two depending on the model): water turtles need to come out of the water from time to time. The supplied platform can be positioned as desired using a powerful suction cup that holds it perfectly.

Karapas Aqua habitats are available in size 60cm, 80cm or 100cm.

These different dimensions are available to adapt to the sizes of the animals, the ideal choice being to offer a length 8 to 10 times greater than that of the turtle's breastplate.

Source: <https://www.zolux.com/tout-nouveau-tout-beau/karapas-aquariums-terrariums>

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31 Plan

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