Whale Labs

# Introduction to Bee Venom Collection

Compiled By Whale Labs Pty Ltd

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# Preface

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Information was sourced through readings of historical accounts of bee venom collection, as well as a study of which tools were used. All text is original wording by Whale Labs Pty Ltd, and unless otherwise noted, all figures are also property of Whale Labs Pty Ltd.

Data used within graphs and tables are courtesy of users of the Whale Labs Bee Venom Collector, as well as in-house testing of approximate yields, and observations about behaviours and temperaments of the hives tested on. All information presented in graphs and tables is intended to be used as an approximation or rule-of-thumb guide rather than hard facts about what to expect every time.

Please contact Whale Labs Pty Ltd if you have any questions not answered in this document. This will help us ensure the scope of topics covered within is as wide as possible and the information is as accurate as possible.

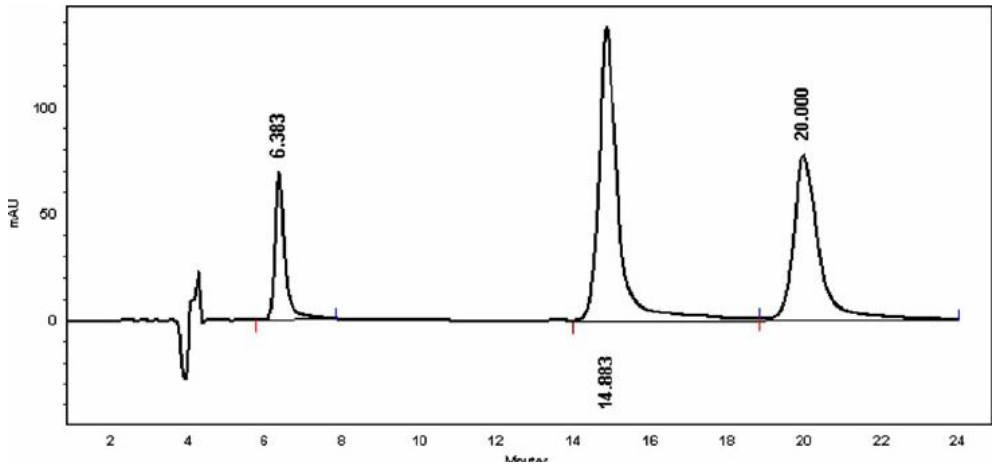
# Purpose

This document is intended as a reference for those interested in collecting bee venom. While this document is prepared by Whale Labs, which retails the BVC Mk1 Bee Venom Collector at the time of issue, the information provided within is intended to be used by any person, regardless of which brand of bee venom collector they purchase. It can be difficult to find sufficient information about the process of collecting bee venom in one place, which this document aims to address.

At the time of issue, Whale Labs provides an HPLC (High-Performance Liquid Chromatography) testing service, regardless of which brand of collector was used to collect the bee venom. This method uses the Water Tunable Absorbance Detector to test the contents and purity of the bee venom, though Whale Labs also can do additional testing upon request, including TLC (Thin Layer Chromatography), Gel Electrophoresis, and PCR analysis and sequencing.

| Substance Group | Component | % of dry weight |
|---|---|---|
| Proteins (Enzymes) | Phospholipase A2 | 10-12 |
| | Phospholipase B | 1 |
| | Hyaluronidase | 1-2 |
| | Phosphatase | 1 |
| | α - Glucosidase | 0.6 |
| Peptides | Melittin | 40-50 |
| | Apamine | 2-3 |
| | MCD peptide | 2-3 |
| | Secapine | 0.5-2 |
| | Tamine | 1-3 |
| | Minimine | 2 |
| | Adolapine | 0.5-1 |
| | Procamine A, B | 1-2 |
| | Protease inhibitor | 0.1-0.8 |
| | Tertiapine, cardiopep, melittin F | 1-2 |
| Phospholipids | | 1-3 |
| Biogenic amines | Histamine | 0.5-2 |
| | Dopamine | 0.2-1 |
| | Noradrenalin | 0.1-0.5 |
| Amino acids | Aminobutyric acid, α-amino acids | 1 |
| Sugars | Glucose, fructose | 2-4 |
| Volatiles (pheromones) | Complex ethers | 1-0 |
| Minerals | P, Ca, Mg | 3-4 |

Example readout from an HPLC analysis

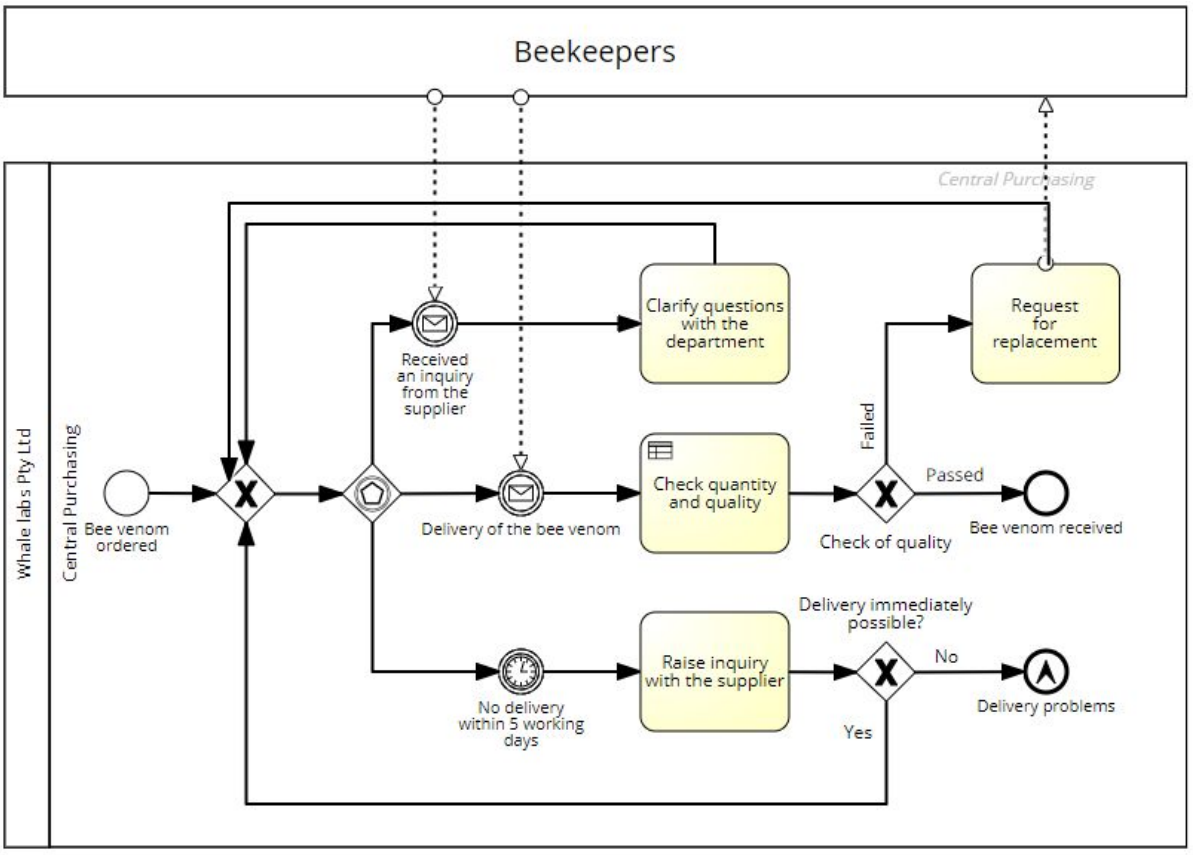



Example graph from an HPLC analysis

Certain cosmetics and pharmaceutical buyers of bee venom require a specific level of purity (with pharmaceutical requiring the highest) and the most comprehensive way of conveying this information is through a report compiled after an HPLC test. This is because they need to guarantee they are purchasing bee venom with a certain melittin/apamin content with minimal contamination from pollen or dirt. It is typically not necessary to test every batch of bee venom once a purchasing contract has been arranged.

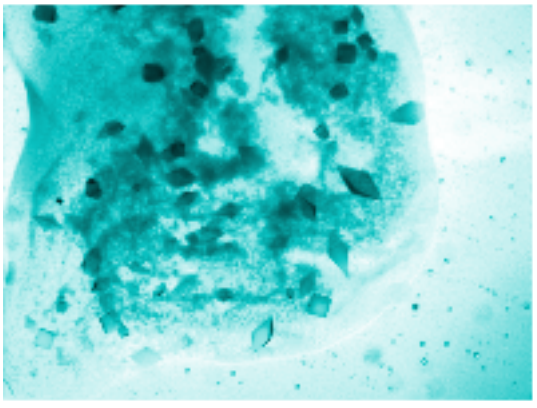
Also, at the time of issue, Whale Labs is the only retailer of a bee venom collector which periodically buys bee venom back (within Australia). Again, it is not a requirement for the venom to have been collected with a Whale Labs bee venom collector. This is an extra assurance for those who get involved with collecting bee venom but cannot line up suitable buyers for it. Currently this is not a steady purchasing stream, rather operating through work orders which are then able to be fulfilled. This demonstrates that Whale Labs truly believes in the applications of bee venom and is not retailing a product which ultimately has no end use.

If you collect bee venom and would like to inform Whale Labs of your intention to sell, please notify the company at *business@whalelabs.com.au* to be able to be considered for future work orders. The typical process we use for buying bee venom is illustrated below.




## What is bee venom?

Bee venom, known as apitoxin, is a group of polypeptides and proteins in a clear colourless fluid which is produced in the venom sac of the *Apis mellifera. W*hen the bee stings a human's skin, the venom sac is separated from the body creating a vacuum, causing the venom to be deposited.



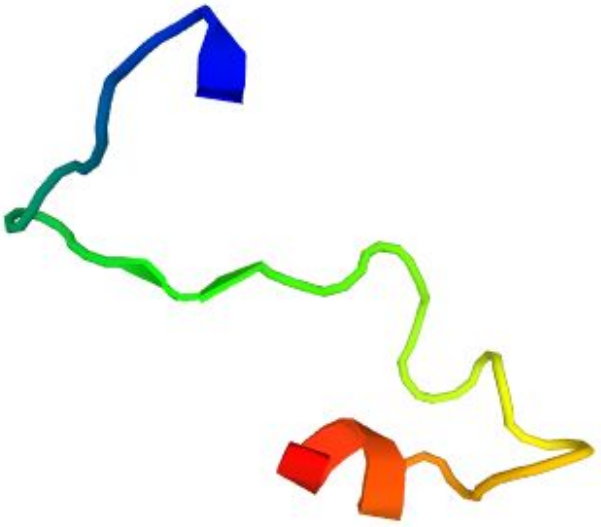
Apitoxin Crystallised

# What are apitoxin's uses?

**Pharmaceutical**

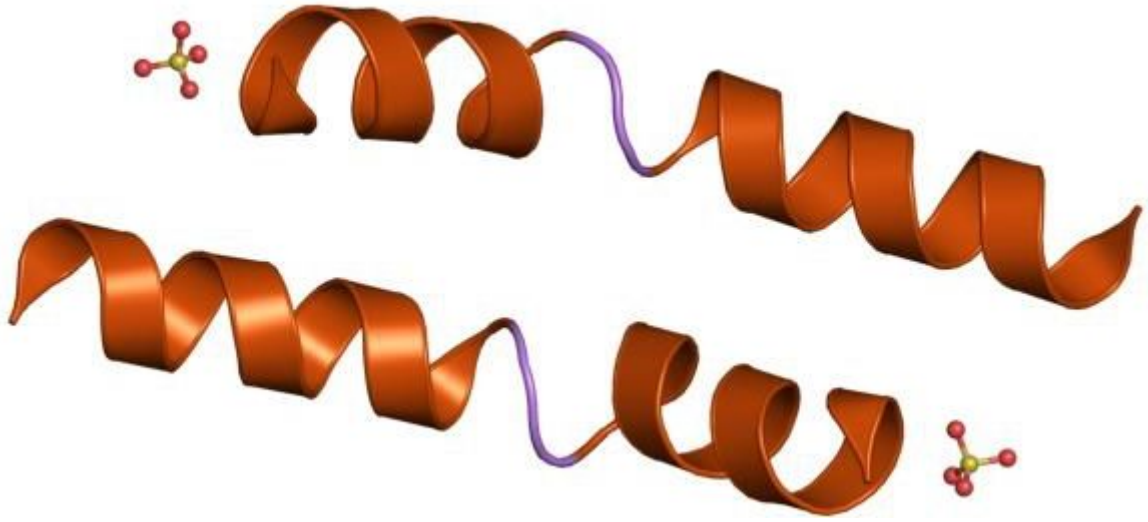
The pharmaceutical benefits of individual compounds in apitoxin are well known. The small polypeptides within apitoxin  are a well-researched group used in everything from dementia research to potentially being a smart drug which can increasing learning and memory by a factor 1.  The larger proteins such as melittin are used for tumour suppression.

Within pharmacy, different compounds of bee venom are currently being used in research for treatments of dementia and HIV.  One of these compounds is apamin. Apamin is a short 18-length amino-acid which is small enough to cross the blood brain barrier. Apamin docks with SK channels in the brain leading to higher neuron activity. The SK channel is expressed throughout the body and they are in control of excitability of neurons and synaptic transmission. When these channels are blocked by apamin, they lead to more excitability. When the channels are open, they lead to the neuron activity being slowed. This lead to conditions such as cardiovascular disease(4)(Mingxia Gu,2018) and neurological disease (5(A M Dolga, 2014) the significant role of this Ca2+-activated K+ channel can not be understated.



The process of using honey bee products within medical treatment is known as Apitherapy, and this includes using bee venom.

The most common use of bee venom within apitherapy is for treatment of arthritis. Adolapin is useful for treating arthritis.



**Cosmetic**

Apitoxin is hemotoxic, meaning it reduces blood clots and results in more blood flow. For this reason it has been shown to have a regenerative effect in in damaged tissue.

Bee venom can be included in cosmetic products in very low quantities to provide a beneficial effect with minimal risk of causing negative reactions. These cosmetics can include creams, soaps, lip balms and more.

The use of bee venom as a cosmetic dates as far back as Hippocrates (460-370 BC), Aristotle (384-332 BC) and Galen (150-200 AD), who prescribed the use of bee venom as a cure for baldness. The ancient Greeks considered bee venom as a medicine and believed that bee venom taken regularly resulted in human life being prolonged. Earlier thinkers such as Homer, Pythagoras, Ovid, Democritus, Hippocrates, and Aristotle mentioned that people should eat honey and use bee venom to preserve their health and vigour.

## Biological product legislation

Currently within Australia (as at June, 2019), there is no legislation restricting the collection, or the import/export of a biological product matching the definition of bee venom.

However, this should not understate that bee venom in its concentrated form is very toxic and poses a health hazard if ingested, inhaled or absorbed. Precautions need to be taken to ensure the safe handling of bee venom, which is covered later in this document.

# History of collecting bee venom

The oldest form of collecting bee venom for later use was to hold a bee with a pair of tweezers and force their barb into a rubber mat, causing it to embed, then pulling the bee away to tear the barb off. This is an extremely inhumane process and guarantees the death of the bee in exchange for only approximately .1mg of venom (1/10000$^{th}$ of a gram).

The wire grid method later made an appearance, though still the rubber mat was used and the bees' barbs were still ripped out. This made the collection of bee venom more efficient, but also resulted in devices that were more efficient at *killing* the bees.

The wire grid method works by having alternating positive and negative wires, and when a bee crosses the two wires, they complete the circuit and receive a shock. Initially it was decided that much harsher voltages were more suitable for collecting venom, as they solicited far more aggressive responses from the bees.

Later the rubber mats were replaced with rigid plates, typically glass. This provided the much needed benefit of not allowing the bees to embed their barbs into anything, though also resulted in less venom being collected. As the barb does not embed, and the bee's venom sac is not ripped out, there is no vacuum created to deposit all of the venom. The bee will instead only deposit a tiny fraction of its venom when it stings.

The widely accepted solution to this was to simply subject the bees to harsher shocks to try to get them to rapidly sting the rigid plates, though this places incredible stress on the bees. Long term this can cause high die-off rates, sometimes pushing over half of the hive's population.

Similarly, some devices were placed inside of the hive, as this was a way of guaranteeing more bees landed on it. This turned out to be very damaging to the health of the hives, as the pheromone which triggered the defense response from the bees would linger, even when the devices were switched off, causing great stress on the bees. Some of these devices are still widely used in Asia.

It is recommended that a bee venom collection device be external to the hive, as this allows the device to be removed once collection is complete, reducing the exposure a single hive will have to the pheromone.

Several products on the market now recognise that the health of the bees needs to be preserved, but do not factor in just how many individual deposits of venom a bee needs to make to get the most out of a collection session - they will only safely operate for around 45 minutes, which is often not enough time needed for the bees to deposit their venom.

By ensuring a device can safely operate for 1 to 2 hours per hive, or as long as is required for the bees to lose interest in defending the hive from the device and depositing venom, much more venom can be collected.

# Safest practice of collecting bee venom

The health of the bees cannot be compromised in the process of collecting bee venom, otherwise the hive's overall productivity will diminish. At the same time, it is important to consider methods that will allow you to achieve the highest collection rates of bee venom possible.

The ***Whale Labs BVC Mk1*** is designed to induce as little stress in the bees as possible. By subjecting the bees to only very weak pulses, you are able to allow them to sting the collector for a longer period of time.

This is crucial in ensuring the highest rates of venom collection are achieved, as a bee only deposits a tiny fraction of its venom when it stings a rigid plate. A bee needs to sting the plate many times to deposit all of its venom, and by allowing them to do this over an extended period of time, rather than over a quick burst in a stressed frenzy, their health is preserved over many collection periods throughout the year.

**Negative stresses on bees**

While the goal is to ensure the bees are subjected to as little stress as possible, it is true that no matter the method for collecting venom, the bees will undergo some stress. The pheromone which sends the hive into a defence mode naturally will cause the bees to stress. The electric currents subjected to the bees are also another factor in causing stress.

For this reason not only is it important for a bee venom collector to be removeable after it has been used on a hive (rendering all internal or permanently-installed devices unsuitable) it is also crucial to regulate the electric currents subjected to the bees.

The ***Whale Labs BVC Mk1*** does not emit a permanent electric output, rather powers on for approximately 5 seconds and switches off for 5 seconds before powering on again. This allows the bees to have short breaks between pulses, regulating their level of stress.

# Collecting bee venom with a BVC Mk1 (or any other collector)

**What to expect**

It can be said that as a superorganism the honey bee is predictable and methodical, but every hive is unique and will react to different factors in different ways. Introducing a hive to venom collection methods is no different and in some cases a little bit of extra effort will be necessary to convince them to interact with a collector.

**Methods for getting the hive to interact with a bee venom collector**

The most crucial factor in collecting bee venom is the pheromone. When a new, clean and unused collector is placed in front of a hive, with no pheromone on it, bees likely

will not pay any attention on it. (Refer to *Taking care of your bee venom collector* on how to clean the device without removing the pheromone.)

In order to get the pheromone onto the collector, first bees must land on it. If the bees are currently doing pollinating routes, they may simply enter the hive directly without ever landing on the collector, even if it is placed right against the entrance to the hive.

You can create an artificial bottleneck at the entrance of your hive, by blocking part of the entrance, in order for a greater amount of bees to accumulate outside. This will typically cause a few bees to land on the collector to rest. Once a hive has started interacting with the bee venom collector sufficiently, it's recommended to unblock the entrance to allow more bees to exit the hive (while wearing sufficient protective equipment).

Additionally, though not recommended and not always effective, tapping/knocking on a hive can cause more bees to leave the hive and subsequently land on the collector. This is not typically as effective as restricting entrance to the hive and can have a negative effect on the temperament of the bees. Knocking on the hive can also potentially have other unforeseen side effects such as the queen bee getting crushed or the cracking of cells.

Sometimes the first five bees to land on the collector will be enough to release a sufficient amount of pheromone to entice the rest of the hive to attack, sometimes it may take 100 or more. The effect can happen within the first 30 seconds, or it could take up to 20 minutes. If it's taking more than 20 minutes, the extra methods such as bottlenecking must be employed.

**Hive sizes & venom quantity**

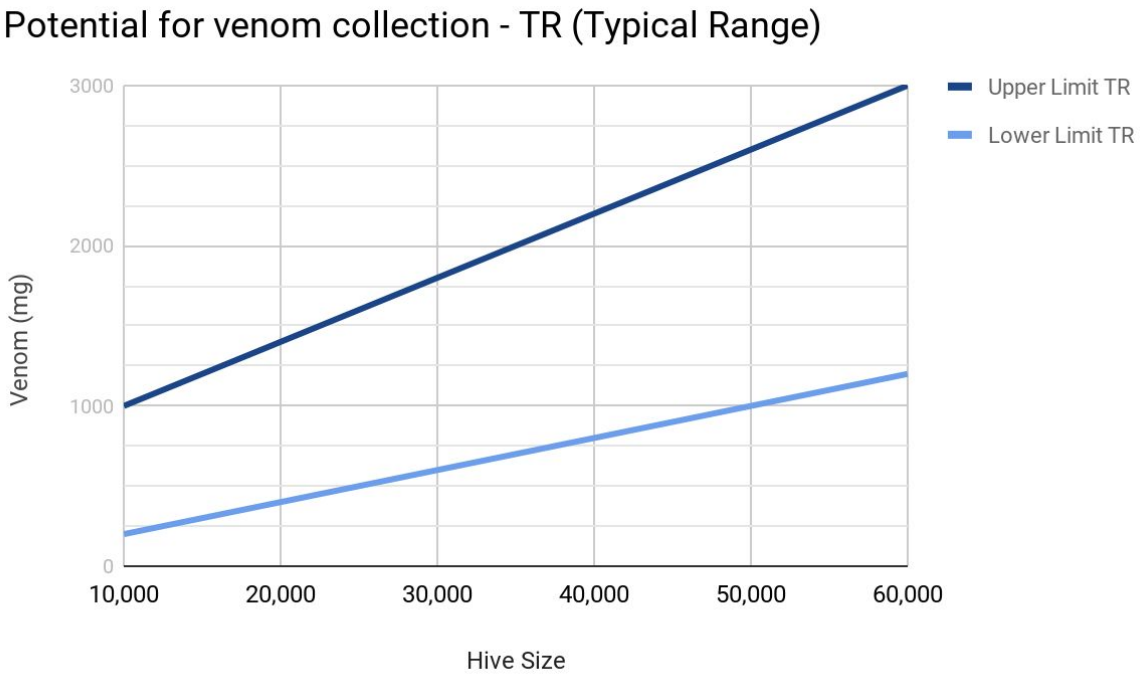
'Typical' honey bee hives have between 10,000 and 60,000 bees in them. The number of bees in a hive will directly influence how much venom it is possible to collect.

It should be kept in mind that these numbers are based off of honey bee hives within Australia and the numbers are subject to change depending on the season, as covered in the following segment.

An average honey bee holds around .1mg of venom, or 0.0001 grams. This means it will take 10,000 bees to fully deposit their venom to create a full gram. The more likely rule of thumb to use is that a bee will only deposit a third to half of its venom over the course of collection, requiring about 20-30,000 bees to create a full gram. Sometimes the bees, particularly in summer, are able to deposit closer to their full venom stores - on hives containing approximately 15-20,000 bees, Whale Labs has managed to collect a full gram at times during the summer, where the bees have deposited venom in excess of two hours.

However, some hives do not maintain engagement in a collector for two hours, rather may only deposit venom for 45-60 minutes. This can essentially halve the amount of venom collected.

A hive should be able to be collected from around twice a week, though again, this is subject to the rate at which a hive can replenish its venom. You can still use the collector 2-3 times a week, but this may not prove any more profitable than simply waiting longer between collection sessions, due to the bees requiring more time to replenish their venom. If you have more hives which have not been collected from during that week, it is recommended to collect from those hives rather than revisiting the first hives.




The above graph demonstrates the approximate weight of venom hives of respective sizes are able to deposit if they retain engagement with a bee venom collector for two hours. Some other commercially available bee venom collectors collect 100mg before switching off around the 45-minute mark. This is an acceptable quantity of venom to be collected in this time, but there is a missed opportunity to collect more venom if the hive was to show interest in depositing venom for longer.

Essentially, a bee venom collector of a modern design is guaranteed to cause the bees to *deposit* venom once it solicits a reaction from them, but it is not guaranteed that the bees will deposit a certain *amount* of venom. The graph above is a guide only.

Every manufacturer of a bee venom collector will claim a typical collection rate, but no one is able to guarantee the exact numbers will be met on every hive due to differences between them.

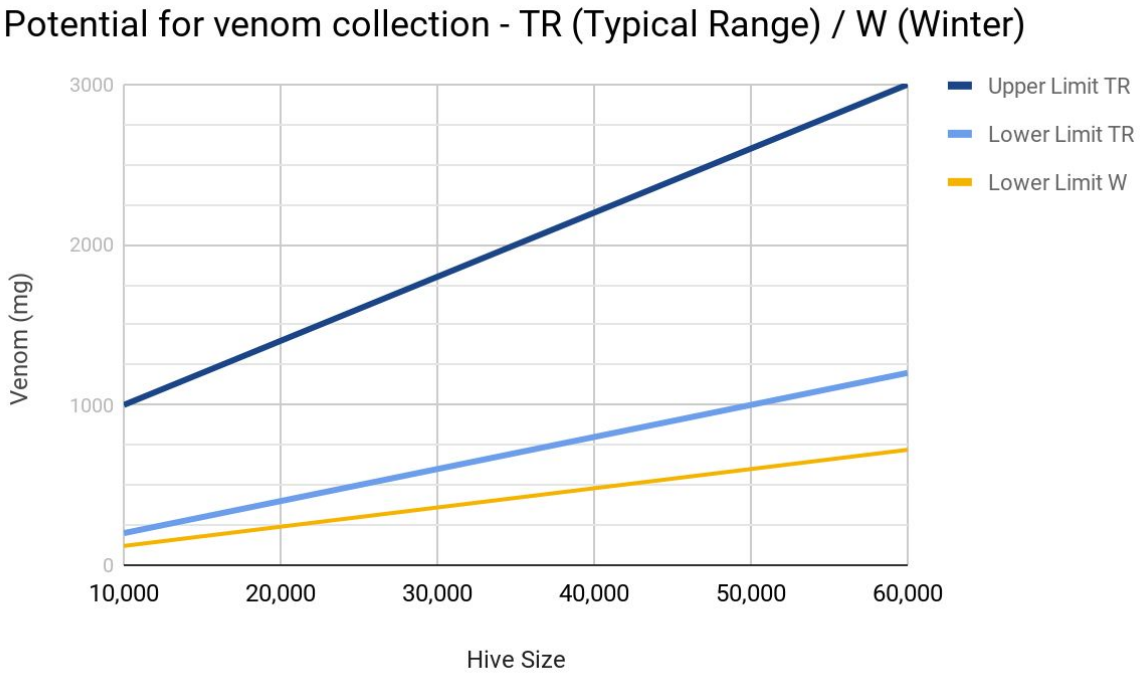
**Seasonal variance & countries**

It is much easier to collect bee venom from a hive during the warmer months of the year. Countries and regions where it is typically warm throughout the year tend to have the best year-round yields. This includes arid, tropical, sub-tropical and temperate climates.

However, droughts may have an adverse effect on collections. In countries and climates where frost forms or the hives become inactive, it may be unsuitable or damaging to collect venom during the cooler months.

Typically within Australia, it is possible to collect venom throughout the year, but there are a few things to keep in mind. First, bees tend to focus more on their pollination runs and little else other than keeping the hive stable during cooler weather, meaning they are less likely to interact with a bee venom collector. Typically if a bee venom collector has been used prior and has pheromone on it, and the hive is used to being collected from, this is not an issue. If it is an issue, please refer to the section titled *Methods for getting the hive to interact with a bee venom collector***.**

Additionally, bees tend to deposit less venom and replenish their venom slower during the cooler months of the year. This could see a collection rate drop by as much as a third.




## Bee death as a result of collecting venom

There are two types of death of bees that could result from collecting bee venom.

The first type, *long term*, is practically nonexistent in modern models of bee venom collectors. This type of death results from the significant stress imposed on the bees from not being able to escape the pheromone that causes the hive to defend against a threat. Similarly it can also be caused by collectors which subject the bees to too harsh a shock, if the effect accumulates over time. Modern external collectors negate the pheromone issue by being able to be removed from a hive, and typically do not operate at too high of voltages.

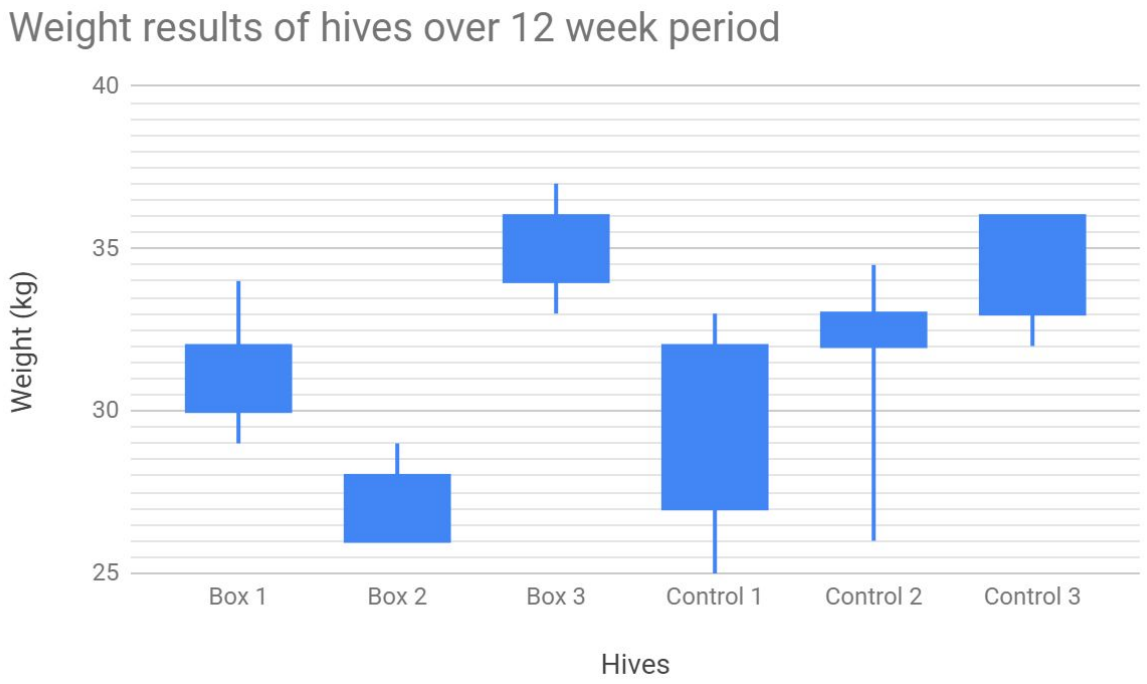
The lack of long term death can be demonstrated through weight tests on a hive over a period of time. The hives can be weighed throughout a particular season, and factoring in any honey or wax collected throughout that period, the weight of the hive can be observed to not drop. Below is a graph displaying weight test data of 3 hives throughout summer.

| | Box 1 | Box 2 | Box 3 | Control 1 | Control 2 | Control 3 |
|---|---|---|---|---|---|---|
| 1 | 30.0 | 26.0 | 34.0 | 32.0 | 26.0 | 33.0 |
| 2 | 32.0 | 29.0 | 33.0 | 33.0 | 32.0 | 36.0 |
| 3 | 29.0 | 27.0 | 35.0 | 25.0 | 32.0 | 32.0 |
| 4 | 34.0 | 26.0 | 37.0 | 27.0 | 34.5 | 36.0 |
| 5 | 32.0 | 28.0 | 36.0 | 27.0 | 33.0 | 34.0 |

Weight results of hives (in kilograms)

Notes:

1. Hive data was collected each 3 week period (total of 12 weeks during summer)
2. Controls were chosen as they had similar weights
3. Box 2 is a plastic box, all other hives are wood
4. Bee venom was collected on average twice per week
5. Only small amounts of bee venom were harvested from Box 2



The second type of death, *immediate*, is where the bees die during the process of venom collection. While most modern bee venom collectors do not currently operate at voltages which cause death in healthy bees, and no current designs result in a bee's barb being ripped off, some bees which are weaker could die as a result of collection. Typically a collection session will not result in any deaths, though it is not uncommon for up to 4 bees per 10,000 bees (0.0004%) in a hive to die as a result. Note that this is fewer bees that typically are crushed as a result of hive manipulation to check for honey.

Similarly, one phenomenon which can be observed during collection is the occasional fights two bees may pick with each other. Occasionally a bee may accidentally sting another bee, which will cause those two bees to spar, but it is not often this results in the death of one or both of the bees.

## Temperament of the bees as a result of collecting venom

As collecting bee venom causes the bees to release a pheromone which sends the entire hive into a defence mode, the hive as a whole will display extra aggression during collection. Typically you are able to get close to the hive (within a metre) without gaining the attention of the bees during collection, and Whale Labs has not observed a swarm of bees pursue someone while being collected from, but it is not recommended to get too close or to interact with the collector.

You are usually able to place the collector in front of the hive without wearing protective gear, and move the collector around or restrict access to the hive up until the point the bees start reacting. You will need to be wearing protective gear when switching a device off.

There is no documented long term effect on the temperament of the bees, but for a period of time after collection has completed (up to around a half hour) the bees may show slightly higher aggression, until the pheromone has sufficiently dispersed.

## Taking care of your bee venom collector

Please refer to the documentation provided with the brand of bee venom collector you purchased.

It is very important to remember that the main factor in enticing the bees to use a bee venom collector is the presence of a pheromone. As the case/body of a bee venom collector is not a functional aspect of the collection process, it is best not to clean it too thoroughly, as this may remove the pheromone. A quick wipe with a damp cloth may be necessary at times to remove dirt buildup.

The wires will also get dirty and it is recommended to wipe the wires with a cotton ball or cloth soaked in isopropyl alcohol or another form of alcohol to remove surface contaminants. Different collectors will use different gauge wires, therefore will accumulate surface dirt at different rates. It is common for the wires to be made from stainless steel, though it is not recommended to clean them with water.

Wires of bee venom collectors are often delicate and will deform if weight is placed on them, so bee venom collectors need to be stored with no weight on them, no items can be dropped on them, and the wires should not be compressed while holding the device.

## Effect on honey and wax production

Honey and wax production would be affected through misuse of bee venom collectors, or the use of collectors which are improperly designed. These would cause a drop in bee populations or affect the bees' health to the point where they do not function as part of the hive.

As demonstrated through the graphs displayed earlier in this document, as the hive's weights haven't been observed to drop, the hive is still operating at its full capacity and will produce as much honey as before.

## Health considerations & handling of bee venom before storage

Even in those who are not allergic to bees (specifically their venom), fewer than 200 bee stings (20mg/0.02g) is typically enough to cause death, though at times this could be far fewer. In those who are allergic, death could happen with even a much lower quantity.

## Unvalidated beneficial and negative effects

**More bees as a result of bee venom collection**
It has been claimed that, due to the queen believing that the hive is under attack more frequently than usual, and with the knowledge that bee population will drop during attacks, the queen will produce more offspring as a result. This, however, is unsubstantiated, with weight tests suggesting that populations (and therefore honey and wax productions) are staying relatively stable.

**Lower production of royal jelly**
It may be possible that the stress pheromone may cause a reduce in royal jelly production. This is why it is important that a bee venom collector is removable and external to limit the pheremone's prevalence within the hive.

# Storing Bee Venom

Bee venom should be stored in a glass jar/vial and placed in a freezer for long term storage. Bee venom does oxidise and degrade if left in the open, but if it is left out for no longer than 2 hours the effect is minimal. The bee venom should be scraped from the collection plate after each hive it has been placed on

The health precautions below should be taken, including when storing bee venom in a freezer. However it is understood that bee venom may not always be stored in its own freezer, meaning it should be clearly labeled and unable to be mistaken as a food product.

A standard freezer temperature of around -18deg celcius is sufficient to store the bee venom for several years.

# Health precautions when handling bee venom

Take extreme caution when handling bee venom To do this, follow some of the following precautions:

1. Use gloves and eye/mouth protection.
2. Do not ingest or inhale.
3. Cover any exposed cuts or grazes on your hands or body.
4. Handle the Bee Venom Collector with care as remnants of venom will be left on the device after collections.
5. Do not place the collector on any surface used for food preparation.
6. When storing bee venom in a freezer, keep away from food products if possible
7. Do not allow children or pets to come in contact with venom or a bee venom collector

Manufacturers of bee venom collectors accept no liability for the mishandling of bee venom, or any damage which may be caused from bee venom being placed within consumer products.

# FAQ’s

**How much venom can I collect from a single hive during a collection session?**

Please refer to *Hive sizes and venom quantity*. A general rule of thumb to use is to third a hive’s population, so a hive with 10,000 bees could produce 330mg to a gram, while a hive with 30,000 could easily create a full gram or more. The amount of venom actually collected depends on the collector you use and it is not uncommon for a small hive to deposit as little as 100mg/hr or 200mg/2hr, especially during cooler weather.

**How long should I collect venom for?**

This depends on the model of bee venom collector you are using. Several devices currently on the market only run for 45 minutes to 1 hour, but you should refer to the documentation provided with the product you purchase.

The Whale Labs BVC Mk1 is intended to be switched on for 1-2 hours, as long as the hive is still reacting - it should be switched off if they stop reacting to the collector before the 2 hours. Even if the bees are still reacting to the device past the 2 hour point, it should be switched off to not detrimentally affect the bees’ pollination runs.

Each hive is different and there is no guarantee that just because one hive will react to a collector for 2 hours, that another hive will lose interest after 1 hour.

**How many times can I collect each week?**

Once a week is optimal, though you can often get away with collecting twice per week throughout the year, with some people suggesting you can collect three times per week in summer. Depending on the individual hives, the amount of bee venom collected by each subsequent collection session per week is likely to diminish.

If collecting once a week, the bees are likely to have fully replenished their venom, assisted by the fact that they likely hadn’t deposited all of their venom during the initial collection session that week anyway. The amount of venom collected from each session after the first should be closely assessed to determine if collecting additional times each week does not have reduced commercial viability.

For this reason, if you have more hives to collect from during the week before returning to the first, it is by far recommended to collect on these hives first, to ensure the first hive has plenty of time to replenish.

**Can I collect during inclement weather?**

When it is very windy, bees may have difficulty landing on a bee venom collector, so it is best to not collect from a hive unless the hive is well sheltered from the wind in these conditions.

If it is misty/foggy, not only may this cause condensation on or inside a bee venom collector, it may also prevent the bee venom from drying. The moisture within the bee venom needs to evaporate in order for it to crystallise.

If the venom does not evaporate at a normal rate, it is much more likely for pollen, dirt and other contaminants to land in the liquid venom and result in a higher percentage of contaminants. Typically in regular weather where the bee venom can dry at a sufficient rate, minimal contaminants are able to get trapped within it.

Will collecting venom kill my bees?

In most cases, no. It is possible that an occasional bee will perish during collection of venom, but please refer to *Bee death as a result of collecting venom* for figures.

**Have more questions not answered here?**

Please email your questions to business@whalelabs.com.au - we will get back to you with as comprehensive of an answer as we can, and make sure this document is updated to reflect this.