# APPLICATION FOR THE APPROVAL OF MANUKA HONEY WITH ADDED BEE VENOM

Regulation (EC) No 258/97 of the European Parliament and the Council of 27th January 1997 concerning novel foods and novel food ingredients.

**ADMINISTRATION DATA**


**Name and Address of Applicants/Manufacturers**

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## SUMMARY

Nelson Honey & Marketing (NZ) Ltd seeks European Union Novel Food approval for Manuka honey with added bee venom. This product is based on Manuka honey, with added honeybee venom, and is registered in New Zealand as a dietary supplement under the provisions of the Dietary Supplement Regulations 1985.

Components of honeybee venom have an anti-inflammatory effect and market feedback has shown that customers derive benefits from taking Manuka honey with added bee venom to alleviate symptoms of arthritis. The active ingredient, the honeybee venom (20 μg venom/g honey), does contain known allergens, principally melittin and phospholipase $A_2$, consequently in this application an emphasis has been placed on showing that Manuka honey with added bee venom is safe at the suggested consumption level. An intake of two teaspoons of Manuka honey with added bee venom per day is equivalent to 20 g honey and 400 μg honeybee venom per day. This dosage (5.3 μg/kg/day) is close to the dose that would be expected to be effective for a 75 kg adult from reported clinical studies.

Manuka honey with added bee venom has been marketed to the arthritis segment of the New Zealand natural health market since its development in 1996. During this time, total sales of more than 13,437,000 individual 20 g doses of Manuka honey with added bee venom has been made. Over this 13 year period reported incidences of adverse reactions to Manuka honey with added bee venom have been extremely low. Toxicological data from a range of human and animal studies is reported. These data show that at the suggested level of consumption Manuka honey with added bee venom is safe.

## INTRODUCTION

Approval is sought under Regulation (EC) No 258/97 of the European Parliament and the Council of 27th January 1997 concerning novel foods and novel food ingredients. Article 1(2) defines Novel Foods as:

> - foods and food ingredients which have not hitherto been used for human consumption to a significant degree within the Community (before 15th May 1997) and which fall under the following categories ….
>
> (e) foods and food ingredients consisting of or isolated from plants and food ingredients isolated from animals ……

Manuka honey with added bee venom is a honey-based product that has had additional honeybee venom added. It is marketed to the natural health food market for it's anti-arthritic properties. While honey and bee products may already contain small amounts of honeybee venom, this product has had additional bee venom added directly to the honey and is therefore considered a novel food.

Bee venom therapy has been utilized to relieve pain and to treat inflammatory diseases such as rheumatoid arthritis in humans and experimental animals (Kwon et al. 2002; Kim et al. 2004; Adriano et al. 2005; Goggs et al. 2005).

Manuka honey with added bee venom is marketed to the arthritis segment of the New Zealand natural health market since its development in 1996. These are small target markets where the product retails in specialist natural health shops and pharmacies. The product label clearly states that bee venom has been added and directions are given for an effective dose. The label also has a warning that people with allergies to honey or bee venom should seek medical advice prior to use, and that honey should not be given to infants under 12 months of age.

Market feedback on the product has shown that customers derive benefits from taking Manuka honey with added bee venom to alleviate symptoms of arthritis. The active ingredient, the honeybee venom, does contain known allergens, principally melittin and phospholipase $A_2$, consequently in this application an emphasis is placed on showing that with the low level of added bee venom Manuka honey with added bee venom is unlikely to cause adverse reactions at the suggested consumption level.

Production processes are described in detail that ensure the marketing of a consistent product with specific conformance criteria, especially with respect to bee venom concentration in the final product.

Toxicological data from a range of human and animal studies is reported. These data show that at the suggested level of consumption Manuka honey with added bee venom is unlikely to cause adverse reactions at the suggested consumption levels.

We seek approval for Manuka honey with added bee venom.

Section 4 of the Commission Recommendation of 1997 outlines recommendations made by the Scientific committee for Food (SCF) pertaining to the "Scientific Classification of Novel Foods for the assessment of wholesomeness, which facilitates the safety and nutritional evaluation of a given food/food ingredient. Under section 4, Manuka honey with added bee venom (Manuka honey with added honeybee venom) would meet the definition of:

> Class 2.1: Complex Novel Food from non-genetically modified (GM) source. The source of the Novel Food has a history of food use in the Community.

The essential information requirements for submission under this class are:

I Specifications of the Novel Food
II Effect of the production process applied to the Novel Food
III History of the organism used as the source of the novel Food

IX Anticipated intake/extent of use of the Novel Food

X Information from previous human exposure to the Novel Food or its source

XI Nutritional information on the Novel Food

XII Microbiological information on the Novel Food

XIII Toxicological information on the Novel Food

# Contents

# I SPECIFICATIONS FOR MANUKA HONEY WITH ADDED BEE VENOM

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- Is appropriate analytical information available on potentially toxic inherent constituents, external contaminants and nutrients?

- Is the information representative of the novel food when produced on a commercial scale?

- Is there an appropriate specification (including species, taxon etc for living organisms) to ensure that the novel food marketed is the same as that evaluated?

Manuka honey with added bee venom is a commercial product of honey containing honeybee venom (up to 20 μg venom/g honey). It is currently marketed in New Zealand as a dietary Supplement under the provisions of the Dietary Supplement Regulations 1985. At the suggested consumption level (two teaspoons equivalent to 20 g of Manuka honey with added bee venom per day) this concentration of honeybee venom has been found to be low enough to minimise potential allergic effects while still providing a therapeutic benefit for an average adult.

### *a) Specification of the Manuka Honey*

The honey is a creamed blend of honey derived from the native New Zealand "Manuka" tree (*Leptospermum scoparium*). For Manuka honey to be marketed as such in New Zealand and the EU the Manuka component of the honey must constitute greater than 70% of the raw material before processing as verified by pollen analysis for each batch.

The industry specification for Manuka honey is that it has a moisture content less than 21%, more than 60% reducing sugars and a pH less than 4.2. Whereas, typical actual measured values for moisture and water activity are 18.4% and 0.66. A mean pH for New Zealand Manuka honey has previously been reported as 3.9 (Weston 2000).

### *b) Specification of the Honeybee Venom*

Venom is extracted from the bees, dried and added into the honey as described in Section II. The venom is extracted exclusively from healthy European honeybees (*Apis mellifera*). The venom is accompanied by an Apiarist and Beekeeper Statement, also called a Harvest Declaration (see Attachment 1 for a copy of this statement). The venom is considered to be the active ingredient in the product. Table Ia gives specifications for the dried bee venom.

**Table Ia. Specification for dried honeybee venom. Melittin and phospholipase values represent minimum values for any batch of venom.**

| Component | Concentration or Activity |
|---|---|
| Melittin | ≥ 45% |
| Phospholipase $A_2$ | ≥ 100 μM/mg/min |
| Moisture Content | ≤ 5% |

### *c) Composition of the Honeybee Venom*

The composition of honeybee venom has been studied extensively and been found to be reasonably consistent. Table Ib shows the components of honeybee venom. The main components of toxicological significance are melittin (50%) and phospholipase $A_2$ (10%) of which melittin is the dominant toxic component (Schmidt 1995).

As discussed in Section XIII, the very low level of honeybee venom added to Manuka honey with added bee venom (20 μg/g) means the toxicological risks associated with consumption of the product are minimal.

**Table Ib. Composition of dried honeybee venom.**

| Class of Molecules | Component | Concentration (%) |
|---|---|---|
| **Enzymes** | Phospholipase $A_2$ | 10-12 |
| | Hyaluronidase | 1-3 |
| **Other proteins and peptides** | Melittin | 50 |
| | Apamine | 1-3 |
| | Mast Cell Degranulating Peptide (MCD) | 1-2 |
| | Secapin | 0.5-2.0 |
| | Procamine | 1-2 |
| | Small peptides (less than 5 amino acids) | 13-15 |
| **Physiologically active amines** | Histamine | 0.5-2.0 |
| | Dopamine | 0.2-1.0 |
| | Noradrenaline | 0.1-0.5 |
| **Amino acids** | γ-aminobutyric acid | 0.5 |
| | α-amino acids | 1 |
| **Sugars** | Glucose and fructose | 2 |
| **Phospholipids** | | 5 |
| **Volatile compounds** | | 4-8 |

From (Dotimas et al. 1987).

### *d) Method for Determining Honeybee Venom Concentration*

The principle method that has been used for determining venom concentration in dried venom and in honey-based products in this application has been a Western blot assay.

The Western blot assay comprises of SDS-PAGE electrophoresis followed by immunoblots using commonly available bee venom antiserum (Sigma) coupled to either a fluorescent or enzyme-linked assay. For quantitative analysis bee venom standards are used. This method is highly sensitive, specific to honeybee venom and can be used with harvested venom or to determine venom content of honey-based products.

### *e) Specification of Manuka Honey with Added Bee Venom*

Manuka honey with added bee venom has 20 μg venom/g honey added, at this low level of venom addition the proximate composition is not affected significantly, consequently the other specifications for Manuka honey with added bee venom are the same as for the bulk honey. That is, moisture less than 21% and pH less than 4.2.

# II EFFECT OF THE PRODUCTION PROCESS APPLIED TO MANUKA HONEY WITH ADDED BEE VENOM

Based on the SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to the specifications of the novel food:

- Does the novel food undergo a production process?
- Is there a history of use of the production process for the food?
- Does the process result in a significant change in the composition or structure of the novel food compared to its traditional counterpart?
- Is information available to enable identification of the possible toxicological, nutritional and microbiological hazards arising from use of the process?
- Are the means identified for controlling the process to ensure that the novel food complies with its specification?
- Has the process the potential to alter the levels in the novel food of substances with an adverse effect on public health?
- After processing is the novel food likely to contain microorganisms of adverse public health significance?

Manuka honey with added bee venom is produced by mixing dried honeybee venom with commercial Manuka honey. We believe that addition of high quality honeybee venom to honey does not significantly alter the microbiological hazards associated with standard honey manufacture. Toxicological aspects are discussed in Section XIII.

Manufacture of creamed honey involves standard processes that have been developed over a long period by the honey industry. The key differences for Manuka honey with added bee venom compared to honey is the harvesting of the venom from bees and addition of a small quantity of the dried venom to bulk honey (20 g venom per tonne honey). Because honey is highly viscous at room temperature, considerable effort has been spent on developing effective mixing processes to ensure uniform mixing of bee venom to give a consistent final product composition.

Production is done under risk management (RMP) and hazard analysis critical control point (HACCP) plans approved by the New Zealand Food Standards Authority. Products comply with the New Zealand Food Standards Code 2.8.2.

### *a) The Production of Bee Venom*

Venom is harvested from healthy bees (*Apis mellifera*) by the use of an electrical milking apparatus. This apparatus is commercially available and used in many countries around the world. It is placed into hives and uses low amperage electrical impulses to stimulate worker bees to sting through a latex film onto a glass collector plate. The use of a latex film excludes contaminating substances and allows the collection of pure venom. The venom is then gently air dried to a final moisture content of 5% (± 2.0%) and removed from the glass plate for addition to the honey.

### *b) The Production of Manuka Honey with Added Bee Venom*

The addition of dry venom to honey to produce Manuka honey with added bee venom ensures precise control of the honeybee venom concentration in each batch of the final product (20 μg/g). Accurately weighed dry venom is thoroughly mixed into a small amount of pre-warmed honey prior to slow addition of this concentrate to the bulk honey. Venom is added immediately after the creaming and filtering processes. The gentle heating (less than 35°C) reduces viscosity of the honey and the extended mixing time associated with the creaming process ensures thorough mixing and even distribution of the venom throughout the bulk honey. Mixing is continued for 24 hrs with gentle stirring. During this time, the honey-venom mixture is given additional mixing by vertically circulating the bulk honey from the bottom and reintroducing it into the top of the tank.

### *c) Packaging*

Manuka honey with added bee venom is packed into standard polypropylene jars for distribution and retail sale. These ensure there is no uptake of moisture during storage, and the product remains stable and free from contamination during distribution and sale.

### *d) Manuka Honey with Added Bee Venom is Shelf Stable for Up to 3 Years*

Honey is nectar collected by bees from a variety of plants. It is concentrated by evaporation of water to form a saturated or super-saturated solution of sugars, typically consisting of about 18% water, 38% fructose, 31% glucose, 10% other sugars, and a wide range of micronutrients (vitamins, amino acids, and minerals), with a pH below 4.0.

For New Zealand Manuka honey, the moisture content is typically close to 18% and the pH has been shown to average 3.9 (Weston 2000). Measurements of production Manuka honey show the water activity to be low at 0.66. With these low water activity and pH values Manuka honey is therefore recognised as shelf stable at room temperature for at least 3 years when stored in its unopened container.

New Zealand Manuka honey has antibacterial properties that have been the subject of extensive research over recent years (Weston et al. 1999; Weston 2000; Weston et al. 2000; Snow et al. 2004; Patton et al. 2006). In addition, honeybee venom also has antimicrobial properties (Perumal Samy et al. 2007) that would help further stabilise the Manuka honey with added bee venom and prevent microbial growth during storage.

The concentration of honeybee venom in honey stored at 20°C has been determined using Western blot analysis. There was a small decrease from 100% in the initial product to 94.9% after 1.5 years and to 92.4% after 2.5 years showing the bee venom was stable over this period.

# III HISTORY OF THE ORGANISM USED AS THE SOURCE

Based on the SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the history of the source organism.

- Is the novel food obtained from a biological source (i.e. a plant, animal or microorganism)?

- Is the source organism characterised?

- Is there information to show the source organism and/ or foods obtained from it are not detrimental to human health?

### *a) Bee Venom Therapy is Accepted in Many Countries*

The therapeutic benefits of honeybee venom have been known for a long time, particularly for treatment of arthritic and rheumatic conditions in humans (Goggs et al. 2005) and in animals (Kwon et al. 2002; Kim et al. 2004; Park et al. 2004). It is a well known treatment for many types of afflictions and is commonly used in some countries, in particular China, Korea, Czech Republic and Romania, where entire clinics have been established for apitherapeutic treatment.

In Eastern Europe and many Asian countries bee venom has been used in official medical treatment of a large variety of ailments for a considerable length of time. Application methods for venom include natural bee stings, injections, ointments, inhalations and tablets. There is now strong scientific interest in the pharmacological properties of bee venom. Studies have shown that in experimental animals the induction of arthritis is successfully suppressed by long-term bee venom treatment (Eiseman et al. 1982; Hadjipetrou-Kourounakis et al. 1984). In further studies it has been shown that the therapeutic effect of bee venom therapy can only be reproduced by the water-soluble fraction of the bee venom

(Kwon et al. 2002). Other pre-clinical studies on rodents using high dose tests (200-fold or 1,000 μg/kg) have demonstrated that bee venom can be used as a safe antinociceptive and antiinflammatory agent (Kim et al. 2004). The study of Kim et al. (2004) is discussed further in Section XIII.

Venom immunotherapy is practiced in many European countries and in the USA. It is highly effective for reducing allergic sensitivity (local and systemic) in people over time and has been shown to reduce the risk of systemic reactions in people with honeybee sting allergies by more than a 95% (Moffitt et al. 2004). Specific immunotherapy with bee venom can result in an almost complete protection against adverse (or allergic) reactions from stings in the great majority of cases (Severino et al. 2008).

Typically, venom immunotherapy uses much lower doses of bee venom than when treating subjects for arthritis and treatment usually consists of an induction (or up-dosing) phase with increasing amounts of allergen followed by a maintenance phase where amounts of up to 200 μg venom are injected subcutaneously (Moffitt et al. 2006).

Recent studies have shown that sublingual immunotherapy with the introduction of honeybee venom under the tongue prior to swallowing is safe, and can significantly reduce reactions in people allergic to bee stings (Passalacqua et al. 2008; Severino et al. 2008). Much higher allergen doses (eg 22 to 500 times) are commonly used in sublingual immunotherapy than in subcutaneous immunotherapy, however with less side effects (Cox et al. 2006). From these and other studies it appears that sublingual immunotherapy can exert local and systemic immunological effects similar to injection-based immunotherapy.

Sublingual immunotherapy is used in many European countries where a major advantage is acknowledged as its very favourable safety profile (Cox et al. 2004) with systemic reactions reported as being rare, severe adverse events are exceptional and the common local side effects are mild and self limiting (Serverino et al. 2008).

Since consuming Manuka honey with added bee venom is similar to the sublingual delivery route for immunotherapy, and considering the excellent safety record and tolerance to sublingual bee venom, it is little wonder there have been very few reported adverse reactions

to the consumption of Manuka honey with added bee venom over the last 13 years (discussed further in Sections X and XIII).

# IX ANTICIPATED INTAKE/EXTENT OF USE OF NOVEL FOOD

Based on SCF guidelines, the following questions must be addressed to ensure sufficient information pertaining to the effect of the production process applied to the novel food:

- Is there information on the anticipated uses of the novel food based on its properties?
- Is there information to show anticipated intakes for groups predicted to be at risk?
- Will introduction of the novel food be restricted geographically?
- Will the novel foods replace other foods in the diet?
- Are any of the replaced foods significant nutritional sources?
- Does the probable level of substitution have a nutritional significance for any population groups?

The anticipated food uses of Manuka honey with added bee venom is novel only to the extent that the honey contains a very low level (20 μg/g) of added honeybee venom. As a consequence, the honey will not differ appreciably in nutrient content from normal table honey. However, consumers of Manuka honey with added bee venom would be expected to be taking the product to gain a specific health benefit and not to replace honey in their normal diet.

At the maximum consumption levels suggested on the product label "Start with ¼ teaspoon per day and increase to one or two teaspoons per day as required", and using two teaspoons full as equivalent to 20 g of Manuka honey with added bee venom the maximum consumption of bee venom would be 400 μg per day.

# X INFORMATION FROM PREVIOUS HUMAN EXPOSURE

Based on SCF guidelines, the following questions must be answered in the affirmative to ensure sufficient information pertaining to previous exposure to the novel food:

- Is there information from previous direct, indirect or intended human exposure to the novel food or its source which is relevant to the EU situation with respect to production, preparation, population, lifestyles and intakes?

- Is there information to demonstrate that exposure to the novel food is unlikely to give rise to mitochondrial, toxicological and/or allergenicity problems?

Manuka honey with added bee venom has a reasonable history of consumption of 13 years and has had very few reported incidences of reaction to the product.

### *a) Previous Consumption History*

Manuka honey with added bee venom has been marketed since 1996, that is, for 13 years in New Zealand. During this time, total sales of more than 280,000 units (individual packs) have been made. This is equivalent to more than 13,437,000 individual 20 g doses of Manuka honey with added bee venom.

### *b) Very Low Incidence of Adverse Reactions Reported*

Over this 13 year period reported incidences of adverse reactions to Manuka honey with added bee venom have been extremely low. In fact, only three adverse reaction reports where Manuka honey with added bee venom has been causally associated have been made to the Centre for Adverse Reactions Monitoring (CARM) in New Zealand. Two reports were in 1998 and the last was in 2005 and involved a 91 year old with a number of other conditions.

In a recent CARM report discussing these incidents, Nelson Apiaries were advised "that whilst in the one report where only Manuka honey with added bee venom is suspect this is likely to reflect a strong causal association, in the other two reports you will note that there are a number of other products co-administered. On both of these reports there are two products which were jointly assessed as being potentially associated" (see Attachment 2 for a copy of the CARM report). In the 1998 incidence where there was a strong causal association the person had a known allergy to bee products and they recovered without sequelae.

### *c) Commissioned Studies*

Further human studies reporting toxicological evidence are presented in Section XIII. These show that at the anticipated consumption level of Manuka honey with added bee venom the product is safe.

# XI NUTRITIONAL INFORMATION ON MANUKA HONEY WITH ADDED BEE VENOM

Based on the SCF guidelines, the following question must be answered in the affirmative to ensure sufficient nutritional information pertaining to the novel food:

- Is there information to show that the novel food is nutritionally equivalent to existing foods that it might replace in the diet?

The anticipated food uses of Manuka honey with added bee venom are novel only to the extent that the honey contains a very low level (20 μg/g) of added honeybee venom. As a consequence the honey will not differ appreciably in nutrient content from normal table honey. However, consumers of Manuka honey with added bee venom would be expected to be taking the product to gain a specific health benefit and not to replace honey in their normal diet. The nutritional information for Manuka honey with added bee venom is presented in Table XIa.

**Table XIa. Nutritional information for Manuka honey with added bee venom**. Figures are for average quantities and the serve size is 10 g, equivalent to one heaped teaspoon. This information is on the product label.

| | **Per Serve** | **Per 100g** |
|---|---|---|
| **Energy (kJ)** | 136 | 1360 |
| **Protein (g)** | <0.1 | 0.4 |
| **Fat (g)** | 0.0 | 0.0 |
| **Carbohydrate (g)** | 8.0 | 79.6 |
| **Sodium (mg)** | 1.2 | 12 |

### *a) Relationship of Daily Intake of Manuka Honey with Added Bee Venom to Total Energy Expenditure*

Two teaspoons of Manuka honey with added bee venom (ie 20 g) represents about 2 – 4% of an average adult's energy expenditure per day (approx. 272 kJ), using Australian values for males of 11.5 – 14 MJ per day, and 7 – 9.5 MJ per day for females.

# XII MICROBIAL INFORMATION ON MANUKA HONEY WITH ADDED BEE VENOM

Based on the SCF guidelines, the following question must be addressed to ensure sufficient microbiological information on the novel food:

- Is the presence of any microorganisms or their metabolites due to the novelty of the product/process?

The microbial hazards associated with Manuka honey with added bee venom do not differ from those associated with standard Manuka honey, except that as already mentioned in Section II the honeybee venom may confer increased antimicrobial properties to the product.

Vegetative forms of bacterial pathogens (e.g. *Salmonella* spp., *Listeria monocytogenes*) have not been detected in honey. However, bacterial spores (e.g. *Bacillus* spp., *Clostridium* spp.) are likely to occur in honey. Although, these spores will not grow in honey, when it is used as an ingredient in another food the bacterial spores could be introduced into, and grow in that food. As Manuka honey with added bee venom is meant to be consumed on its own or with food and not used as an ingredient the possible presence of bacterial spores is unlikely to cause a problem. However, as a precautionary measure against possible infant botulism, which could arise from the presence of *Clostridium botulinum* spores, a warning is included on the product label stating *"honey should not be given to infants under 12 months of age"*.

# XIII TOXICOLOGICAL INFORMATION ON MANUKA HONEY WITH ADDED BEE VENOM

Based on SCF guidelines, the following questions must be addressed to ensure sufficient toxicological information pertaining to the novel food:

- Is there a traditional counterpart to the novel food that can be used as a baseline to facilitate toxicological assessment?

- Is there information from a range of toxicological studies appropriate to the novel food to show the novel food is safe under anticipated conditions of preparation and use?

- Is there information which suggests that the novel food might pose an allergenic risk to humans?

Honey has been used as a food for many centuries, and Manuka honey especially is increasingly being recognised for its therapeutic properties (Weston et al. 1999; Weston 2000; Weston et al. 2000; Snow et al. 2004). Although honey itself can be allergenic, this section will focus on the toxicological assessment of Manuka honey with added bee venom using human and rodent studies undertaken specifically on Manuka honey with added bee venom. We will also review existing toxicological information on honeybee venom and its use in venom immunotherapy. This information will show that the low level of honeybee venom in Manuka honey with added bee venom makes this product safe under anticipated conditions of use.

### *a) Suggested Daily Intake of Honeybee Venom From Manuka Honey with Added Bee Venom is Low*

A suggested intake of two teaspoons of Manuka honey with added bee venom per day is equivalent to about 20 g honey and 400 μg honeybee venom per day. This dosage (5.3 μg/kg/day) is close to the dose that would be expected to be effective for a 75 kg adult from

reported clinical studies. In these studies subcutaneous or intradermal doses of 5 μg/kg body weight were effective for treatment of arthritis in rodent models (Kim et al. 2004). Even lower venom doses of 0.8 and 1.6 μg/kg body weight were found to be antiarthritic in the study of Park et al. (2004).

### *b) Bee Venom is Found Naturally in Honey*

Low levels of bee venom is found naturally in honey. Results of tests on New Zealand honey are shown in Table XIIIa. According to the results of Passalacqua et al (2008) and Severino et al. (2008), who used a total cumulative dosage of 525 μg per month in their sublingual immunotherapy studies, a concentration of 1 – 2 μg venom/g honey would be sufficient to confer immunological benefits to a person consuming 10 – 20 g per day of normal commercial Manuka honey.

**Table XIIIa. Naturally occurring bee venom concentration in commercial New Zealand honeys**.
Venom concentration was determined using Western blot analysis.

| Honey Variety | Bee Venom Concentration (μg venom/g honey) |
|---|---|
| Uncreamed Manuka | 1.3 |
| Creamed Manuka | 1.7 |
| Active Manuka | 1.5 |
| Multifloral | 1.1 |

These data that show normal commercial honey contains bee venom is supported by comments made by bee-keepers who have observed bees stinging directly into honey within hives. The reason bees do this is unexplained at this time.

Data from a study commissioned by Nelson Apiaries with Auckland University, New Zealand (Attachment 3), where two separate assay methods for bee venom showed that the

control Manuka honey gave a similar response to that for Manuka honey with added bee venom also provide evidence that normal honey contains bee venom.

The first method used a bioassay to assess the ability of bee venom to induce the release of prostaglandins from a cell line (WISH) in culture. Manuka honey with added bee venom was only slightly more potent than the control Manuka honey in inducing $PGE_2$ production. Other honey samples (no added venom) tested were also slightly more potent than the control Manuka sample.

The second method used a liposome assay in which the rate of melittin-induced release of alkaline phosphatase encapsulated in phospholipid micelles (liposomes) was measured through release of a fluorescent product. Once again, the results indicated that Manuka honey with added bee venom products were similar to, or only slightly more potent than the Manuka control.

### *c) Results From a Human Trial to Test the Effectiveness of Manuka Honey with Added Bee Venom Show It Is Safe*

As part of a random, double-blind, placebo controlled cross-over study in osteoarthritis and rheumatoid arthritis study, the safety of administering Manuka honey with added bee venom was evaluated (Doube (2001), Attachment 4). The principal investigator for this study was a Consultant Rheumatologist and was supported by pharmacologists and toxicologists based at Auckland University.

Prior to starting, the study was reviewed and approved by the Waikato Ethics Committee, Hamilton, New Zealand.

The study was also reviewed by the New Zealand Health Research Council Standing Committee on Therapeutic Trials (SCOTT) and an exemption pursuant to Section 30 of the Medicines Act 1981 was approved by the Director-General of Health.

Ninety-four patients suffering from osteoarthritis or rheumatoid arthritis were treated in two six-week treatment phases, separated by a four-week wash out period. Patients took two teaspoonfuls of Manuka honey with added bee venom per day, equivalent to 20 g per day.

At the conclusion of the study the investigator reported that adverse events were recorded for seven (7.4%) of the patients entered into the study – four on Manuka honey with added bee venom and three on placebo honey. All these events were considered to be non-serious, though the event caused the patients to discontinue treatment in four cases.

Laboratory results of blood tests for all patients were reviewed and no clinically relevant changes to laboratory parameters were noted for any patient in the study.

It was concluded that "no serious adverse events occurred. Rash occurred in both active and placebo patients but the overall occurrence of side effects was low. There were no abnormal laboratory findings. Manuka honey with added bee venom appears safe [providing bee and bee product allergy is excluded], with a side effect profile similar to the placebo" (Doube 2001).

### *d) Rodent Toxicity Studies on Manuka Honey with Added Bee Venom*

Two rodent studies were commissioned with the Medical School, Auckland University, New Zealand by Nelson Apiaries to report on potential toxicity of Manuka honey with added bee venom. A copy of the full report is in Attachment 3.

**i) Acute Toxicity Study**

Forty mice (five males and five females per group) received Manuka honey with added bee venom at 0, 5, 50, or 500 mg/kg by gavage. The mice were then observed for overt toxicity for 48 hours, after which they were killed by cervical dislocation. Internal organs were inspected for signs of pathological changes. These tests were performed with two forms of Manuka honey with added bee venom: one with original liquid honey, and one in concentrated freeze-dried honey.

No animals showed any signs of overt toxicity and internal organ inspection did not reveal any abnormalities, even at the highest dose of 500 mg/kg for each product.

**ii) Sub-acute Toxicity Study**

Ten mice (five males and five females per group) received the product in drinking water for three months. Honey or freeze-dried product was dissolved to a final concentration of 100 μg/mL. This concentration was based on water consumption of 2 mL/day giving a daily dose of 200 μg venom/day. For a 30 g mouse this is equivalent to 6.67 mg/kg/day, equivalent to 500 mg/day for a 75 kg human. Mice were allowed *ad libitum* access for 3 months. Animals were checked daily for general welfare and weighed weekly. At the conclusion of the experiment, animals were killed by carbon dioxide euthanasia and the internal organs inspected for macroscopic changes.

During the course of the experiment, no animals appeared to suffer from any distress, as assessed by disturbances in behaviour, and all animals gained weight as expected. There were no significant differences between the groups fed Manuka honey with added bee venom honey or freeze-dried product at any time point. At the end of the experiment, internal inspection did not reveal any abnormalities in any organ.

**iii) Conclusions from the Rodent Studies**

Based on the toxicity tests performed, Manuka honey with added bee venom appears to have no acute or sub-acute toxic effects in both liquid and freeze-dried forms. Animals gained weight, were observed to behave normally, and showed no signs of change in internal organ form or function.

### *e) Published Rodent Toxicity Studies on Bee Venom*

Kim et al. (2004) investigated the general pharmacological effects of bee venom and venom extracts on a range of physiological parameters of the central nervous system, digestive, cardiovascular and respiratory systems in rodents. A single clinical dose of bee venom was taken as 5 μg/kg when administered by intradermal or subcutaneous route to human patients. They administered bee venom to mice, rats and rabbits in doses up to 200-fold the effective clinical dose (i.e. 1,000 μg/kg). Using a variety of indicators the results of this study showed that treatment with whole bee venom (at a dose 200 times the recommended clinical dose) did

not produce any significant effect on the central nervous system (as determined by general behaviour, sleep induction time and duration, spontaneous activity, motor function, body temperature, or drug-induced convulsions). Bee venom was a potent antinociceptive agent without the side effects associated with many narcotic drugs. Bee venom treatment did not affect motor activity, intestinal peristaltic function or gastric function. Additionally, bee venom did not alter blood pressure and heart rate in rats nor respiratory rates in rabbits.

### *f) Level of Bee Venom in Manuka honey with Added Bee Venom is Very Low Compared to a Lethal Dose*

Human toxicity data has been reported for bee venom from bee stings and this data can be used to compare lethal levels of bee venom to those found in Manuka honey with added bee venom. However, it should be noted that all of this published toxicity data relates to transdermal venom delivery, that is, resulting from bee stings.

From the discussion of sublingual immunotherapy using bee venom (and other allergens) in Section III it is clear that comparing data from transdermal delivery to sublingual or oral delivery will be highly conservative and will overestimate the toxicity of bee venom consumed orally. This is due to the much higher doses of bee venom (and other allergens) used in sublingual immunotherapy, and its better safety profile, compared to subcutaneous immunotherapy (Moffitt et al. 2004; Cox et al. 2006; Serverino et al 2008).

Cox et al. (2006) have reviewed studies comparing efficacy and dose levels of allergens used in subcutaneous and sublingual immunotherapies. They found that sublingual doses could be more than 500 times the customary dosage used in subcutaneous therapy. Furthermore, in sublingual studies comparing response to different doses there was greater improvement in symptom-medication scores with higher doses. Severino et al. (2008) note that for honeybee venom subcutaneous immunotherapy 100 or 200 μg doses are considered effective but that for other allergens efficacy of sublingual immunotherapy has been shown using doses up to 500 times those used in subcutaneous courses of immunotherapy.

The median lethal dose ($LD_{50}$) for honeybee venom has been reported in a number of reports (Schumacher et al. 1989; Schumacher et al. 1990; Schmidt 1995) as 2.8 mg venom/kg body weight for intravenous and 3.8 mg venom/kg body weight for intraperitoneal delivery in mice. We will use the lower value of 2.8 mg venom/kg body weight value for comparison. For a 75 kg person, the value of 2.8 mg venom/kg body weight is approximately 530 times higher than the daily consumption of bee venom found in 20 g per day of Manuka honey with added bee venom. To put it another way, for this person to consume 2.8 mg venom/kg body weight in Manuka honey with added bee venom they would need to consume more than 10.5 kg of the product. Considering the high intrinsic level of monosaccharide carbohydrates in honey this would unlikely be possible for an average person.

### *g) Risk of Manuka Honey with Added Bee Venom to Those Who May be Allergic to Bee Venom*

Consumption of Manuka honey with added bee venom may pose an allergenic risk to those allergic to bee products. To reduce this risk the label for these products clearly states:

*"Special Manuka Honey*
*With Added Bee Venom"*

*"WARNING: People with allergies to honey or bee venom should seek medical advice prior to use."*

Consistent with established venom immunotherapy protocols (Cox et al. 2006) an induction phase (or up-dosing phase) where dosage is started low and increased is recommended. To reduce the risk of side reactions consumers are requested on the label under "*Directions for use*" to start with a small amount first and to build up to one to two teaspoons full per day.

"*Start with ¼ teaspoon per day and increase to one to two teaspoons per day as required.*"

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## Attachment 1. Copy of an Apiarist and Beekeeper Statement for the Harvest of Honey or Other Bee Products for Human Consumption

## Attachment 2. Centre for Adverse Reactions Monitoring Report on Manuka honey with Added Bee Venom

Note: Nectar-Ease is the trade name for Manuka honey with added bee venom.

# Attachment 3. Final Report on Manuka honey with Added Bee Venom Toxicological and Potency Assays

Note: Nectar-Ease is the trade name for Manuka honey with added bee venom.

# Attachment 4. Summary of a Random, Double-blind, Placebo Controlled Cross-over Study in Osteoarthritis and Rheumatoid Arthritis – A Final Study Report

Note: Nectar Ease is the trade name for Manuka honey with added bee venom.