RISK PROFILE:
CLOSTRIDIUM BOTULINUM
IN
HONEY

Prepared as part of a New Zealand Food Safety Authority contract for scientific services

by

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HONEY

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SUMMARY

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management. Risk profiling may result in a range of activities e.g. immediate risk management action, a decision to conduct a quantitative risk assessment, or a programme to gather more data. Risk Profiles also provide information for ranking of food safety issues. This risk assessment is focused on the food/hazard combination of Clostridium botulinum spores and honey.

The Clostridium botulinum types involved in infant botulism belong to Group I and produce type A, B and to a lesser extent type F toxins. When spores of the organism are ingested by infants (who have an incomplete intestinal microflora) they may become established and grow. The neurotoxin produced in vivo may then cause the rare disease known as infant botulism. To date, there have been no reported cases of infant botulism in New Zealand. Worldwide there have been over 1000 reported cases since the disease was recognised as a clinical entity in 1976. Approximately one third of these cases have been associated with honey consumption. The majority of infections are thought to be contracted directly from the environment, for example from soil, dust, vacuum cleaner dust etc.

Honey is the only laboratory confirmed dietary source of the organism in infant cases. Generally, the susceptible age group is less than 12 months of age, with the majority of cases (94%) occurring in those under 6 months. The spectrum of clinical manifestations of these infections is wide, from asymptomatic through to death. A controversial link between SIDS cases and infant botulism has been postulated, although the available evidence is mixed.

Worldwide, the level and frequency of spores found in honey is generally low, although testing methodology improvements are generating higher prevalences and counts in honey. A relationship has been observed between the geographical prevalence of type A, B and F spores and toxin types identified in human cases. Honey contamination may be caused by bee food, water, soil, airborne dust, the bee intestinal tract and dead bees in the hive. Due to the sporadic nature of the contamination and clustering of spores in honey, surveys must involve large numbers of samples.

There is no acceptable means of inactivating or removing spores from honey. Vegetative cells are not present due to low water activity and inhibitory properties of honey.

There are no data concerning the presence of C. botulinum spores in New Zealand honey. However, as environmental contamination is the source of spores, and the types involved in infant botulism cases appear to reflect types present in the environment, the status of New Zealand with respect to toxigenic C. botulinum is important.

Between 1996 and 1998, AgResearch obtained more than 250 isolates of C. botulinum from chilled meats and meat plant environments in the South Island, including the hides, faeces and tonsils of slaughtered animals, soil, vegetation and mud. The isolates were all non-proteolytic (Group II) types B, E or F. The typing assignment was based on restriction fragment length polymorphism (RFLP) analysis and DNA sequencing of the 16S rRNA genes. However, none of the isolates carried botulinal neurotoxin genes.
While the 16S rDNA genes of New Zealand *C. botulinum* isolates share 100% similarity with toxigenic non-proteolytic *C. botulinum* types B, E or F, their genome hybridises at <60% with that of the reference strains (i.e. has <60% similarity). This means that some New Zealand isolates are true non-toxigenic *C. botulinum* while others need to be classified as different species.

However, in experiments between 2000 and 2002, AgResearch scientists detected fragments of the botulinal neurotoxin genes by PCR in DNA isolated from samples taken from farm environments (without isolation of bacteria). The DNA sequences of the fragments identified them as neurotoxin types B, E or F sequences.

The identification of fragments of B, E and F toxin genes in these samples raises the possibility that Group I (proteolytic) toxin B or F producing *C. botulinum* are present, despite not being identified amongst the isolates obtained in the earlier experiments.

During 1999-2000 Crop and Food Research collected 498 sediments samples from around the New Zealand coastline. The samples were enriched and tested for botulinum biotoxin by mouse bioassay. No positive samples were found. Marine environments are associated with Type E, which is not linked to infant botulism.

The risk to New Zealand infants (or adults with altered “at risk” gastrointestinal status) from *C. botulinum* spores in honey appears to be extremely low. The absence of diagnosed cases of infant botulism (from any source), and the lack of conclusive evidence for toxigenic Group I Type A or B species of *C. botulinum* from the New Zealand environment support this conclusion. However, there are a number of difficulties in using this information to properly assess the risk.

Potentially there may be undiagnosed infant botulism cases among the approximately 50 SIDS cases reported annually in New Zealand, or the illness may be attributed to other causes (see Section 4.1). The isolates of *C. botulinum* examined by AgResearch were obtained from the South Island only, not all of them were from the environment. There was also evidence for the presence of (partial) toxin genes in DNA extracted directly from environmental samples, although whether this truly indicates the presence of viable bacteria is unclear.

Honey imports are tightly controlled (although not specifically for *C. botulinum* contamination), and apparently only small amounts are imported at the present time. The situation with the recent Import Health Standard being issued for Australian honey (on 11th July 2006) may change this. Thus, the presence or absence of toxin producing *C. botulinum* in the Australian and New Zealand environment is a key point, given that environmental contamination, either directly or indirectly via bees, is the source of the spores in honey, and the types found in honey reflect those found in the environment. There are insufficient data at present to adequately assess this issue.

The New Zealand recommendation that honey is not fed to infants under six months of age is different to that in many other developed countries, where 12 months is the recommended age limit. The New Zealand recommendation appears to be based on a 1981 reference which asserts that 98% of cases occur in infants under 6 months of age. More recent information suggest that the percentage is lower than that, perhaps 90%. The regulation changes in 2006...
allowing importation of honey from Australia suggest that a reconsideration of New Zealand recommendations for feeding of honey to infants would be worthwhile.

The data gaps identified in this Risk Profile are:

- Prevalence and types of *C. botulinum* in the New Zealand environment;
- Prevalence of *C. botulinum* spores in honey in New Zealand; and
- Consumption of honey by infants in New Zealand.
1 INTRODUCTION

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. The place of a Risk Profile in the risk management process is described in “Food Administration in New Zealand: A Risk Management Framework for Food Safety” (Ministry of Health/Ministry of Agriculture and Forestry, 2000). Figure 1 outlines the risk management process.

Figure 1: Risk Management Framework

Figure reproduced from “Food Administration in New Zealand. A risk management framework for food safety” (Ministry of Health/Ministry of Agriculture and Forestry, 2000).

In more detail, the four step process is:

1. Risk evaluation

- identification of the food safety issue
- establishment of a risk profile
- ranking of the food safety issue for risk management
- establishment of risk assessment policy
- commissioning of a risk assessment
- consideration of the results of risk assessment
2. **Risk management option assessment**

- identification of available risk management options
- selection of preferred risk management option
- final risk management decision

3. **Implementation of the risk management decision**

4. **Monitoring and review.**

The Risk Profile informs the overall process, and provides an input into ranking the food safety issue for risk management. Risk Profiles include elements of a qualitative risk assessment. However, in most cases a full exposure estimate will not be possible, due to data gaps, particularly regarding the level of hazard in individual foods. Consequently the risk characterisation part of a risk assessment will usually rely on surveillance data. The Risk Profiles also provide information relevant to risk management. Based on a Risk Profile, decisions are made regarding whether to conduct a quantitative risk assessment, or take action, in the form of gathering more data, or immediate risk management activity.

This Risk Profile concerns *Clostridium botulinum* in honey. Illness is caused by contamination of honey with bacterial spores, which can colonise the gut and produce toxin. The group at highest risk are infants, as their lack of a well-established gut microflora means that the bacteria are more likely to become established.

The sections in this Risk Profile are organised as much as possible as they would be for a conventional qualitative risk assessment, as defined by Codex (1999).

**Hazard identification, including:**

- A description of the organism
- A description of the food group

**Hazard characterisation, including:**

- A description of the adverse health effects caused by the organism.
- Dose-response information for the organism in humans, where available.

**Exposure assessment, including:**

- Data on the consumption of the food group by New Zealanders.
- Data on the occurrence of the hazard in the New Zealand food supply.
- Qualitative estimate of exposure to the organism (if possible).
- Overseas data relevant to dietary exposure to the organism.
Risk characterisation:

- Information on the number of cases of adverse health effects resulting from exposure to the organism with particular reference to the food (based on surveillance data)
- Qualitative estimate of risk, including categorisation of the level of risk associated with the organism in the food.

Risk management information:

- A description of the food industry sector, and relevant food safety controls.
- Information about risk management options.

Conclusions and recommendations for further action
2  HAZARD IDENTIFICATION: THE ORGANISM

The following information is largely adapted from a data sheet http://www.nzfsa.govt.nz/science/data-sheets/clostridium-botulinum.pdf prepared by ESR under a contract for the Ministry of Health (and now kept on the NZFSA website). The data sheet is intended for use by Regional Public Health Units.

2.1  Clostridium botulinum

2.1.1  The organism/toxin

*C. botulinum* bacteria are straight to slightly curved, Gram positive rods. They form oval, subterminal heat-resistant endospores which can distend the cell (Szabo and Gibson, 2003). The organism can produce the most potent biological neurotoxins known, which if ingested, result in ‘botulism’. The amount of type A toxin required to cause death in humans varies between 0.1 and 1.0 mg (Szabo and Gibson, 2003). A lower lethal dose is estimated at 1ng/kg body weight by the European Commission, 2002.

There are eight recognised antigenically distinct toxins, designated as types A, B, C\(_\alpha\), C\(_\beta\), D, E, F and G (Austin, 2001). All are neurotoxins except C\(_\beta\) which is an ADP-ribosylating enzyme. *C. botulinum* type G has been renamed *C. argentinense*. Most isolates are single toxin producers although some can produce multiple toxins. Four of the types; A, B, E and (rarely) F cause botulism in humans. Type C botulism in humans is very rare. Types C, D and E cause botulism in mammals, birds and fish (WHO, 2002).

It is important to note the difference between intoxication and infection when discussing botulism. Ingestion of the pre-formed toxin (foodborne) or inadvertent injection are intoxications, whereas infections occur in infant botulism, adult infectious botulism and the wound form of illness because the disease results from ingestion or wound infection by spores. Where spores germinate, the vegetative cells produce the toxin *in vivo*. This Risk Profile concerns the infection form of the disease.

**Groups and types**

The species is sub-divided on the organism’s proteolytic ability. Four physiological Groups; I, II, III and IV are currently recognised. Most human botulism outbreaks are caused by Groups I (proteolytic) and II (non-proteolytic).

Group I includes isolates producing types A, B and F toxins and the proteolytic activity generally causes spoilage of food. Group II includes toxin types B, E and F where spoilage is not apparent. Animal disease is usually caused by Group III *C. botulinum*. There are no records of disease in humans or animals from Group IV *C. botulinum* (Szabo and Gibson, 2003).
2.1.2 Growth and survival

Growth:

Temperature: These data are for growth under optimum laboratory conditions. Minimum temperatures will be higher where pH and aw values are lower or where preservatives are added to the food.

<table>
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<tr>
<th>Group</th>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
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<tbody>
<tr>
<td>I</td>
<td>10.0°C</td>
<td>35 – 40°C</td>
<td>45 – 50°C</td>
</tr>
<tr>
<td>II</td>
<td>3.0°C</td>
<td>25 – 30°C</td>
<td>40 – 45°C</td>
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1 The ICMSF (1996) and Szabo and Gibson (2003) cite 3.3°C as the minimum growth temperature for Group II. Work by Graham et al. (1997) found growth and detected toxin production at 3°C in 5 weeks. In a review by Peck (2006), growth was also reported at 3°C after 7 weeks, at 3.1°C after 6 weeks and at 3.2°C and 3.3°C after 5 weeks.


For the proteolytic Group I strains, growth below 10°C has not been reported and at 15°C it is slow. Proteolysis generally produces unpleasant odours during growth.

The non-proteolytic nature of Group II means that foods can appear unaltered with no unpleasant accompanying odours. Group II’s ability to grow at refrigeration temperatures means they are psychrotrophic organisms.

Note also that at the minimum temperatures, toxin production may take several weeks to occur (Szabo and Gibson, 2003). In the scientific literature, measures of growth are often reported as ‘time to detectable toxin’.

pH: All strains of C. botulinum grow and produce toxin down to pH 5.2 (when other conditions are optimal). Group I grow slowly down to pH 4.6. Group II strains grow slowly down to pH 5.0. At this point, cells generally undergo sporulation. In some circumstances, germination and growth can take place below an initial pH of 4.6 (Szabo and Gibson, 2003). These generally involve another organism such as a mould or bacterium being present, which raises the pH levels. There are occasional recorded instances of toxin production at lower pH values, e.g. potatoes adjusted to pH 4.83, and an outbreak linked to canned tomato juice where initially the food had a pH less than or equal to 4.6 (Szabo and Gibson, 2003).

Atmosphere: Clostridium botulinum is considered to be an anaerobe, but it can grow in air if the redox potential (Eh – the overall balance of oxidising and reducing agents present) is low. For example, a foodborne outbreak of botulism occurred when C. botulinum spores were trapped between the skin and the foil in baked potatoes (Angulo et al., 1998). It has been suggested that the metabolic activity of high numbers of spores germinating lowers the Eh to allow clostridial bacterial growth (Szabo and Gibson, 2003). The presence of up to 20% oxygen in packaged foods has been shown not to prevent growth of the bacterium. Carbon dioxide concentrations above 75% have retarded growth of C. botulinum, but toxin production by the Group II organisms in fish (under temperature abuse conditions) still...
occurs under 100% carbon dioxide. Modified atmosphere packaging together with refrigeration did not prevent growth and toxin production by Group II organisms in cooked turkey.

**Water activity:** *C. botulinum* can grow at water activity ($a_w$) values of 0.9353 (NaCl ≈10%) for Group I and 0.9707 (NaCl=5%) for Group II. Interactions with additional factors such as pH can influence the NaCl concentrations required to permit growth. Growth of Group I *C. botulinum* occurred at pH 5.2 in the presence of 3.95% NaCl, while at 4.67% NaCl, growth occurred at pH 5.6 (Szabo and Gibson, 2003). Toxins can be produced at $a_w$ values which permit growth.

Group I *C. botulinum* could not grow at 3.95% NaCl at a pH of 5.0. At 4.67% NaCl, growth was inhibited at pH 5.4 (Szabo and Gibson, 2003).

**Growth enhancing micro-organisms:** The growth of acid-tolerant moulds such as *Cladosporium* spp., *Penicillium* spp. can result in pH increases in foods permitting the growth of *C. botulinum* (Austin, 2001).

**Survival:**

**Temperature:** Reductions in numbers of vegetative cells under frozen storage are poorly understood. All types of spores and toxins are resistant to freezing. Studies have demonstrated that frozen storage does not reduce the activity of preformed botulinum neurotoxin in food (ICMSF, 1996).

**Water activity:** Spores can survive drying. Botulinum toxin type A can be preserved and used in medicine by drying with human serum albumin (stabiliser) at pH 7.3, although a substantial loss in toxicity occurs (up to 50 - 90%) (Schantz and Johnson, 1992).

2.1.3 Inactivation, Critical Control Points and Hurdles

Note that in microbiological terms “D” refers to a 90% (or decimal or 1 log cycle) reduction in the number of organisms. Details of thermal inactivation of *C. botulinum* have largely been derived from work for the food canning industry.

**Temperature:** At temperatures at or below 3°C, germination and growth of both Group I and Group II *C. botulinum* will not occur. This can be used as a control point for storing products under deep chill conditions without any additional controlling factors (Betts, 1996). All vegetative cell types of *C. botulinum* are readily killed at pasteurisation temperatures. Destruction of spores requires higher times and temperatures. For spores of Group I strains, the ‘botulinum cook’ was developed. This well known food processing procedure for low-acid (pH>4.6) canned foods is based on the destruction of Group I heat resistant spores. The ‘12D’ process, as it is also known, equivalent to exposure to moist heat at 121°C for 3 minutes, is sufficient to reduce the population of spores by a factor of $10^{12}$. At 100°C, it takes 25 minutes to inactivate 90% of spores. Group II spores are less resistant to heat than Group I. At 100°C, 90% of spores are destroyed in less than 0.1 minute (ACMSF, 2005).

Recent papers have challenged the log linear nature of spore inactivation which is the conventional model for bacterial inactivation by heat, and suggest a Weibull model.
distribution curve to represent the relationship more accurately (Van Boekel, 2002). Application of this model to C. botulinum spores has been reported (Peleg and Cole, 2000) but the implications for commercial sterility requirements are not resolved. All neurotoxins can be inactivated at time/temperature combinations ranging from 65°C for 1.5 hours to 85°C for 1 minute. Alternative time/temperature combinations of 80°C for 30 minutes to 100°C for 10 minutes have been suggested (Slovis and Jones, 1998).

**pH:** Since a pH of 4.6 is considered the demarcation point at or beneath which Group I C. botulinum is not able to germinate and grow, high acid canned foods (pH< 4.6) are not required to undergo the full ‘botulinum cook’. Similarly, the low pH formed by fermentation in certain meat products prevents growth.

Neurotoxins are inactivated at pH 11 and above.

**Water activity:** Growth of Group I C. botulinum strains is inhibited; when NaCl in water exceeds 10% (a\(_w\) =0.9353). Growth of C. botulinum type E is inhibited for 13 weeks at 10°C by the presence of 5% NaCl (Graham et al., 1997). At temperatures less than this, the concentration of NaCl required to inhibit growth at a given pH reduces such that at 5°C, 3.5% NaCl results in a similar inhibition.

**Stability in water:** In tap water, the toxicity of toxin types A and B are reduced by 80% at room temperature for 1 to 3 days, compared to 2 to 4 days for type E toxin (Szabo and Gibson, 2003).

**Preservatives:** Preservatives such as nitrites, sorbic acid, parabens, phenolic antioxidants, polyphosphates and ascorbates inhibit C. botulinum growth. Interactions between reduced water activity, pH and temperature are usually used in combination with preservatives to achieve several microbial hurdles. Curing salts, particularly sodium nitrite, are used in meat preservation specifically to control C. botulinum. Smoking of meat and meat products has little effect on spores although adding liquid or generated smoke reduces the inhibitory level of NaCl in aqueous phase from 4.6 to 2.8% for type A spores and from 3.7% to less than 2% for type E spores (Szabo and Gibson, 2003).

**Competitive micro-organisms:** The acid produced by lactic acid bacteria such as Lactobacillus, Pediococcus and Streptococcus spp. is inhibitory to growth of C. botulinum due to reduced pH, and some of these organisms also produce bacteriocins (research cited in Austin, 2001). Inhibition of growth of type E C. botulinum by naturally occurring Bacillus spp. has been observed in cooked surimi nuggets (Lyver et al., 1998).

**Radiation:** To inactivate C. botulinum spores in honey, the D values ranged from 1.91 to 12.8 kGy (Huhtanen, 1991). All spores are relatively resistant. Resistance is greater below about −80°C than at ambient temperature. To inactivate C. botulinum spores in foods and neutral buffers, at a temperature below 10°C, the D values for group I strains vary between 2.0 and 4.5 kGy. Group II, type E spores require marginally less radiation at 1.0 to 2.0 kGy (Szabo and Gibson, 2003). The toxins, like all proteinaceous toxins, are not inactivated by the levels of irradiation used by the food processing industry (ICMSF, 1996).

**Disinfectants:** The sporicidal nature of chlorine and iodophor compounds and their common use in the food industry as disinfectants is potentially useful, although optimum effectiveness
is determined by the type of compound, concentration, exposure time, temperature and presence of organic matter. Chlorine is more effective at low pH (3.5) than at neutral or high pH. Group I spores are more resistant than those from Group II organisms. Spores are inactivated in ozone and chlorine dioxide.

In ethylene oxide spores are inactivated although the mechanism is unknown. Similar issues such as temperature, relative humidity, exposure time, concentration etc, must be considered.

With hydrogen peroxide, levels up to 35% are required for spore inactivation (usually together with a raised temperature combination) (Szabo and Gibson, 2003).

**Pressure:** Spores are reported to be very pressure resistant in contrast to vegetative cells. However, under low pressure spore inactivation can be more rapid and complete. This is explained by the process taking place in two stages. First, the pressure causes the spores to germinate, then further pressure (or high temperature) inactivates the germinated cell. Throughout the whole pressure range, there is a strong synergy with heat. The inactivation of *C. botulinum* is extremely important in an expanding range of pressure-pasteurised products including those with high pH and high water activity (Peck *et al.*, 2004).

To summarise, the canning industry have developed procedures which destroy *C. botulinum* spores. Where a ‘botulinum cook’ is not practical, hurdles are used, mainly in the form of temperature controls (to inhibit germination and growth), lowered pH (acidification) and reduced water activity through the manipulation of salt levels.

**2.1.4 Sources**

In approximately 85% of cases, the vehicle of botulism infection is unknown and could be foodborne or environmental.

**Human:** *C. botulinum* is not a normal part of the healthy human adult or infant intestinal flora. A study of the faeces of 160 healthy infants age-matched to infant botulism cases found that *C. botulinum* were not normally resident in the microflora (Arnon, 1980b). The normal infant microflora contains mainly *Bifidobacterium* and *Bacteriodes* spp., that in *vitro*, can inhibit the multiplication of *C. botulinum*.

**Animal:** The disease in animals is usually caused by pre-formed ingested toxin, although spores of *C. botulinum* have been found in the intestinal tracts of fishes, birds and mammals and in decomposing carrion. Many predator and scavenger vertebrates feeding from carrion are assumed to have developed immunity by selection. Animals particularly affected are cattle and birds, and to a lesser extent, horses, sheep, pigs and zoo animals. The main types involved in are toxins C and D, and to a lesser extent, toxin types F and G. An outbreak of *C. botulinum* type C in wild waterfowl in 1971 demonstrated that the genus occurred in New Zealand. In 1986, the first confirmed case of botulism in a dog in New Zealand (intoxication - type C) occurred in Hamilton (Wallace and McDowell, 1986).

**Food:** Many surveys have identified *C. botulinum* spores in food, especially fish, meats, honey and vegetables/mushrooms. Spores have been repeatedly isolated from leftover honey fed to infected infants with the toxin types matching isolates from the stools (Dodds, 1993).
Environment: *C. botulinum* is found worldwide, although quantitative spore surveys have found unexplained concentrations in certain areas. For example, type A, B and F spores are distributed widely in soils and sediments. However, there is a recognised divide in North America. Soil west of the Mississippi river in the USA is predominantly contaminated with type A spores while soil east of the river is predominantly type B. Sediments from the Great Lakes region contain type E only [E is associated with marine environments] while wetlands soil from Saskatchewan, Canada yield type C spores. The ICMSF (1996) reports that type B spores are more prevalent in European soils. Despite its ubiquitous nature, the level of spore contamination is often low or very low.

Smith (1978) reported that type A strains were isolated from neutral to alkaline soils (pH 7.5 average) while type B strains were from slightly more acidic soils (average pH 6.25). The reasons for this are unknown.

In New Zealand, types C and D (which are not linked to human botulism) have been isolated in lake and waterway sediments in the Auckland area (Gill and Penney, 1982).

Transmission Routes: Person-to-person transmission of botulism does not occur (WHO. 2002). Infant botulism may be caused by exposure to organisms in soil and dust, or spores contaminating honey.

2.2 *Clostridium botulinum* Isolation and Typing

Direct plating of food or faeces onto suitable agars rarely yields *C. botulinum* because of the presence of other competitive organisms. Heat treatments to isolate spores followed by subculturing from enrichments are the preferred method. Incubation is generally 5 to 10 days at 26 to 35°C. Identification of neurotoxin types after enrichment is carried out by mouse bioassay protection tests (takes 48 hours) or ELISA (enzyme-linked immunosorbent assays), both techniques are detailed in Szabo and Gibson, (2003). A table of the various detection methods (mouse lethality, ELISA, endopeptidase, various PCR methods, fluoroimmunoassays, fibre optic-based biosensor, evanescent wave immunosensor) can be found along with type of neurotoxin detectable and associated bibliographic references in European Commission (2002).

Molecular techniques have determined the gene sequence of the bacterium. Comparative analysis of the 16S and 23S rRNA gene sequence have identified sequences which are characteristic of each physiological group (I, II, III and IV) and confirm how close genetically *C. botulinum* is to non-neurotoxin producing clostridia (Szabo and Gibson, 2003).
3 HAZARD IDENTIFICATION: THE FOOD

Honey is the only food source linked with infant botulism. Spores have also been detected in corn syrup in the USA, although there have been no confirmed cases linked to corn syrup (Kautter et al., 1982). Syrups in New Zealand are predominantly based on sugar cane juice (golden or cane syrup) or molasses. Imported syrups are generally in the form of maple (tree) syrup. None of these forms of syrups have been implicated in cases of infant botulism.

3.1 Relevant Characteristics of the Food: Honey

Honey is defined by Codex (1987) as “the natural sweet substance produced by honeybees from the nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature”. The same definition is used by Food Standards Australia New Zealand (FSANZ) (2002) and the European Union in Council Directive 2001/110/EC (European Commission, 2001).

As the definition suggests, there are two types of honey according to the flora from which it originates;

- Blossom or nectar honey, obtained from nectaries of plants, and
- Honeydew honey, obtained from excretions of plant sucking insects (Hemiptera) on the living part of plants or secretions directly from the living parts of plants (European Commission, 2002).

The main components are carbohydrates and water followed by trace constituents. Carbohydrates constitute the bulk of honey at 73 to 83% (European Commission, 2002). The main carbohydrates are fructose (30.9 – 44.3%) and glucose (22.9 – 40.8%). The water content of honey is usually between 14.5 and 18.5%. Higher values can allow fermentation to occur. Codex (1987) stipulates that honeys must not contain more than 21% moisture content.

Invert sugar is the sum of monosaccharides glucose (dextrose) and fructose, and includes these sugars that might be produced by the hydrolysis of any sucrose present. The sum of “inverted sugar” is lower in honeydew honeys (Codex Standard, not less than 60%) compared to blossom honeys (not less than 65%).

Other minor constituents include organic acids (0.6%, primarily glucoronic acid), nitrogenous compounds (0.4%, mainly proteins), minerals (0.1%), and vitamins, lipids and aromatic substances. The pH of honey ranges from 3.4 to 6.1 with an average of 3.9 and water activity varies between 0.5 and 0.6 (Snowden and Cliver, 1996).

Production of honey in New Zealand is based upon the introduced white clover (Trifolium repens). The honey bee was introduced into New Zealand in 1839 and is the dominant pollinator of white clover. New Zealand has a relatively small indigenous flowering plant population, there are currently 1813 species known, 84.4% of which are endemic (Godley, 1979).
Worldwide, no investigation to date has found preformed toxin in the honey (foodborne botulism) (Midura, 1996). Despite the anaerobic conditions due to high viscosity, the high sugar content, low pH, low protein concentration as well as oxidases and other antimicrobial substances in honey, mean that the spores cannot germinate and grow to produce toxin (Snowden and Cliver, 1996).

Research in the USA inoculated bees with *C. botulinum* spores (Huhtanen et al., 1981) to investigate whether they carried the spores into the honey. The bees were fed a 50% solution of water and sugar containing $1.6 \times 10^5$ spores (of 20 different isolates; 11 type A and 9 type B). After two weeks, collected honey had 1100 spores/g and after 5 weeks, 50 spores/g. Quantitative estimates found that all of the spores had been incorporated into the honey. However there was no multiplication and the organism was not found two weeks later in the intestinal or rectal contents of the bees. This suggests that the bees do not harbour these micro-organisms.

Spores are not normally distributed uniformly throughout honey but appear as clusters. The reason for this is unknown. Where high numbers of vegetative bacteria are found, this indicates recent secondary process (post-harvest) contamination (Snowden and Cliver, 1996).

Several papers have researched the effect of time and temperature on the spore prevalence in honey. Kokubo *et al.*, (1984) found that when they inoculated *C. botulinum* spores (and *B. cereus* and *C. perfringens*) into commercial honey, after 4 months at 25°C (~120 days), the spore count remained the same.

However, decreasing spore prevalence in honey from apiaries through to retail packaging was found by Nakano *et al.*, (1990). Spores were more common from apiary samples (23% positive) than from samples in drums (18%) and retail packages (5%). The authors also found a decrease in viable spores after honey was stored for a year at 25°C with the number of spores decreasing after 100 days (Nakano *et al.*, 1989) (in contrast to the Kokubo *et al* study above). At 4°C however, the spore population remained the same, even after 12 months. The authors also found that after mild heat treatment at 65°C for five days, no spores were detected. Nakano *et al.*, (1990) suggest that the routine processing and storage of honey in warehouses, which can take months under warm conditions, might explain the low prevalence of spores at retail and that a time/temperature matrix for spore survival exists.

The trend of decreasing spore prevalence was confirmed by Nevas *et al.*, (2005b). The authors found higher spore prevalence in honeycomb rather than extracted and retail packaged honey, i.e. numbers decline during processing.

According to the National Honey Board in the USA if honey is stored in a sealed container it can remain stable for decades [http://www.nhb.org/download/factsht/shelf.pdf](http://www.nhb.org/download/factsht/shelf.pdf). However physical and chemical changes during storage darkens the honey and it looses flavour and aroma. Practically, a two year shelf life is often stated. Processed honey should be stored between 18 and 24°C. In considering the survival of spores in honey during long term storage, the European Commission (2002) see no reason why spores of *C. botulinum* cannot survive in honey for years.
3.2 The Food Supply in New Zealand: Honey

New Zealand honey producers have founded a co-operative of over 100 beekeepers; http://www.nzhoney.co.nz/. To the 20th June 2005, there were 2,911 beekeepers, 19,281 apiaries and 294,886 hives. Poor climatic conditions in 2002 together with the impact of the varroa mite had a dramatic effect on honey production in that year (total production 4,682 tonnes), although the following year was very productive (12,252 tonnes). Total production in 2004 was 8,888 tonnes (MAF, 2005). Twelve percent of commercial beekeepers own 94% of the hives (MAF, 2002a).

It is difficult for New Zealand honey exporters to compete with bulk honey producing countries; China exports 75,000 tonnes a year, followed by Argentina (60,000) and Mexico (30,000) (European Commission, 2002). Australia and Canada are also bulk producers. Therefore, New Zealand concentrates on added value honey products as the main exports.

The average annual honey production in New Zealand is usually above the domestic consumption figure of approximately 5000 tonnes. The surplus of 3000 – 3500 tonnes of honey a year is exported or placed into storage. There is also a small trade in other bee products such as live bees in bulk (exported to Canada, Korea, Japan and Germany), royal jelly and propolis. Bees collect tree resin to make propolis, a substance used to encase the hive, line each cell in the honeycomb and used extensively at the surface of the hive.

3.2.1 Imported food

The border controls regarding bee products into New Zealand are strict. It is prohibited to bring into New Zealand any honey, used hive equipment, live bees or other apiary products. There are some exceptions for certain countries, namely;

- Pitcairn Island,
- Niue,
- Solomon Islands,
- Tonga,
- Tuvalu, and
- Western Samoa.

To this list, Australia can now be added with the advent of an import health standard dated 11 July 2006 (Anon, 2006). This health standard can be found at the following website address; http://www.biosecurity.govt.nz/imports/animals/standards/beeproic.aus.htm. The Veterinary Certificate that must accompany such imports is attached in Appendix 1.

The Pitcairn Islands have their own import health standards (IHS) for honey and propolis into New Zealand. The IHS can be found at the following website; http://www.biosecurity.govt.nz/imports/animals/standards/beehonic.pit.htm.

For the remaining Pacific Islands, there is an IHS for the import of specified bee products which includes honey in liquid, comb, propolis or any other form; http://www.biosecurity.govt.nz/imports/animals/standards/beeproic.all.htm.

There is no mention in the IHS of $C.\ botulinum$ spores.
In the last two years, only Pitcairn Island, Niue and Western Samoa have exported honey to New Zealand (Leone Basher, MAF, personal communication, 04.11.05).

3.2.2 Processing

There are three methods of processing defined by Codex (1987). These are:

- Extracted honey; obtained by centrifugation of decapped broodless combs,
- Pressed honey; obtained by pressing broodless combs with or without moderate heat, and
- Drained honey; obtained by draining decapped broodless combs.

The main stages of processing are nectar or honeydew collection, harvesting, purification by straining or decantation, pasteurising (optional), ripening, packaging and storage. None of these processes remove or inactivate Group I \textit{C. botulinum} spores because of their heat resistance and small size (European Commission, 2002).

Honey is minimally processed for table use. Spoilage by fermentation is a major concern with honey as the water activity may allow osmophilic yeasts to grow.

The ICMSF (1998) and the European Commission (2002) describe the processing of honey as follows: Once the bees have been smoked out, the upper part of the hive honeycomb is removed. The thin wax caps must first be removed by scraper or a heated knife. Honey is extracted from the cappings by centrifuging or melting. Centrifuging to remove spores is not feasible because pollen would also be removed, an essential component for honey identification and labelling. The honey can be purified by straining or decantation (or filtration).

Straining is where the honey is heated to 30-35°C and passed through a strainer (a 150µm screen). This removes bee fragments, propolis, splinters etc, but does not remove spores.

Decantation is where the honey is placed into ripening containers, and maintained at 25°C. Air bubbles, wax and other impurities (except pollen and \textit{C. botulinum} spores) either settle or rise to the surface and can be removed (European Commission, 2002).

Filtration (honey heated to 60°C, filtered through ceramic or diatomaceous filters, with pore sizes less than 50µm) removes all pollen grains and extraneous solids. Under European law, no pollen or other individual ingredient of honey is allowed to be removed unless it is inevitable to remove foreign matter, consumers must be informed of the filtering process. Removing the pollen makes it impossible to determine the floral origin of the honey and risks exceeding the 80 or 40 mg/kg HMF Codex and European Directive limit (hydroxyl-methyl-furfural) described below. Subsequently, most countries do not filter honey. \textit{C. botulinum} spores may be removed where they are adhering to these particles, but the size of filter would need to be 0.5µm to remove spores and this would be impractical as a control method.

The ripening of the liquid honey takes two weeks at 15°C. It is then drawn off by pump and placed into containers.
The effect of heat is cumulative and must be limited. Heat can be used at extraction and during the handling of the honey but excessive heat must be avoided as it can darken the honey and reduce its quality (hexoses lose 3 molecules of water to produce 5-hydroxy-2-furaldehyde or HMF). Standard 3.10 of Codex (1987) states that honey shall normally not exceed 40 mg/kg HMF.

Pasteurisation of honey is not common although it can destroy sugar-tolerant yeasts preventing fermentation and delay crystallisation. In addition, pasteurisation removes vegetative bacteria but the process has no effect on botulinum spores. The European Commission (2002) states that a common pasteurisation treatment is 77°C for 2 minutes followed by rapid cooling (usually by plate heat exchanger) to 54°C. However a subsequent time/heat combination equivalency range is cited as between 60°C for 30 minutes to 71°C for 1 minute.

Descriptions of processed honey styles reflect its state. Codex (1987) categorises styles as follows;

- Liquid or crystalline form or a mixture of the two,
- Comb honey,
- Chunk honey, honey which contains one or more pieces of comb honey,
- Crystallized or granulated honey, a natural process of solidification, and
- Creamed honey.

Creamed (creamy or set) honey has undergone an induced and controlled crystallisation process to produce very fine crystals. This is achieved by mixing liquid honey (90%) with fine crystallised honey (10%) at 25 – 27°C and after a few hours (to allow decantation) the honey is placed into containers and stored at 14°C where complete crystallisation occurs within 4 to 5 days (European Commission, 2002). Creamed honey accounts for approximately 80% of the honey sold in New Zealand.

A Code of Practice “Processing of bee products” has been produced by the NZFSA: http://www.nzfsa.govt.nz/animalproducts/publications/code-of-practice/Bee/part-3/bee-cop-part-3.pdf. Within this document are the principles of Good Manufacturing Practice, and generic HACCP plans regarding the extraction, processing and packaging of honey. Bacterial spores of Clostridium spp. are identified under bacterial hazards but no control measures are identified.

### 3.3 Clostridium botulinum in the Environment (Soil, Sediments, Bees Intestines, Flowers, Dust)

Primary microbial sources of contamination of C. botulinum into honey possibly include pollen, dust, air, soil, nectar, flowers and digestive tracts of bees (Snowden and Cliver, 1996; European Commission, 2002).

#### 3.3.1 Soil

Nevas et al. (2005b) regards the prevalence of spores in honey as a reflection of the overall spore count in the environment. The authors cite the research on cultivated farmland soil in Denmark (41% positive, mostly type B) (Huss, 1980) and the significant prevalence of
mostly type B spores in the honey (26%), see Table 1. Similar observations are made in Sweden regarding type E spores.

In Argentina, from 1982 to 1997, there were 146 laboratory confirmed cases of infant botulism, all less than one year old. In general, the toxin type involved in disease (type A isolated from all 146 stool cultures) was reflected in the predominance of type A toxin types in the soil (Centorbi et al., 1999; Fernández et al., 1999).

A summary of the environmental distribution data of *C. botulinum* collected overseas can be found in Bell and Kyriakides (2000). In Table 1 below, only the data relating to soils are reproduced.

**Table 1: Summary of *C. botulinum* toxin types in overseas soils**

<table>
<thead>
<tr>
<th>Country</th>
<th>Sites or samples positive (%)</th>
<th>Toxin types</th>
<th>Percentage of sites or samples positive</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>33.8%</td>
<td>A B C/D E F/G</td>
<td>67% 20% 0% 0% 5% / 1%</td>
<td>Hauschild, 1989</td>
</tr>
<tr>
<td>Denmark</td>
<td>41.0% cultivated farmland</td>
<td>B</td>
<td>&gt;90%</td>
<td>Huss, 1980</td>
</tr>
<tr>
<td>England &amp; Wales</td>
<td>13.1</td>
<td>B and untyped</td>
<td>55.5%</td>
<td>Meyer and Dubovsky, 1922</td>
</tr>
<tr>
<td>Great Britain</td>
<td>10.0%</td>
<td>B</td>
<td>100%</td>
<td>Smith and Young, 1980</td>
</tr>
<tr>
<td>Poland (Baltic coast)</td>
<td>31.5%</td>
<td>A B C/D E</td>
<td>3% 0% 0% 97%</td>
<td>Hauschild, 1989</td>
</tr>
<tr>
<td>Switzerland*</td>
<td>35.2%</td>
<td>B and untyped</td>
<td>72.7%</td>
<td>Meyer and Dubovsky, 1922</td>
</tr>
<tr>
<td>USA (Eastern)</td>
<td>19.0%</td>
<td>A B C/D E</td>
<td>12% 64% 12% 12%</td>
<td>Hauschild, 1989</td>
</tr>
<tr>
<td>USA (Western)</td>
<td>28.0%</td>
<td>A B C/D E</td>
<td>62% 16% 14% 8%</td>
<td>Hauschild, 1989</td>
</tr>
<tr>
<td>Country</td>
<td>Sites or samples positive (%)</td>
<td>Toxin types</td>
<td>Percentage of sites or samples positive</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------</td>
<td>-------------</td>
<td>----------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Former USSR (Europe)</td>
<td>9.4%</td>
<td>A</td>
<td>8%</td>
<td>Hauschild, 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/D</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
<td>73%</td>
<td></td>
</tr>
<tr>
<td>Former USSR (Asia)</td>
<td>12.6%</td>
<td>A</td>
<td>9%</td>
<td>Hauschild, 1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/D</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E</td>
<td>44%</td>
<td></td>
</tr>
</tbody>
</table>

* Soils and vegetables

No information was found in the literature regarding *C. botulinum* in the environment or in honey for the Pacific Islands.

The presence of the *C. botulinum* spores in Australia is relevant, given the potential future imports of honey from there. Surveys have been carried out in Queensland, New South Wales (NSW), Victoria and Tasmania. In 1947, Eales and Gillespie (cited in Szabo and Gibson, 2003), examined 183 soil samples from mountainous regions above 3000 feet in Victoria. Four of the samples contained *C. botulinum* type A.

In 1957, Ohye and Scott (cited in Szabo and Gibson, 2003) studied 22 marine muds from Tasmania and NSW. Two of the samples were positive for type B neurotoxin.

Between 1965 and 1970, Christian (cited in Szabo and Gibson, 2003) examined 528 muds, cultivated soils, fish intestines and potato washings from NSW, Tasmania and Queensland. Specifically looking for *C. botulinum* type E, this study did not detect any toxin type in any the samples.

A survey by Gibson et al. (1994) examined 368 samples from ship ballast waters, harbour sediments, port sediments and estuarine sediments. Type C neurotoxin was detected in one ballast sample from a ship docked in Queensland.

The presence of *C. botulinum* (types B, C and D) in the Australian environment are indicated by the numerous accounts of animal botulism outbreaks, particularly in horses and cattle (Szabo and Gibson, 2003).

In a thesis by Broughton (1993), cited in Szabo and Gibson (2003), PCR methods were used to test for the botulinum neurotoxin genes. Samples from 136 soils and vegetables were collected within a 100 km radius of Brisbane (vegetables included onions, carrots, celery and broccoli. Thirty four soil samples and three vegetable samples were positive. Some samples contained more than one toxin type; 22 x type A, 13 x type B and 5 x type F gene products, making 40 toxin types in total from 37 positive samples. No further details were given.

### 3.3.2 Bee food, their intestines and pupae

Work carried out on bees’ intestines (cited in Snowden and Cliver, 1996) found that they contain 29% Gram-positive bacteria including *Clostridium*, *Bacillus* and *Strepococcus* spp. 70% Gram-negative or Gram-variable bacteria and 1% yeast-shaped microbes.
Samples of sugar products intended to be used as bee food (unrefined sugar and corn syrup) in Japan were tested for the presence of *C. botulinum* (Nakano *et al.*, 1992). Three of the 56 sweetener samples, or 5%, were positive for the spores, while 8/217 (4%) of sweeteners not destined for the bees were positive. Work also carried out by Nakano *et al.*, (1994) found that when dead bees were inoculated with *C. botulinum* spores ($10^2$-$10^3$) per bee and incubated aerobically for ten days, the organisms replicated to $10^4$-$10^5$ per bee. There were similar findings in bee pupae but not in bee larvae (which appeared to dry despite wet cotton being placed in the Petri dish). Larvae under proper humid conditions may grow *C. botulinum*. The authors suggest that the heavy contamination of spores sometimes encountered during surveys is associated with contamination from dead bees.

In summary, *C. botulinum* spores in honey are thought to originate from environmental sources such as airborne dust, the digestive tracts of bees, dust on pollen, the legs of bees, contaminated bee food, and water sources. The sometimes very high concentrations of spores found in honey may be due to the growth of the organism in dead bees in the hive which then contaminate the honey.
4 HAZARD CHARACTERISATION: ADVERSE HEALTH EFFECTS

In humans, there are five recognised clinical forms of botulism (WHO, 1999):

- **Infant**: ingested spores that survive the acidity of the stomach and germinate, colonise and produce neurotoxin \textit{in vivo} in the intestinal tracts of infants below 12 months of age,
- **Adult infectious**: affects adults with altered gastro-intestinal anatomy (e.g. abdominal surgery) and microfloras, e.g. conditions such as Crohn’s disease. Similar disease mechanism to infants, ingestion of spores and colonisation of the intestines producing toxaemia,
- **Foodborne**: food contaminated with preformed botulinum neurotoxin, the toxins associate with non-toxic proteins and the resulting toxin is then protected through the gastric acid conditions of the stomach,
- **Wound**: where spores grow and produce neurotoxin in the wound, associated with intravenous and subcutaneous drug use, and
- **Inadvertent botulism**: suspected where patients have a history of ‘botox’ injections. The toxin is administered into large muscle groups for a systemic effect or as a suicide attempt. A marked clinical weakness is observed along with electro-physiologic abnormalities.

This Risk Profile concerns the first two of these.

4.1 Intestinal toxaemia botulism

Infant and adult infectious botulism are sometimes referred to as ‘intestinal toxaemia botulism’.

Adult intestinal toxaemia cases are very rare. Only ten cases have been described in the USA up to 1995 (Arnon, 1995; Chia \textit{et al.}, 1986; Bartlett, 1986). Predisposing conditions were peptic ulcer, ileo-jejunal bypass, gastric bypass, stomach surgery, cancer treatment, Crohn’s disease and bowel cancer. Two cases had no known predisposing conditions. One of these cases (ileo-jejunal bypass) was the first adult case with a history of honey ingestion. The 51 year-old Californian woman ate honey two weeks before onset (two samples of honey were negative for spores). She had also taken oral penicillin and an antacid for a week before onset. \textit{C. botulinum} type B was detected in the patient’s faeces (Arnon, 1995).

Since 1976, there have been over 1500 cases of infant botulism reported in more than 15 countries worldwide. Of all the various potential sources of spores (soil, dust etc.), honey is the only dietary source that has been linked to the disease through both laboratory and epidemiological studies. There have been cases of infant botulism in infants fed corn syrup although no spores have been subsequently found in the syrup (Kautter \textit{et al.}, 1982).

Most infant patients inhale \textit{C. botulinum} spores carried by dust that sticks to saliva and is swallowed. Such cases are not considered preventable. The only avoidable source of botulinum spores for infants is dietary, and honey has been identified as a vehicle (Arnon, 1995).
Historically, many home remedies for constipation, croup and colic contained honey. It is known overseas that honey has been put onto dummies to encourage infants to take them, and this has led to the disease. Cases linked to dummy use have been reported in Argentina (Centoibi et al., 1999) and Australia (McMaster et al., 2000). In the Australian case, the mother had dipped the dummy into commercial honey. The 11 week old was hospitalised and a variety of different diagnoses made until a culture of the infant’s stool tested positive for the vegetative cells and toxin of \textit{C. botulinum}. A jar of honey was tested but no toxin or spores were found. Other honey jars from which the infant had been fed were no longer available.

The types of \textit{C. botulinum} implicated in infant botulism caused by the ingestion of honey are Group I (proteolytic) types A and B. It has been suggested that because Group I spores are more heat resistant and growth is optimum around the human body temperature, they are at a competitive advantage in the human intestine (ACMSF, 2005).

In children, the intestinal tract lacks the protective bacterial flora and clostridium-inhibiting bile acids found in healthy adults’ intestines. The neurotoxins are synthesized and carried from the intestinal tract by the bloodstream to the neuromuscular endings. WHO (1999) makes the assumption that the toxin is released when the bacterial cell undergoes lysis. The neurotoxins are not critical to the growth of \textit{C. botulinum}.

All toxins share the same pathogenic mechanism, essentially because they are based on the same structure. However, type A has the greatest affinity for nerve tissue (Midura, 1996).

Infant botulism can be difficult to recognise. The most common misdiagnosis is suspected sepsis, other examples of mis-diagnoses in infants are pneumonia, hypotonia of unknown etiology, failure to thrive, myasthenia gravis, poliomyelitis, Guillain-Barré syndrome, brain stem encephalitis, meningitis, hypothyroidism and disorders of amino acid metabolism (Midura, 1996).

### 4.2 Symptoms

*\textbf{Incubation:}\text* The time for the spores to germinate and colonise the intestines is unknown, although periods from 3 to 30 days before symptoms are exhibited have been suggested. In adult infectious botulism cases, the interval between bowel surgery or food exposure and onset of clinical symptoms can be one or more months (WHO, 1999). In the case of both infant and adult infectious botulism, it is likely that the disease results from a single exposure (WHO, 1999).

*\textbf{Symptoms:}\text* The large intestine is thought to be the site of \textit{C. botulinum} spore colonisation. Constipation (3 or more days without defecation in an infant previously defecating at least every other day) is usually the first and most common symptom of infant botulism.

The numbers of \textit{C. botulinum} cells in faeces can rise to $10^3 – 10^8/g$ before the clinical symptoms occur (ICMSF, 1996). Diagnosis is confirmed by detecting \textit{C. botulinum} or its neurotoxin in the infant’s stools or enema fluids, since toxin levels in the serum are often too low to allow detection (Szabo and Gibson, 2003).
There is a wide spectrum of severity; from asymptomatic cases where little toxin is absorbed through to paralysis and even sudden death.

Extreme forms of infant botulism have been controversially linked by some to misdiagnosed Sudden Infant Death Syndrome (SIDS) because of the similarity in respiratory arrest and the virtually identical age distribution of cases (WHO, 1999; ACMSF, 2005). There are still mixed views in the medical and research groups regarding this.

**Condition:** Infant botulism and adult infectious botulism. (Intestinal Toxemia). Commonly known as ‘floppy baby syndrome’.

**Toxins:** Spores of *C. botulinum* germinate and colonise the alimentary tract of infants, producing *in vivo* neurotoxins which are then distributed in the bloodstream. Healthy adult gut flora is fully developed at a density of $10^{11} – 10^{12}$ anaerobes/g of faeces, out-competing the clostridium spores (Arnon, 1995). Bile is also inhibitive to establishment. In infant botulism and adult infectious botulism, the neurotoxins are not preformed in the food (unlike foodborne botulism).

**At Risk Groups:** Infants between 1 week and 12 months of age (Midura, 1996). The youngest case has shown symptoms at 6 days, (Arnon, 1995). In 1990, a 2 year old was diagnosed with the disease (Dodds, 1993).

Controversial evidence links differences in formula-fed and breast-fed babies (see section 6.2.3). Onset of infant botulism occurs sooner in formula-fed babies (7.6 weeks) compared to breast-fed babies (13.7 weeks) (Arnon et al., 1982). The ACMSF (2005) conclude that formula-fed children are at greatest risk in the first few weeks of life. Arnon (1995) suggests that the formula-fed infant lacks immune factors such as secretory immunoglobulin type A (s-IgA), leukocytes, lactoferrin, lysozyme etc, which are present in human milk. Mothers’ colostrums have also been shown to contain antibodies to organisms that include *C. botulinum* (Arnold et al., 1982). Breast fed babies are at most risk during the weaning stage, possibly due to the dietary changes allowing clostridial spores to colonise the gut. There is also a gap in immunology development between maternal antibodies and the infant’s own antibody production during this time.

In regard to age of infant botulism cases, the statistics from Europe and the USA are in close agreement, see Table 2.

**Table 2:** Age distribution and infant botulism cases in Europe and USA

<table>
<thead>
<tr>
<th>Age of infants</th>
<th>Percent of cases</th>
<th>Europe</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;16 weeks</td>
<td></td>
<td>77%</td>
<td>72%</td>
</tr>
<tr>
<td>16 – 32 weeks</td>
<td></td>
<td>21%</td>
<td>25%</td>
</tr>
<tr>
<td>&gt;32 weeks</td>
<td></td>
<td>2%</td>
<td>3%</td>
</tr>
</tbody>
</table>

(Source: ACMSF, 2005)

A graph of the US cases in the report indicates that perhaps 10% of cases occurred in infants aged 26 weeks or older (ACMSF, 2005). The ACMSF report states that 99% of cases occur
in children less than 1 year old, although 94% of cases occur in children less than 6 months old.

Adults with major intestinal complications, for example abdominal surgery, chemotherapy, Crohn’s disease and antibiotic use, are at risk of adult infectious botulism.

To date there has been no evidence that the fetus is at risk of neonatal botulism. It appears that the toxin cannot cross the placental barrier where the mother has acquired botulism during pregnancy (Cherington, 1998).

**Long Term Effects:** Where the illness progresses rapidly, the disease can be difficult to distinguish from SIDS. Death usually occurs from complications such as respiratory arrest and not as a direct result of the toxin (Arnon, 1995). The mortality rate is around 5% compared to foodborne botulism where it is around 10% (ACMSF, 2005). However, where the illness is sufficiently gradual enough to allow hospital intervention, the prognosis can be very good and supportive intensive care can bring mortality rates down. All botulinum neurotoxins interfere with neurotransmitters, which is temporary and neurotransmission is eventually restored when new motor endplates are regenerated, usually there are no long-term effects. *C. botulinum* toxin and organism can still be excreted long after the recovery of the infant (Midura, 1996). Toxin has been detected in a patient for 138 days, and organisms 158 days after onset of illness (Turner et al., 1978). However, *C. botulinum* is eventually cleared from the intestine.

There have been no reports of an infant who has recovered from the disease contracting a second episode of the illness. This may be due to development of immunity, maturation of the intestinal tract, or the rarity of the disease. However, relapses have been reported (Arnon, 1995).

**Treatment:** Hospitalisation has been reported in up to 80% of cases with the average stay approximately 4 to 5 weeks differing with toxin type. For infant botulism, high quality supportive care is required and in 25% of cases, mechanical ventilation and gavage feeding is required. Botulism immunoglobulin (BIG, a human-derived antitoxin) has been used for treatment. BIG is composed of immunoglobulin G (IgG) antibodies and antitoxins A and B that inactivate any circulating neurotoxin before it can bind to nerve endings. It is not given to patients with selective immunoglobulin A (IgA) deficiency because of the risk of anaphylactic reactions (Tanzi and Gabay, 2002).

### 4.3 Types Causing Infant Botulism

Toxin types A and B are generally implicated in cases of infant botulism. Exceptions have occurred in the USA, Mexico and Italy (Hatheway, 1995 cited in WHO, 1999; Midura, 1996). The cases in the USA and Mexico were caused by *Clostridium baratti*, producing a neurotoxin similar to type F. The first two Italian cases reported were identified as *C. butyricum* producing a type E toxin (Midura, 1996). There has also been a case of a 6-month old baby with type C infant botulism in Japan, but honey consumption was not involved (Oguma et al., 1990).

Type G has not been isolated from any food although it has been isolated from a SIDS case in Switzerland from a necropsy specimen (see section 6.2.3.1). Dodds (1993) noted that type G
cases may be undiagnosed because type G does not produce a positive lipase reaction on egg yolk agar (which is commonly used to screen for presumptive C. botulinum) and type G produces small amounts of toxin in culture and takes 7 days (longer than the 4 day standard incubation).

The different serotypes found appear to reflect the environmental distribution rather than ability to infect (European Commission, 2002).

4.4 Dose Response

The infective dose of spores for human infants is not known and information from the literature on the dose-response for infant botulism is sparse. In a review by Austin (2001) the author states that a minimum dose for infant botulism has not been established. He cites two separate studies (Dodds, 1993) that examined honey samples implicated in cases of infant botulism. The levels of $8 \times 10^3$ and $8 \times 10^4$ spores/kg in implicated honey samples were 1000-fold higher than random honey samples. The author pointed out that babies consume very little honey so that high infective doses should not be assumed.

Arnon (Arnon et al., 1979; 1992) has estimated a widely cited figure of 10 to 100 spores as being capable of causing infection. These figures were calculated from honey samples involved in actual infant botulism cases (Midura et al., 1979) and are based on the exposure of human infants to spore-containing honey i.e. six samples containing 5-25 spores/g honey and in another case 5 to 70 spores/g of honey, although it is not known how much honey was consumed. The highest dose estimated not to cause infant botulism is 7 spores per 25g honey (Sugiyama et al., 1978).

The lowest estimate of the minimum number of cells needed to cause disease was obtained from a sample of honey from Canada, which contained 1 spore/g (Hauschild et al., 1998).

Work on mouse models by Sugiyama et al. (1978) demonstrated that the microflora of adult mice prevented colonisation. When $10^6$ type A spores were administered there was no colonisation. However, when the mice were treated with oral antibiotics (erythromycin and kanamycin) for 2.5 days, half the mice were colonised by $2 \times 10^4$ spores. In addition, adult germ-free mice were colonised by just 10 type A spores. The 50% infective dose (ID$_{50}$) for type A spores, in normal infant mice (7 to 13 days old) was 700 spores (170-3000 spores, 95% confidence range), a dose much smaller than antibiotic-treated adults. In one experiment, with an infant mouse population with normal distribution of susceptibilities to infection, a few mice were infected with a dose of 10-20 spores. Susceptibility peaked between days 8 and 11 which the authors stated corresponds with 2 to 4 months in human infants. Extrapolation to human babies was considered by the authors and concluded that the corresponding timeframe could not be ruled out. This work assumed that the dose of spores would be present in one feed or in a series of closely spaced feedings. The ID$_{50}$ in infant rats has also been determined at 1,500 spores (Moberg and Sugiyama, 1980).

The number of vegetative cells of C. botulinum secreting a lethal amount of botulinal toxin for a 7 kg infant has been calculated at $2.6 \times 10^6$ to $2.6 \times 10^9$, details of the calculation can be found in Arnon et al. (1981).
The (ICMSF, 1996) states that the numbers of *C. botulinum* cells in faeces can rise to $10^3 - 10^8$/g before the clinical symptoms show. Healthy human infant faeces usually contains $10^{10} - 10^{11}$ of bacteria per gram. Arnon (1980b) observed that it is not known whether the large number of vegetative *C. botulinum* cells in faeces from a case are infectious to other infants or adults by the faecal-oral route, and therefore usual precautions during hospitalisation are recommended.

In summary, the weight of evidence suggests that ingestion of only a few cells can result in infection in infants. The majority of the texts cite Arnon’s figure of 10 to 100 spores.
5 EXPOSURE ASSESSMENT

5.1 The Hazard in the New Zealand Food Supply: *Clostridium botulinum* in Honey

Apiculture statistics and forecasts are available on the MAF website (MAF, 2002b; MAF, 2005).

5.1.1 *Clostridium botulinum* in honey

Information on the prevalence of *C. botulinum* spores in New Zealand honey is minimal.

Surveys of honey available in Finland and Japan have included New Zealand produced honey. In Finland, Nevas *et al.* (2002) tested 76 imported honey samples, 2 of which originated from New Zealand. Both samples were negative for *C. botulinum*.

In Japan, a survey of domestically produced and imported honey during the period 1986 to 1990, examined three samples from both New Zealand and Australia. All of these samples were negative for *C. botulinum* (Nakano *et al.*, 1990).

5.1.2 *Clostridium botulinum* in soil and sediments

An outbreak of type C botulism in wild waterfowl in 1971 demonstrated that the organism was present in New Zealand. This led to an environmental study looking for the presence of *C. botulinum* in New Zealand pond and waterway sediments (Gill and Penney, 1982). The samples were incubated and the media tested for toxin. Types C and D (neither implicated in foodborne botulism) were detected in 11 of 20 sites in the Auckland area. Samples from other urban North Island sediments were negative. The authors concluded that although the survey in Auckland was limited, the failure to detect toxins of other types indicates their rarity in New Zealand.

Between 1996 and 1998, AgResearch obtained more than 250 isolates of *C. botulinum* from vacuum-packed chilled meats and meat plant environments in South Island, including the hides, faeces and tonsils of slaughtered animals, soil, vegetation and mud. The isolates were all non-proteolytic (Group II) types B, E or F. The typing assignment was based on restriction fragment length polymorphism (RFLP) analysis and DNA sequencing of the 16S rRNA genes. However, none of the isolates carried botulinal neurotoxin genes (Broda *et al.*, 1998).

While the 16S rDNA genes of New Zealand *C. botulinum* isolates share 100% similarity with toxigenic non-proteolytic *C. botulinum* types B, E or F, genomes of some of these isolates hybridise at <60% with that of the reference strains (i.e. has <60% similarity). This means that some New Zealand isolates are true non-toxigenic *C. botulinum* while others need to be classified as different species (Dorata Broda, November 2005, unpublished data). The scientists involved have argued that such non-toxigenic isolates should be renamed to reflect their lack of risk (Broda *et al.*, 2001).

However, in experiments between 2000 and 2002, AgResearch scientists detected fragments of the botulinal neurotoxin genes by PCR in DNA isolated from samples taken from farm
environments (without isolation of bacteria). The DNA sequences of the fragments identified them as neurotoxin types B, E or F sequences (Dorata Broda, AgResearch, November 2005, personal communication).

The identification of fragments of B, E and F toxin genes in these samples raises the possibility that Group I (proteolytic) toxin B or F producing C. botulinum are present, despite not being identified amongst the isolates obtained in the earlier experiments. Alternatively, the carriage of these toxin genes by Clostridium species other than C. botulinum (e.g. C. baratii, C. butyricum) and/or by another yet un-cultured species may also be possible.

During 1999-2000, Crop and Food Research collected 498 sediments samples from around the New Zealand coastline. The samples were enriched and tested for botulinum biotoxin by mouse bioassay. No positive samples were found. These samples have since been archived at –85°C and are undergoing further testing. This will involve anaerobic enrichment to grow any C. botulinum spores, followed by extraction of DNA from the enriched samples and PCR analysis to identify any toxin-producing genes (Graham Fletcher, Crop and Food, personal communication, April 2006).

5.1.3 Conclusions

The available data are limited and somewhat ambiguous. The apparent absence of toxin-producing Group I species of C. botulinum from South Island meat, animal and environmental samples suggests that the risk of honey contamination by toxin producing spores from the environment is low. However, the results identifying toxin genes amongst DNA from other farm samples requires further investigation. The marine sediment results are probably not relevant in relation to potential honey contamination.

5.2 Food Consumption: Honey

For this Risk Profile the most important consumption data concerns infants. However, adult consumption of honey will also be considered, in view of the potential for infection of individuals with altered intestinal status.

With respect to infant consumption, overseas risk assessments have used the figure of 25% of infants consuming honey. Taking an approximate mean at 57,000 infants in New Zealand in any one year (Statistics New Zealand, Births and Deaths website; www.stats.govt.nz), a 25% exposure to honey relates to 14,250 infants potentially ingesting honey. However, the Ministry of Health’s Food and Nutrition Guidelines for Healthy Infants and Toddlers (Aged 0-2 years) (MoH, 2000) highlights the issue of infant botulism and, as a precautionary measure, recommends that honey not be introduced into the infant diet until after six months of age.

While no specific information is available on the consumption of honey by infant New Zealanders, dietary guidelines and demographic trends suggest that consumption of honey by this group is likely to be low. This is consistent with European expert opinion, that speculated that infant (<1 year of age) honey consumption is probably less than 1% of adult consumption (European Commission, 2002).
For adults, honey may be consumed as a spread (on bread or toast), a sweetener (in tea or coffee) or as an ingredient in processed foods. The cereal and baking industries are the two largest commercial users of honey. The honey content of some breakfast cereals may be as high as 8%, but the honey content of most processed foods will be less than 1%. Other products which contain honey are condiments (salad dressings, sauces, mustards), dairy (flavoured yoghurts, ice cream), meats (honey cured bacon, ham, pâté, sausages), drinks, snacks (peanut butter) and sweets (Snowden and Cliver, 1996).

Information on honey consumption by the general population can be obtained from a number of sources. Food Balance Sheets published by FAO (http://faostat.fao.org/) give an annual per capita consumption of honey by New Zealanders of 0.6 kg/person/year (1.6 g/person/day). However, this figure appears to be low as production figures suggest that approximately 5,000 tonnes per annum goes to domestic consumption (3.4 g/person/day based on a population of four million). The New Zealand Honey Producers Cooperative estimate that 88% of New Zealanders consume honey at an average of 2 kg a year (http://www.nzhoney.co.nz/) (accessed 04.11.05).

For exposure assessment purposes, WHO have defined 13 ‘cluster diets’. These represent average diets across a group of countries with similar dietary patterns. Regional cluster diets proposed by WHO give a figure for honey consumption of 1.5 g/person/day for cluster M, the cluster including New Zealand. This cluster also includes Argentina, Australia, Canada, Chile, United States and Uruguay (http://www.who.int/foodsafety/chem/gems/en/index2.html). Daily intakes for other cluster diets are in the range 0.03 (West Africa) to 2.0 (Mediterranean) g/person/day.

Analysis of 24-hour dietary recall records from the 1997 National Nutrition Survey (NNS; covering the adult population 15 years and over), gives a population average consumption of 2.6 g/person/day, with approximately 16% of the population consuming honey on any given day (Russell et al., 1999). A Food Standards Australia New Zealand (FSANZ) analysis of the same data set gave a slightly higher estimate of 2.9 g/person/day. The difference will be due to differences in assumptions made around the frequency and level of honey in processed foods.

Analysis of 24-hour dietary recall records from the 2002 Children’s Nutrition Survey (CNS) (Ministry of Health, 2003a), covering New Zealanders in the age range 5-15 years, suggests that this age group consumes much less honey than adult New Zealanders, with an average of 0.6 g/person/day, and only 5.9% of respondents consuming honey on any day. This observation is supported by demographic analysis of the 1997 NNS data, that suggests that consumption of honey increases with age and is most frequent amongst the over 65s (31% consuming on any day).

5.3 Qualitative Estimate of Exposure

5.3.1 Number of servings and serving sizes

No information is available on the frequency of honey consumption and serving sizes for infant New Zealanders.
5.3.2 Frequency of contamination

No data could be found on the prevalence of Group I *C. botulinum* spores for honey on sale in New Zealand. A survey of honey in Finland (Nevas *et al.*, 2002), tested 76 imported honey samples, 2 of which originated from New Zealand. Both New Zealand samples were negative for *C. botulinum*.

A survey in Japan (Nakano *et al.*, 1990) tested 3 samples of honey from New Zealand and 3 samples from Australia. All these samples were negative for *C. botulinum*.

In an Australian risk assessment, the probability of honey contamination was estimated at 0.0001% (Sumner, 2002).

5.3.3 Predicted contamination level at retail

No quantitative information could be found on the predicted *C. botulinum* spore count in honey on retail sale in New Zealand. In the Australian risk assessment, there was no quantitative data on how many spores would be in the 0.0001% contaminated.

5.3.4 Growth rate during storage and most likely storage time

There is no vegetative cell multiplication of *C. botulinum* in honey.

New Zealand labelling requirements do not provide directions for use and storage of honey (http://www.nzfsa.govt.nz/animalproducts/publications/info-pamphlet/bee-products/honey-label.htm).

5.3.5 Heat treatment

Honey is not usually cooked before being fed to infants and it is very unlikely that if spores were present in honey that they would undergo a ‘botulinum cook’. Normal cooking temperatures would destroy vegetative cells, although these would not be expected to be present in honey in the first instance. Commercially available honey may be pasteurised but this process is not sufficient to destroy the spores.

5.3.6 Exposure summary

It is not possible to conduct a qualitative exposure assessment for this food/hazard combination. Absence of data on consumption and hazard prevalence prevent such an assessment.

5.4 *Clostridium botulinum* in Honey Overseas

Due to the low prevalence and clustering of spores in honey, statistically designing a sampling plan requires an extremely large number of samples, and a low chance of successful detection. There is also no appropriate indicator (European Commission, 2002).

A study of 86 honey samples representing the range of honey available in Australia has been carried out (Murray, 1980). However, there is no information whether any of this honey
originated in New Zealand. Two enrichment culture methods were used and the supernatants from these broths inoculated into mice. None of the 86 samples demonstrated toxin production by this method. The broths were also plated out and any anaerobic Gram positive rods were identified. A variety of Clostridium species were present but none were C. botulinum.

A survey of honeys imported into the USA examined a sample of Australian eucalyptus honey (Huhtanen et al., 1981). The sample was positive for type B C. botulinum spores.

More recently, among 76 imported honeys in a Finnish study (Nevas et al., 2002), out of the seven samples originating from Australia, two were positive, with a mean number of spores of 36/kg. One isolate was type A and the other had both types A and B. In the same study, two of the samples originated from New Zealand and both were negative for C. botulinum spores.

Worldwide surveys of honey up to 1991 have shown a markedly higher number of spores in honey associated with illness compared to randomly derived samples (reviewed in Dodds, 1993). Surveys reviewed by Nevas et al. (2005b) found a less marked difference; non-illness related honey ranged from <1 spore to 60 spores/g, while in honeys associated with illness the number ranged from 5 to 70 spores/g.

Nevas et al. (2002) suggest that the low prevalence of spores in earlier studies is most likely due to inadequate concentration and growing methods for the organism, the limited number of samples, low numbers of spores involved and uneven distribution leading to false-negatives. As detection methods improve, the numbers of spores in random samples appears to be increasing. Nevas et al. (2002) found a 1 log\textsubscript{10} higher count in honey samples not associated with illness compared to earlier studies.

In a review of 15 microbial surveys of honey worldwide (Snowden and Cliver, 1996) from 1978 to 1991, of the 2033 total samples tested 104 (5.11%) contained C. botulinum. Many studies failed to detect spores. The authors highlighted the variation in analytical technique, (one survey reported a prevalence of 62%) and concluded that most studies found botulinum spores in 5 to 15% of samples, typically at levels below 1 spore per gram of honey. The ACMSF (2005) reported the presence of clostridial spores in 0% and up to 20% of samples worldwide. They put the number of spores at between 2.5 and 80,000 MPN per kilogram (ACMSF, 2005). Table 3 summarises the reported prevalence of C. botulinum in honey surveys worldwide up to 1993, while Table 4 summarises those surveys worldwide reported since 1993 and those before 1993 not reported by Dodds (1993).

Table 3: Reported prevalence of C. botulinum in honey: overseas (up to 1993)

<table>
<thead>
<tr>
<th>Country</th>
<th>Sample size (g)</th>
<th>% positive samples</th>
<th>MPN (spores) per kg</th>
<th>Toxin types identified</th>
<th>Association with illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>20</td>
<td>15</td>
<td>8</td>
<td>A,B,C</td>
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</tr>
<tr>
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<td>20</td>
<td>14</td>
<td>7</td>
<td>A,C</td>
<td>No</td>
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<tr>
<td>North America</td>
<td></td>
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<tr>
<td>Canada</td>
<td>75</td>
<td>0</td>
<td>&lt;0.2</td>
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</tr>
</tbody>
</table>

\textit{Risk Profile: Clostridium botulinum in Honey}
<table>
<thead>
<tr>
<th>Country</th>
<th>Sample size (g)</th>
<th>% positive samples</th>
<th>MPN (spores) per kg</th>
<th>Toxin types identified</th>
<th>Association with illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>75</td>
<td>100</td>
<td>$8 \times 10^2$</td>
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</tr>
<tr>
<td></td>
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<td>7</td>
<td>8-28</td>
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<tr>
<td></td>
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<td>0.4</td>
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<tr>
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<td>100</td>
<td>$8 \times 10^4$</td>
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<td>&lt;0.8</td>
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**Central & South America**

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of samples</th>
<th>% positive samples</th>
<th>MPN (spores) per kg</th>
<th>Toxin types identified</th>
<th>Association with illness</th>
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<tbody>
<tr>
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<td>9</td>
<td>5</td>
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</tr>
<tr>
<td></td>
<td>30</td>
<td>50</td>
<td>23</td>
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<td>Mexico</td>
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**Europe**

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of samples</th>
<th>% positive samples</th>
<th>MPN (spores) per kg</th>
<th>Toxin types identified</th>
<th>Association with illness</th>
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<td>Germany</td>
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</tr>
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<td>&lt;0.4</td>
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<td>No</td>
</tr>
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<td>9</td>
<td>A,C</td>
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<td>Italy</td>
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<td>&lt;0.9</td>
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<td>30</td>
<td>0</td>
<td>&lt;0.5</td>
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<td>NR</td>
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<td>United Kingdom</td>
<td>20</td>
<td>0</td>
<td>&lt;0.4</td>
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</table>

(source: Dodds, 1993)

From Table 3, it appears that surveys in the USA, Europe and Asia, where honey was not related to illness, the typical spore count was around <1 to 10 per kg, whereas those samples in the USA and Canada that were associated with illness yielded a very high number of spores per kg in comparison (Dodds, 1993).

**Table 4: Reported prevalence of *C. botulinum* in honey: overseas (mainly since 1993)**

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of samples</th>
<th>% positive samples</th>
<th>Toxin types</th>
<th>Approx. spores/kg</th>
<th>no. Association with illness</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>45</td>
<td>3 (7)</td>
<td>A</td>
<td>One sample 15,000 spores/kg</td>
<td>Yes</td>
<td>Monetto <em>et al.</em>, 1999</td>
</tr>
<tr>
<td></td>
<td>177</td>
<td>2 (1.1%)</td>
<td>A &amp; F</td>
<td>2 samples &lt;1000 spores/kg</td>
<td>No</td>
<td>DeCenorbi <em>et al.</em>, 1997</td>
</tr>
<tr>
<td>Brazil</td>
<td>100</td>
<td>3 (3)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Rall <em>et al.</em>, 2003</td>
</tr>
<tr>
<td>Brazil</td>
<td>85</td>
<td>6 (7.1)</td>
<td>2A, 1B, 3D</td>
<td>NR</td>
<td>NR</td>
<td>Schocken-Iturrino <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Country</td>
<td>No. of samples</td>
<td>No. (% positive samples)</td>
<td>Toxin types</td>
<td>Approx. spores/kg</td>
<td>no. positive samples</td>
<td>Association with illness</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------</td>
<td>--------------------------</td>
<td>-------------</td>
<td>-------------------</td>
<td>----------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Denmark</td>
<td>112</td>
<td>29 (26)</td>
<td>1 A, 28 B</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Finland (from domestic producers)</td>
<td>190</td>
<td>20 (11)</td>
<td>8 A, 12 B</td>
<td>18 – 140 spores/kg.</td>
<td>No</td>
<td>Nevas et al., 2002</td>
</tr>
<tr>
<td>Imported</td>
<td>76 (imported)</td>
<td>12 (16)</td>
<td>A,B,C,D,F</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Japan</td>
<td>270</td>
<td>23 (8.5)</td>
<td>A,B,C,D,F</td>
<td>Most samples; 1 spores/g</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>- 58</td>
<td>6 (10)</td>
<td>A,C</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>- 76</td>
<td>9 (12)</td>
<td>A,B,C,A,D,F</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>- 15</td>
<td>3 (20)</td>
<td>A,C**</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>- 12</td>
<td>1 (8)</td>
<td>A</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>- 2</td>
<td>1 (50)</td>
<td>C</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>- 2</td>
<td>1 (50)</td>
<td>C</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>- 31</td>
<td>2 (7)</td>
<td>C</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Norway</td>
<td>112</td>
<td>12 (10)</td>
<td>7B, 4E, 1F</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Sweden</td>
<td>61</td>
<td>1 (2)</td>
<td>1E</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Taiwan</td>
<td>152</td>
<td>2 (3%)</td>
<td>NR</td>
<td>NR</td>
<td>No</td>
<td>Du et al., 1991</td>
</tr>
<tr>
<td>Turkey</td>
<td>48</td>
<td>6 (12.5)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Küplülü et al. (in press)</td>
</tr>
<tr>
<td>USA</td>
<td>90</td>
<td>9 (10)</td>
<td>2 A, 7 B</td>
<td>5-25/g; 1 sample 70-80/g.</td>
<td>Yes</td>
<td>Midura et al., 1979</td>
</tr>
<tr>
<td>California (late 1970s)</td>
<td>60</td>
<td>8 (13)</td>
<td>NR</td>
<td>NR</td>
<td>No</td>
<td>Tanzi &amp; Gabay, 2002</td>
</tr>
</tbody>
</table>

NR = not reported.
* Because of unusually high spore counts in an Argentinian clover honey sample, further samples from the same brand were examined. The authors suggested that during the honey ripening stage, *Bacillus alvei* were detected and this organism may have stimulated growth of *C. botulinum.*
** One sample contained two types of *C. botulinum* spores.

The Japanese survey (Nakano et al., 1990) included 3 samples of imported honey from New Zealand and 3 samples from Australia, no spores were detected in these samples.
In more recent research (Nevas et al., 2005b) in Nordic countries (Denmark, Norway and Sweden), a total of 294 honey samples were examined for types A, B, E and F using multiplex-PCR methodology. The samples consisted of honeycombs direct from the hives and also extracted honey (from several hives and apiaries). Type B was the predominant toxin detected. There was significant variation between the countries, Danish honey had significantly higher frequency of *C. botulinum* spores than Norway or Sweden. Overall there was a trend towards higher spore prevalence in honeycomb rather than extracted honey but this was not significant \((p > 0.5)\).

The results are presented in Table 5.

### Table 5: Details of Nordic honey surveys

<table>
<thead>
<tr>
<th>Country (origin)</th>
<th>No. of samples</th>
<th>No. (%) positive samples</th>
<th>Detected types of <em>C. botulinum</em></th>
<th>No. and type of <em>C. botulinum</em> isolates*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark, total extracted</td>
<td>112</td>
<td>29 (26)</td>
<td>1 A: 28 B</td>
<td>1 A: 20 B</td>
</tr>
<tr>
<td>honeycomb</td>
<td>58</td>
<td>14 (24)</td>
<td>14 B</td>
<td>13 B</td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>15 (28)</td>
<td>3 A: 9 B: 3 E</td>
<td>1A: 7 B</td>
</tr>
<tr>
<td>Norway, total extracted</td>
<td>122</td>
<td>12 (10)</td>
<td>7 B: 4 E: 1 F</td>
<td>3 B</td>
</tr>
<tr>
<td>honeycomb</td>
<td>100</td>
<td>9 (9)</td>
<td>5 B: 4 E</td>
<td>1 B</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>3 (14)</td>
<td>2 B: 1 F</td>
<td>2 B</td>
</tr>
<tr>
<td>Sweden (all extracted)</td>
<td>61</td>
<td>1 (2)</td>
<td>1 E</td>
<td>Not isolated</td>
</tr>
</tbody>
</table>

* all isolates confirmed as proteolytic.  
(Source: Nevas et al., 2005b).

It has been suggested that the significantly higher prevalence in Denmark is due to water source. Honeybees prefer a dirty source of water to a cleaner one. Johansson and Johansson (1978) state that this may be due to its odour and salt content. The water used by the bees may therefore be contaminated with manure. Denmark has an extensive pig farming trade and pig waste is used as manure. Dahlenborg et al. (2001) found that 62% of pig faecal samples collected from abattoirs in Denmark contained *C. botulinum*. In addition, water is often sprayed over the combs inside the hives, to regulate temperature and humidity (Johansson and Johansson, 1978).

It appears that contamination of honey with *C. botulinum* spores is widespread, with evidence accumulating as laboratory methodology improves. The types causing infant botulism are not always found however, and this may well reflect the types present in environmental sources.
6 RISK CHARACTERISATION

6.1 Adverse Health Effects in New Zealand

6.1.1 Incidence

There have been no notifications of human botulism in New Zealand through the notifiable diseases surveillance system since records began in 1987.

To date, there has been one published report (Flacks, 1985) involving two patients (sisters) with foodborne botulism in Rotorua, New Zealand. The incident occurred in February 1984 before surveillance records began and both cases were linked to the consumption of home-preserved Tiroi made from watercress and boiled mussels. The husband of one of the sisters also ate the food but developed no symptoms and a third person who ate the food developed only diarrhoea. Botulism was confirmed in the sisters and trivalent ABE antitoxin given intramuscularly. Blood from one of the patients contained type A botulinum toxin. It appears that the boiling of the mussels rather than steaming may have destroyed inoculating fermentative organisms that would have otherwise rendered the food safe (Hudson et al., 2001). A “very suspicious organism” was cultured later from the food but could not be confirmed as *C. botulinum*. The author states that earlier cases of botulism may have gone unrecognised in New Zealand because of the reticence in diagnosing a previously unrecorded disease.

There have not been any releases of botulinal anti-toxins to treat cases of botulism since the Rotorua cases in 1985 (Pam Raynel, Vaccine Supplies, ESR – personal communication, August 2006).

In the Annual Report for Notifiable Diseases for 2004 (ESR, 2005), hospital discharge data for one case of botulism in 1989, two cases in 1994 and one case in 1995 were mentioned. Subsequent investigations (for this Risk Profile) with the Ministry of Health and relevant District Health Boards revealed that these discharge records had been miscoded for botulism.

6.1.1 Sudden Infant Death Syndrome (SIDS)

SIDS is the major cause of death in the post-neonatal period (40.6% of such deaths in 2000). Overall, 17.5% of all infant deaths in 2000 were attributable to SIDS (Ministry of Health 2004b). A controversial link has been made overseas between Sudden Infant Death Syndrome (SIDS) and infant botulism. The latest Ministry of Health figures in New Zealand for the year 2000 show there were 65 SIDS cases that year. Of these, 5 occurred in the neonatal period (less than 28 days old), 59 in post-neonatal and 1 death occurred after 12 months of age. The SIDS death rate in New Zealand is falling. The rate was 1.1 per 1000 live births for the year 2000, 63.4% lower than the rate recorded in 1990 (Ministry of Health 2004b).

The most up to date figures for post-neonatal mortality (28 days – 12 months) in New Zealand come from Child and Youth Mortality Review Committee Statistics (CYMRC, 2005). These statistics record Sudden Unexpected Death in Infancy (or SUDI) and is limited to infants found dead after being placed to sleep. SUDI encompasses SIDS and other similar deaths that fall outside of the SIDS definition. The SUDI figures for 2002 and 2003 were 33
and 50 respectively, a rate of 0.61 and 0.89 per 1000 live births. SUDI is the second highest cause of mortality in this age group (37%) following medical causes (48%). Caution must be exercised when comparing rates for SIDS and SUDI numbers.

Dr Martin Sage, the National Forensic Pathology Adviser for New Zealand gave the following statement regarding the controversial link between SIDS and infant botulism in New Zealand.

“...the data from the 80’s and 90’s did not give any convincing support for effects of Clostridia or Staphylococci toxins, despite the theoretical attraction. Practice throughout New Zealand and Australia at the moment is a little varied, but even in the most thorough units, specific culture of faeces for Clostridia is no longer done in the routine list of investigations. In my experience, a recent history of diarrhea and vomiting is uncommon in SIDS and would precipitate a thorough microbiological screen of gastro-intestinal tract samples for pathogens. While the general feeling is that enterotoxic bacteria are not a universal or even minority explanation for SIDS, I guess it doesn’t exclude the possibility of the occasional single case. If proven, the case would automatically be excluded from SIDS diagnosis by definition. I and my colleagues are not aware of any infant fatalities from this cause in New Zealand in the last couple of decades”

Dr. Martin Sage, National Forensic Pathology Adviser, personal communication, August 2006

6.2 Adverse Health Effects Overseas

Since infant botulism was recognised as a disease in 1976, the number of cases has been growing slowly but with increasing speed. This phenomenon may be due to increased awareness, better detection methods or is a genuine result. Up to 1979, worldwide, honey exposure occurred in 34.7% (28/75) of hospitalised botulism cases (Arnon et al., 1979).

Infant botulism has been reported in more than 15 countries worldwide. Africa is the only continent where it has not been reported (WHO, 1999; ACMSF, 2005). Of the more than 1000 cases that have been reported worldwide 90% have occurred in the USA.

6.2.1 Incidence

Data on the incidence of reported cases of infant botulism overseas (other than Europe) up to 1993 have been summarised in Table 6, European data can be found in Table 10.

Table 6: Non-European reports of infant botulism and association with honey where known

<table>
<thead>
<tr>
<th>Country</th>
<th>Cases of infant botulism</th>
<th>Fatalies</th>
<th>Toxin type</th>
<th>Honey implicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible</td>
</tr>
<tr>
<td>Argentina</td>
<td>23a</td>
<td>6</td>
<td>A only</td>
<td>?</td>
</tr>
<tr>
<td>Australia</td>
<td>11</td>
<td>0</td>
<td>A, B</td>
<td>1</td>
</tr>
<tr>
<td>Canada</td>
<td>4</td>
<td>0</td>
<td>A, B</td>
<td>1</td>
</tr>
<tr>
<td>Japan</td>
<td>10b</td>
<td>0</td>
<td>A, C</td>
<td>9</td>
</tr>
</tbody>
</table>
A summary of the percentage of infant botulism cases associated with honey exposure has been compiled in Table 7. The percentages range from 2.7% in Argentina to 100% in Japan and Nordic countries.

### Table 7: Percentage of infant botulism cases associated with honey exposure (worldwide)

<table>
<thead>
<tr>
<th>Location</th>
<th>Period</th>
<th>No. of cases exposed to honey/total cases</th>
<th>Percentage</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>-</td>
<td>4/146</td>
<td>2.7%</td>
<td>calculated from figures</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Fernández et al., 1999)</td>
</tr>
<tr>
<td>California</td>
<td>late 1970s</td>
<td>12/41</td>
<td>29.2%</td>
<td>Arnon et al., 1979</td>
</tr>
<tr>
<td>California</td>
<td>late 1990s early 2000s</td>
<td>-</td>
<td>5– 10%</td>
<td>Midura, 1996; Schechter, 1999;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nevas et al., 2002</td>
</tr>
<tr>
<td>Europe</td>
<td>1978-2002</td>
<td>29/49</td>
<td>59.2%</td>
<td>ACMSF, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30/49 (6 were not specified and 13 were not associated with honey)</td>
<td>61.2%</td>
<td>European Commission, 2002</td>
</tr>
<tr>
<td>Japan</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>Dodds, 1993</td>
</tr>
<tr>
<td>Nordic countries</td>
<td>-</td>
<td>5/5</td>
<td>100%</td>
<td>Nevas et al., 2002</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1978-2001</td>
<td>3/6</td>
<td>50%</td>
<td>ACMSF, 2005</td>
</tr>
<tr>
<td>USA</td>
<td>-</td>
<td>-</td>
<td>15%</td>
<td>Shapiro et al., 1998</td>
</tr>
<tr>
<td>Worldwide</td>
<td>-</td>
<td>28/75 cases</td>
<td>34.7%</td>
<td>Arnon et al., 1979</td>
</tr>
</tbody>
</table>

In the United States, an estimated 15% of infant botulism cases are suspected to be linked to honey ingestion (Shapiro et al., 1998). In a review of US cases (Arnon, 1992) honey was identified as the vehicle for spore ingestion in 26 cases, and toxin types A and B were equally represented. The high rate in the USA may be due to the heightened awareness of the disease by physicians (Midura, 1996).

In 1978, the CDC in the USA reported on the association between honey consumption and infant botulism. They concluded that honey may have been a source of infection in
approximately a third of cases and should be avoided as a food source for infants under 12 months (Arnon et al., 1978).

There were 929 hospitalised infant botulism cases in the USA between 1976-1990 (ACMSF, 2005). There are between 75 to 100 cases diagnosed annually (Arnon, 1995). The USA has 90% of the worldwide cases of infant botulism, around 50% of these cases occurring in the State of California. However once adjustments are made for birth rates, California does not have the highest rate (Arnon, 1995). The ten States with the highest incidence are shown in Table 8. It is noted that eight of these States are located west of the Rocky Mountains, although this may be due to increased physician awareness. Type A predominates in the West while type B predominates in the East reflecting the incidence in the soil. Seasonal fluctuations are limited with a small increase in the Autumn months and a slight decline in winter months.

### Table 8: Cases and rate of infant botulism in ten USA States (ranked top ten)

<table>
<thead>
<tr>
<th>State</th>
<th>No. of Cases</th>
<th>Incidence (per 100,000 births)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delaware</td>
<td>16</td>
<td>10.0</td>
</tr>
<tr>
<td>Hawaii</td>
<td>29</td>
<td>9.7</td>
</tr>
<tr>
<td>Utah</td>
<td>53</td>
<td>8.5</td>
</tr>
<tr>
<td>California</td>
<td>538</td>
<td>7.1</td>
</tr>
<tr>
<td>Pennsylvania</td>
<td>121</td>
<td>4.7</td>
</tr>
<tr>
<td>Washington</td>
<td>40</td>
<td>3.6</td>
</tr>
<tr>
<td>Oregon</td>
<td>24</td>
<td>3.6</td>
</tr>
<tr>
<td>New Mexico</td>
<td>15</td>
<td>3.5</td>
</tr>
<tr>
<td>Idaho</td>
<td>9</td>
<td>3.2</td>
</tr>
<tr>
<td>Arizona</td>
<td>24</td>
<td>2.6</td>
</tr>
</tbody>
</table>

(Source Arnon, 1995).

Rates per 100,000 live births are rarely given in the literature. The USA reported a rate of 3 per 100,000 with higher rates in Delaware, Hawaii, Utah and California. Early epidemiological and laboratory studies in California linked 12/41 (29.2%) of infant botulism cases to the ingestion of honey containing C. botulinum spores (Arnon et al., 1979), however more recently only 5 to 10% have been linked, a possible indication of California’s successful educational program (Midura, 1996; Schechter, 1999). Nevàs et al. (2002) puts the United States figure at more than 20% infant botulism cases caused by exposure to honey.

The yearly number of infant botulism cases exceeds foodborne and wound botulism combined (Midura, 1996). Some commentators have linked this to increased physician awareness.

In the work carried out by Midura et al. (1979), 90 honey samples were obtained from homes of infant botulism cases and homes of healthy infants, grocery stores, small apiaries and commercial processing plants. From the nine samples that were positive, 8 were related to infant botulism cases and the ninth was an unprocessed sample from a processing plant. By food exposure history, honey was significantly associated with type B infant botulism (p=0.005). The breakdown of detail can be found in Table 9 below. Dose response calculations from this research are detailed in Section 4.4.

---

Risk Profile: Clostridium botulinum in Honey

April 2006
Table 9: Details of results from infant botulism cases associated with honey consumption in the USA

<table>
<thead>
<tr>
<th>Honey sample</th>
<th>Honey</th>
<th>Infant</th>
<th>Honey fed to infant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>B</td>
<td>No^a</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>B</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>A</td>
<td>No^b</td>
</tr>
<tr>
<td>9^b</td>
<td>A</td>
<td>-</td>
<td>No</td>
</tr>
</tbody>
</table>

^a None of the honey fed to the infant available. Same brand and size purchased in same store
^b Unprocessed sample obtained at commercial processing plant

Toxin types A and B are the predominant neurotoxins implicated in cases of infant botulism. In the 31 cases reported in the USA up to 1996, where *C. botulinum* was isolated from honey-fed infants, the organism toxin type (A or B) found in the honey has been the same as that causing the illness in the infant. Older family members who ingested the same honey were unaffected (Midura, 1996).

In Japan, all infant botulinum cases have been type A and associated with honey consumption but type E predominates in the environment. However, Japan imports most of its honey from China where type A prevails (Dodds, 1993).

In all countries of the European Union (except Poland), botulism is a statutory notifiable disease. In Europe between 1978 and 2002, there have been 49 reported cases of infant botulism, 29 of which have been linked to honey consumption (ACMSF, 2005) and eight laboratory confirmed with the same *C. botulinum* spores found in both the implicated and of affected infants (Midura, 1996; Arnon et al., 1979). The EU opinion is that a link exists between honey ingestion and infant botulism. However, the setting of microbial criteria and testing regimes have been considered of little value due to the sporadic nature of the disease and the low level of *C. botulinum* contamination in European honey. Midura (1996) suggests that the rarity of the organism makes detection difficult outside of botulism cases which signal their presence.

All 49 cases were all less than 1 year old, 93% were younger than 6 months, the mean onset was at 13 weeks of age and there was a slightly higher prevalence in males. The majority had been breast-fed and 30 had a history of honey ingestion. Table 10 details the 49 notified cases in Europe, with the toxin type where known and history of honey consumption. This table has been sourced from the European Commission report (2002) which in turn has been modified from Aurelia et al. (2002).
## Table 10: Summary of the 49 European cases of infant botulism

<table>
<thead>
<tr>
<th>Country</th>
<th>Strain type (C. botulinum unless otherwise stated)</th>
<th>Age at onset (weeks)</th>
<th>Milk/feeding</th>
<th>History of honey consumption</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czech Republic</td>
<td>B</td>
<td>4</td>
<td>Breast fed</td>
<td>No</td>
<td>Neubauer and Milaceck, 1981</td>
</tr>
<tr>
<td>Denmark</td>
<td>Not typed</td>
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<td>Yes</td>
<td>#</td>
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<td>Country</td>
<td>Strain type (C. botulinum unless otherwise stated)</td>
<td>Age at onset (weeks)</td>
<td>Milk/feeding</td>
<td>History of honey consumption</td>
<td>References</td>
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<td>Jansson <em>et al.</em>, 1985</td>
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<td>Gautier <em>et al.</em>, 1989</td>
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<td>NS</td>
<td>Turner <em>et al.</em>, 1978</td>
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<td></td>
<td>B and F</td>
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<td>NS</td>
<td>Smith <em>et al.</em>, 1989</td>
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<td>Yes</td>
<td>Gilbert and Brett, 1993</td>
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<td></td>
<td>B</td>
<td>20</td>
<td>n.s</td>
<td>NS</td>
<td>Gilbert and Brett, 1994</td>
</tr>
</tbody>
</table>

* honey containing C. botulinum organisms, same type as isolated from cases

NS not specified

# bibliographical reference not available.

All of the six cases in the United Kingdom required assisted ventilation and none of the six were correctly diagnosed on admission. Original diagnoses included failure to thrive, sepsis, dehydration, viral syndrome, encephalitis, meningitis, pneumonia and idiopathic hypotonia. Three of these cases had not consumed honey (ACMSF, 2005).

Italian cases of infectious botulism are characterised by cases of type E toxin infections (from C. butyricum), and have been linked to two adult cases and one infant botulinum case (Eurosurveillance, 1999). Predisposing factors have been noted for type E botulism cases e.g. Meckel’s diverticulum, a congenital abnormality of the small intestine which may provide a favourable niche for colonisation (Fenicia *et al.*, 1999).

There appears to be an association with the predominant toxin type in the soil and the type involved in infant botulism in South America. For example, Argentine soils are predominantly type A and all cases of infant botulism in Argentina have been type A (Giménez, 1990; Fernández *et al.*, 1999). To 1999, of the 146 cases of infant botulism, four (2.7%) had C. botulinum spores detected in implicated honey that had been ingested. However, no spores were detected in honey ingested by cases in the province of Mendoza, an area that has 46 (32%) of the 146 infant botulism cases. The authors suggested that in these cases, environmental dust and soil are more likely transmission routes (Fernández *et al.*, 1999).
6.2.2 Contribution to outbreaks and incidents

There have been foodborne outbreaks of botulism but nothing found in the literature regarding outbreaks of infant botulism.

6.2.3 Case control studies

Spika et al. (1989) carried out a prospective case-control study from April 1985 to March 1987 in the US (excluding California). This involved 68 laboratory confirmed cases and were each matched with two control subjects. They reported the following:

- 68 infants (53% female, 47% male),
- 11 cases consumed honey (5 cases type A, 6 cases type B),
- mean age of disease onset 2 months (0.2 – 11.7 months),
- decrease in bowel movements (<1 in three days) in 69% of cases,
- altered cry in 25% of cases,
- cases more likely to live in rural/farm area (but not significant), and
- Significantly greater number (16% cases vs 2% controls; p<0.004) of cases fed honey before disease onset.

Risk factors identified for infant botulism:

- white,
- mothers of higher mean age, and
- mothers with more formal educational years.

Of most importance was the significant difference between the epidemiology of the disease in the under two month olds and over two months olds. Those under two months had increased risk if they lived in a rural area or on a farm. Breast feeding was not a risk factor. Three risk factors existed for the over two months age group:

- less than one bowel movement daily for at least 2 months,
- breast feeding, and
- the ingestion of corn syrup.

Disturbance of soil, by agriculture, building or earthquakes was another consideration.

The ingestion of corn syrup is a controversial vehicle for the spores. Arguments against corn syrup have been put forward by Arnon, (1992) and Lilly et al. (1991).

Most of these risk factors have been disputed in California. Arnon (1986) studied cases of infant botulism in the States. The author reported that cases occurred equally between males and females, [56% males/44%females Tanzi & Gabay, 2002] across all major racial and ethnic groups and identified risk factors that included the ingestion of honey. Breast-feeding was found to be protective as it slowed the onset of the illness, and diminished the risk of respiratory arrest in cases of the disease. However the author agreed with the Spika et al (1989) study that a slow intestinal transit time (less than one stool a day) was a risk factor.

The role of breast feeding as a risk factor or protective factor is very controversial. In general, infants fed on breast milk have acidic faeces (pH 5.1 – 5.4), large numbers of
Bifidobacterium spp. and low numbers of anaerobic bacteria. In contrast, formula-fed infants produce less acidic faeces (pH 5.9-8.0), lower Bifidobacterium spp. numbers and more anaerobes. Sullivan et al., (1988) found that Bifidobacterium spp. inhibited multiplication of C. botulinum in vitro, supporting the protective theory of breast milk.

6.2.3.1 SIDS Research and the link to infant botulism

Research was first carried out in 1978 (Arnon 1980a; Arnon et al, 1981). Necropsy specimens from SIDS cases were examined for C. botulinum and toxins from 280 Californian infants (68 died of known causes, and 212 SIDS cases). C. botulinum was detected in 10 of the 212 SIDS cases (4.7%) and botulinum toxin detected in 2 of these 10 positive cultures. The case histories and autopsy findings were identical to typical SIDS cases. [NB. no C. botulinum organisms or toxins were found in the 68 known-death infants].

In Utah between 1977 and 1979, 12 cases of infant botulism were diagnosed (Thompson et al., 1980). A case control study was undertaken and isolates of C. botulinum obtained from all 12 cases, ten of which (83%) had been fed honey before onset. For the control group, 87 infants were chosen (32 randomly selected, 42 with non-botulism neurologic disease and 13 with systemic illness (non-botulism)). Surprisingly C. botulinum was isolated from 20/87 control stools (toxin plus the organism from 2 of these). Leaving 18 control stools that yielded the organism only, the categories were 3 from normal infants, 9 with non-botulism neurologic disease and 6 with systemic illness.

Again in California, USA (Arnon et al., 1981) of 205 healthy infants studied since 1976, 72 infants were selected as residence controls (i.e. they lived within a mile of an infant botulism case) to test the hypothesis of similar environmental exposures. All had negative stool samples. Of the remaining 133 control stool samples, one was positive for type A organisms but no toxin was found.

In addition to this research, a further 141 infants with unspecified diseases prompted doctors to request botulinum testing. All stool samples were negative for the organism and toxin.

Arnon (1984) concluded that infant botulism was in a category of unrecognised toxigenic intestinal infections of infancy which collectively could account for cot deaths. He carried out research into toxigenic intestinal infections using infant rhesus monkeys. The monkeys were injected with minute amounts of purified Clostridium difficile toxins A and B. The monkeys died a quiet death within 4 to 10 hours that was clinically and pathologically consistent with human SIDS.

Research in Manitoba, Canada during 1982 discovered the organism C. botulinum type A in a case of SIDS (Hauschild et al., 1983).

Necropsy specimens in Switzerland revealed 9 out of 59 SIDS cases had toxin types A, B, C, F and G.

In contrast, researchers in South Australia (Byard et al., 1992) found no link between SIDS cases and infant botulism after a 10 year study between 1981-1990. A total of 248 small and large intestine samples from SIDS cases were cultured specifically for C. botulinum. No specimens were positive and the authors concluded no link. Midura (1996) argued that,
although South Australia concluded no link between SIDS and infant botulism, there may be a relationship in a small number of cases in Europe and North America.

A subsequent study in New South Wales (Murrell et al., 1993) analysed faecal samples from 123 SIDS cases and 52 age-matched healthy babies who died from other causes. It was concluded that a significantly higher proportion of enterotoxigenic bacteria and their toxins were found in faecal samples of SIDS babies than in control samples. Toxigenic bacteria from SIDS cases were (in order of highest percentage) C. perfringens (54/119; 45.4%), C. difficile (33/119; 27.7%), S. aureus (12/44; 27.3%) and C. botulinum (6/120; 5.0%) compared with 0/53 healthy babies ($\chi^2 = 2.74, p < 0.1$: zero incidence makes chi-squared calculation an estimate only). The number of C. botulinum bacteria present was low. Toxins found in SIDS cases were as follows; C. perfringens (33/96; 34.4%), C. difficile (4/not stated), S. aureus (8/41; 19.5%) and C. botulinum (1/120; 0.83%). The presence of C. botulinum toxin was described as more typical of late recovery phase rather than acute attack stage of infant botulism. The authors therefore concluded that the true significance of the presence of C. botulinum was unknown in this study.

Over a five year period in Central Germany, researchers (Böhnel et al., 2001) examined 75 infant deaths (including 57 SIDS). Fifteen samples were positive for botulinum toxin (11 from the SIDS group). Previously in Germany, no C. botulinum organisms had been detected from 148 SIDS cases.

A case of SIDS in an 11 week old infant in Finland has been reported (Nevas et al., 2005a). Toxin type B was identified by PCR from intestinal contents and the organism was also isolated from the intestines. The strain was indistinguishable from that in vacuum cleaner dust from the infant’s home. The number of spores in the dust was estimated at 10 per kg.

A United Kingdom survey (Berry et al., 1987; Urquhart and Grist, 1976), of over 200 cases of SIDS, concluded that infant botulism was not a significant factor in SIDS.

The evidence for a role of infant botulism in SIDS is therefore mixed. The ACMSF (2005) has recommended that the UK government commission further research into the link between infant botulism and SIDS.

6.2.4 Risk assessment and other activity overseas

The European Commission (2002) have assessed that honey is the most important risk factor associated with infant botulism. A slow intestinal transit time is also identified. Exposure is mainly through:

- Mothers dipping the nipple into honey before placing it into mouth of the infant,
- Parents smearing the lips of the infant with honey, and
- Parents adding honey to formula-fed infants.

In South Australia, a Risk Profile on Honey and Apiary products was compiled as part of a document on primary industries (Sumner, 2002). The major microbial hazard for honey was considered to be C. botulinum and the risk lay in consumption of honey by infants. A risk ranking of infant botulism from the consumption of honey was undertaken and a summary of this work is reproduced in Table 11.
### Table 11: Risk assessment of honey and infant botulism in South Australia

<table>
<thead>
<tr>
<th>Risk criterion</th>
<th>Infant botulism and <em>C. botulinum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose and severity</strong></td>
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</tr>
<tr>
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<tr>
<td>Susceptibility</td>
<td>Neonates</td>
</tr>
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<td><strong>Probability of exposure</strong></td>
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</tr>
<tr>
<td>Frequency of consumption</td>
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</tr>
<tr>
<td>Proportion consuming(^1)</td>
<td>Some (25%)</td>
</tr>
<tr>
<td>Size of population</td>
<td>South Australia neonates (20,000/annum)</td>
</tr>
<tr>
<td><strong>Probability of contamination</strong></td>
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</tr>
<tr>
<td>Probability of raw product contaminated(^2)</td>
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</tr>
<tr>
<td>Effect of processing</td>
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</tr>
<tr>
<td>Possibility of recontamination</td>
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<tr>
<td>Post-process control</td>
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</tr>
<tr>
<td>Increase to infective dose</td>
<td>None</td>
</tr>
<tr>
<td>Further cooking before eating</td>
<td>Not effective in reducing hazard</td>
</tr>
<tr>
<td>Total predicted illnesses per annum in selected population</td>
<td>0.14</td>
</tr>
<tr>
<td>Risk ranking (0-100)*</td>
<td>56</td>
</tr>
</tbody>
</table>

Assumptions:

1. Proportions of babies consuming honey during their first year,
2. Prevalence of *C. botulinum* in honey.

Risk ratings were prepared for hazard-product pairings on a scale of 0-100 (0 = no risk, 100 = everybody eating a meal containing a lethal dose of the hazard every day). A “low” risk equated to <25, “medium” to 26-40 and “high” >40. Because the scale is logarithmic, an increment of 6 in the ranking relates to a 10-fold increase in risk. The pairing of honey and infant botulism was calculated as a high qualitative risk, at 56. The government published a response (South Australian Government, 2003). It recommended that the Department of Human Services provide advice to parents that they should not feed honey to infants less than 12 months old. The advice points out that children over 12 months and adults can safely eat honey.

In the UK, an *ad hoc* group on infant botulism produced a final draft of their report in July 2005 (ACMSF, 2005). A sub-group carried out a risk assessment for *C. botulinum* in infant foods, which was independently peer-reviewed. The risk assessment modelled spore contamination in ‘infant food material’ but did not include honey (ACMSF, 2005). A log-normal distribution was believed to represent the variable spore concentration in food. From the considerations of an expert panel, the distribution of spores in a 1kg food sample was simulated ([http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml](http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml)). The example given was for a 121g pack of food. The overall estimate was “a low risk”. The ACMSF hope that as further information becomes available, a full risk assessment of the addition of honey to infant foods can be undertaken. However better information is required, such as the contamination rate of honey with Group I spores and the dose-response relationship.
In the USA, there is a strong association between the type of spores prevalent in the soil and the type of toxin. Type A spores predominate in soil west of the Mississippi river, and type B in and soil east of the river. The same geographic distribution occurs for the types involved in cases (Midura, 1996).

6.3 Qualitative Estimate of Risk

There is insufficient New Zealand information available to satisfactorily gauge the risk posed by this food/hazard combination. The available data indicate that the risk is extremely low. This conclusion is based on the lack of any confirmed outbreaks or sporadic cases of infant botulism in New Zealand, and the failure of small scale surveys of environmental and marine sediment samples to demonstrate the presence of toxin producing species of *C. botulinum* in New Zealand.

6.4 Risk Categorisation

Botulism was included with a severity rating in the risk categorisation system in the appendices in other Risk Profiles. The proportion of severe outcomes (hospitalisation, long term sequelae, and death) resulting from infant botulism is approximately 80% hospitalisation, death rate 5% (ACMSF, 2005) placing this infection in the highest severity category.

Given the rarity of mortality linked to botulism in the last twenty years, obviously the incidence category would be the lowest.
7 RISK MANAGEMENT INFORMATION

The lack of a critical control point step for preventing *Clostridium* spores from entering honey, or for their elimination/reduction to an acceptable level, means that risk management predominately involves advice to parents of infants. The advice overseas is to abstain from feeding honey, honeyed water or dipping pacifiers into honey for infants less than 12 months of age. This is until their intestinal microflora matures sufficiently to prevent germination and subsequent neurotoxin production.

7.1 Relevant Food Controls

Codex and Food Standards Australia New Zealand (FSANZ) do not describe microbiological standards for honey. There is a FSANZ Food Standards Code (2.8.2) that sets out composition requirements but *C. botulinum* spores are not mentioned in the Standard.

7.1.1 Animal Products Act 1999

Risk Management Programmes (RMPs) are part of the emerging food assurance system in New Zealand. They form part of the Animal Products Act (APA) 1999. These will eventually be aligned with the Food Safety Programmes (FSPs) required by the Food Act 1981.


The Animal Products Act 1999 reforms the New Zealand law that regulates the production and processing of animal material and animal products to:

- manage associated risks; and
- facilitate overseas market access.

The Animal Products Act requires all animal products traded and used to be "fit for intended purpose". This means they must meet New Zealand animal product standards. The New Zealand animal product standards are contained in Part 1 of the Animal Product Regulations 2000, with further detail being provided in specifications.

By 1 July 2006, all animal product primary processing businesses, except those exempt under the Act or under the Animal Products (Exemptions and Inclusions) Order 2000, were required to have a risk management programme. A risk management programme is a documented programme to identify and manage biological, chemical and physical hazards. The programme is to be based on the principles of Hazard Analysis and Critical Control Point (HACCP): identifying the hazards, the systems of control, and demonstrating that the controls are effective. Risk management programmes are to be designed by individual businesses for the animal materials used, the processes performed and the product range produced.

The Animal Products Act 1999 requires that all beekeepers that export bee products or supply for export must have a documented HACCP system in place, based upon a Risk Management Plan or Food Safety Plan. Beekeepers must submit plans to MAF before 2006. [The NZFSA have provided a generic Risk Management Plan to assist this process, see Code of Practice: Processing of bee products, part 3 HACCP application... -
The generic HACCP plan identifies spores of *C. botulinum* as a hazard but because there are no acceptable control steps at the present time, there are no applicable critical control points.

The RMPs will replace annual Certificates of Registration (COR).

### 7.1.2 Control during or after processing

Kokubo *et al.*, (1984) found that when they inoculated *C. botulinum* spores (and *B. cereus* and *C. perfringens*) into commercial honey, after ~120 days at 25°C the spore count remained the same.

Nakano *et al.* (1990) tried to reduce or inactivate *C. botulinum* spores in honey using acids and bases, alcohol, detergents, enzymes, heat shock and sonication. None had any significant effect (reviewed in Snowdon and Cliver, 1996). Instead they proposed ‘long term storage’. The authors added type A and B spores to honey at the rate of $10^4$/ml and kept the honey at 25°C (Nakano *et al.*, 1989). After one year, the viable count of spores had decreased to less than 1%. Another option proposed was mild heating at 65°C for 5 days to eliminate or reduce the number of spores.

### 7.1.3 Labelling of honey


### 7.1.4 Advice to consumers

There are three Ministry of Health documents that provide advice on the feeding of infants in New Zealand;

- Background paper,
- Starting solids, and
- Eating for healthy babies and toddlers.

Only the background paper specifically mentions the link between infant botulism and honey:

“The risk of contracting infant botulism from honey is extremely small. However, as honey may contain *Clostridium botulinum* spores and is not an essential part of the infant’s diet, it is advisable not to introduce honey until the infant is six months of age. This is because 98 percent of the incidence of infant botulism occurs in infants under six months of age (Arnon *et al.*, 1981). This recommendation is a
precautionary measure as no formal studies on the presence of *Clostridium botulinum* in honey have been undertaken in New Zealand.” (Ministry of Health, 2000).

In contrast to the New Zealand ‘6 month’ advice, the publication then states the recommended advice offered by Canada, the USA and Britain. “…honey should not be consumed by infants under 12 months of age.”

The “Starting Solids” advisory leaflet (Ministry of Health, 2003b) provides information on the feeding of 4 to 6 month old infants. The only reference to honey is in the following statement;

> “Don’t add salt, sugar, honey, butter or cream to baby’s food”  
> (Ministry of Health, 2003b)

Finally the “Eating for Healthy Babies and Toddlers” leaflet, which has a question and answer format, asks the question “My baby seems to like sweet foods best” The advice given is

> “…encourage children to like fresh plain foods….don’t add extra sugar or honey to fruit or breakfast cereals” (Ministry of Health, 2004a)

A recently published fact sheet by Canterbury District Health Board gives advice on feedstuffs for vegetarian children. The document is dated October 2005 and mentions honey as an adjunct to the main text under the heading of introducing new foods. The advice is “Honey should not be given to babies under 6 months”. The advice is in parentheses and does not explain the connection to infant botulism. The website address for the factsheet is [http://www.cpublichealth.co.nz/files/NUT0088.pdf](http://www.cpublichealth.co.nz/files/NUT0088.pdf).

A popular publication on baby food by Holst and Holst (2000) produced in New Zealand describes honey in the introduction as one of the foods to be introduced with caution. It states;

> “Honey should not be given to babies under 12 months old. Although it is rare, even pasteurised honey may contain botulism spores which can cause illness.”

### 7.1.5 Risk management studies overseas

Some controls and treatments that have been suggested overseas include;

- effective educational campaigns;
  - targeted towards parents and carers of infants under 12 months of age,
  - to raise awareness of the disease, prevention, symptoms and treatment among professionals involved in pediatric care,
- more research into the development of rapid, sensitive laboratory methods to replace mouse bioassays, and
- to make the human immune globulin more readily accessible.

WHO (1999;2002) state “Since honey has been identified as a food source of infant botulism this food should not be given to infants under the age of one year”. An exception is where
foods containing honey receive a full botulinum cook or equivalent process in order to destroy the botulinum spores.

Most of the developed countries overseas follow the WHO recommendations and do not advocate the feeding of honey to infants less than 12 months of age.

In the European Union, the opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Honey and Microbiological hazards (European Commission, 2002) advocates that honey is not fed to infants under 12 months of age. To promote this message, it advocates that products are suitably labelled and appropriate messages are conveyed in pregnancy clinics etc. It points out that where labelling has been introduced, numbers of cases have fallen, although the numbers of cases is too small to infer a relationship.

**Australia:** A multicultural health communication factsheet produced by New South Wales (www.mhcs.health.nsw.gov.au) on settling babies under 6 months and advocates not dipping dummies into sweeteners such as honey. As described above, the South Australian government has recommended not feeding honey to infant under 12 months of age.

**Canada:** Health Canada advises parents and caregivers not to feed honey to infants less than a year old; [http://www.hc-sc.gc.ca/iyh-vsv/diseases-maladies/botu_e.html#skipall](http://www.hc-sc.gc.ca/iyh-vsv/diseases-maladies/botu_e.html#skipall). The government also provides a website containing information on botulism for health care workers; [http://www.health.gov.on.ca/english/providers/pub/disease/botulism.html](http://www.health.gov.on.ca/english/providers/pub/disease/botulism.html).

**Italy:** The Italian Pediatricians Association warned parents against giving honey to babies under 1 year of age (Eurosurveillance, 1999).

**United Kingdom:** Department of Health and the UK Food Standards Agency both advise not to give honey to infants less than 12 months of age; [www.eatwell.gov.uk/asksam/agesandstages/childrenandbabies/#A219762](http://www.eatwell.gov.uk/asksam/agesandstages/childrenandbabies/#A219762).

The British Honey Importers and Packers Association has advised members to add the following words onto labels ‘Honey should not be given to babies under 12 months’. The labelling is not compulsory.

The ad hoc group (ACMSF, 2005) also reinforced the recommendation of WHO that honey should not be added to foods targeted to infants under 12 months of age unless the food had been treated with a full botulinum cook or equivalent to destroy the spores.

Research into alternative methods of detection of *C. botulinum* is progressing. The current culturing (3-5 days) then testing for toxin in mouse bio-assay is slow, expensive and in many countries requires a licence for animal testing. Alternative molecular or genetic assays are in development but so far are not commercially validated (European Commission, 2002).
7.2 Economic Costs

No New Zealand information has been found on costs of illness. Information on costs is derived from studies in the US.

Hospitalisation has been reported in up to 80% of cases with the average stay approximately 4 to 5 weeks but differs with toxin type. Type A cases have a mean hospitalisation time of 5.4 weeks because it is more severe, whereas type B are 3.8 weeks (Midura, 1996). In California, for the year 1990, mean hospital costs exceeded $US 80,000 per case. Overall, the most protracted illness in 1988, where the patient was hospitalised for 10 months, cost more than $US 635,000 (Midura, 1996), equivalent to $US 890,000 at 1993 prices, (Arnon, 1995).

In California, economic costs for infant botulism were calculated for type A and type B cases.

The results are presented in Table 12.

Table 12: Economic costs of infant botulism in California, USA

<table>
<thead>
<tr>
<th>Toxin type</th>
<th>Cost per case ($US)</th>
<th>Length of stay (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>type A n=226</td>
<td>91,000</td>
<td>50,900</td>
</tr>
<tr>
<td>type B n=155</td>
<td>68,300</td>
<td>57,700</td>
</tr>
</tbody>
</table>

(Source: Arnon, 1995).

Arnon (1995) subsequently calculated an average of $US 2400 at 1993 prices per day based on hospital costs from 1984 to 1993 for all patients.

7.3 Other Transmission Routes

7.3.1 Other transmission routes: food

Many surveys have identified *C. botulinum* spores in food, especially fish, meats, honey and vegetables/mushrooms. A collation of these surveys can be found in Table 13.

Table 13: Prevalence of *C. botulinum* spores in food surveys worldwide

<table>
<thead>
<tr>
<th>Product</th>
<th>Origin</th>
<th>Sample size (g)</th>
<th>% positive samples</th>
<th>MPN per kg</th>
<th>Type(s) identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eviserated whitefish chubs</td>
<td>Great Lakes, USA</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>E,C</td>
</tr>
<tr>
<td>Vacuum-packed frozen flounder</td>
<td>Atlantic Ocean</td>
<td>1.5</td>
<td>10</td>
<td>70</td>
<td>E</td>
</tr>
<tr>
<td>Dressed rockfish</td>
<td>California</td>
<td>10</td>
<td>100</td>
<td>2400</td>
<td>A,E</td>
</tr>
<tr>
<td>Salmon</td>
<td>Alaska</td>
<td>24-36</td>
<td>100</td>
<td>190</td>
<td>A</td>
</tr>
<tr>
<td>Vacuum-packed fish</td>
<td>Viking Bank, USA</td>
<td>-</td>
<td>42</td>
<td>63</td>
<td>E</td>
</tr>
<tr>
<td>Smoked salmon</td>
<td>Denmark</td>
<td>20</td>
<td>2</td>
<td>&lt;1</td>
<td>B</td>
</tr>
<tr>
<td>Salted carp</td>
<td>Caspian Sea</td>
<td>2</td>
<td>63</td>
<td>490</td>
<td>E</td>
</tr>
<tr>
<td>Fish and seafood</td>
<td>Osaka, Japan</td>
<td>30</td>
<td>8</td>
<td>3</td>
<td>C,D</td>
</tr>
<tr>
<td>Raw meat</td>
<td>North</td>
<td>3</td>
<td>&lt;1</td>
<td>0.1</td>
<td>C</td>
</tr>
<tr>
<td>Product</td>
<td>Origin</td>
<td>Sample size (g)</td>
<td>% positive samples</td>
<td>MPN per kg</td>
<td>Type(s) identified</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>--------------------</td>
<td>------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Cured meat</td>
<td>America</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw pork</td>
<td>Canada</td>
<td>75</td>
<td>2</td>
<td>0.2</td>
<td>A</td>
</tr>
<tr>
<td>Cooked, vacuum-packed potatoes</td>
<td>The U.K.</td>
<td>30</td>
<td>0-14</td>
<td>&lt;0.1-5</td>
<td>A,B,C</td>
</tr>
<tr>
<td>Mushrooms</td>
<td>Canada</td>
<td></td>
<td>0</td>
<td>2100</td>
<td>B</td>
</tr>
<tr>
<td>Random honey samples</td>
<td>USA</td>
<td>30</td>
<td>1</td>
<td>0.4</td>
<td>A,B</td>
</tr>
</tbody>
</table>

Source: (Austin, 2001)

It has been suggested that corn syrup is a potential source of spores (Kautter et al., 1982) although there is disagreement (Arnon, 1992). A prospective study in 1977 (Arnon et al., 1977) tested 15 foods and 6 drug items ingested by infant botulism cases, including mother’s breast milk, honey, rice cereal, oatmeal, infant formula, pediatric vitamin preparations, pediatric iron supplements and acetaminophen drops. Only the jar of honey tested was positive for *C. botulinum*.

Research in 1982 (Kautter et al., 1982) tested ten categories of food from Washington DC. The number of samples of each category is given in Table 14.

**Table 14:** Prevalence of *C. botulinum* in various foods tested in Washington DC, USA, in 1982

<table>
<thead>
<tr>
<th>Food type</th>
<th>Number of samples</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant dry cereals</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>Commercial baby formula</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Non-fat dry milk</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Pasteurised whole milk</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>Commercial canned fruits - apricots &amp; tapioca</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Commercial canned fruit juices - apple, prune</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Honey</td>
<td>100</td>
<td>2 x type A</td>
</tr>
<tr>
<td>Corn syrup</td>
<td>40</td>
<td>8 x type B</td>
</tr>
<tr>
<td>Granulated cane sugar</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>Fresh cooked carrots</td>
<td>100</td>
<td>-</td>
</tr>
</tbody>
</table>

Overall, all the samples were negative apart from two samples of honey which contained viable *C. botulinum* type A spores and 8 samples of corn syrup which contained *C. botulinum* type B spores. A nationwide survey was undertaken of corn syrup because it was felt that honey had been extensively surveyed previously. From supermarkets and grocery stores, 961 bottles of corn syrup were collected, representing samples from all manufacturers. Five bottles (0.5%) contained viable type B spores. The MPN was calculated at 1.25 spores per 25g.
7.3.2 Other transmission routes: environment

*Clostridium botulinum* occurs commonly in soil, dust and aquatic environments worldwide (EFSA, 2004). The ubiquitous distribution of *C. botulinum* in the environment must inevitably lead to ingestion of spores through a multitude of transmission routes by adults and children, and in the vast majority of cases, there are no ill effects because of the several defence mechanisms present in the intestinal system.

Approximately 85% of the infant botulism cases worldwide have an unknown vehicle of infection and could be dietary or environmental. The consensus is that environmental sources are responsible for the majority of infant botulism cases. This has led many investigators to examine sources and establish positive associations from environments around the home. Arnon *et al.*, (1979) examined 555 food and environmental samples from field investigations of cases; *C. botulinum* was found in the following; honey (9), yard soil (2), houseplant soil (1), and vacuum cleaner dust (1). Details of environmental associations can be found in the following papers;

- dirt in houseplants (Chin *et al.*, 1979),
- vacuum cleaner dust (Nevas *et al.*, 2005a),
- soil, tank rainwater and vacuum-cleaner dust (Murrell and Stewart, 1983),
- rain tank water (Midura *et al.*, 1979), and
- dust in cribs (Istre *et al.*, 1986).

In the Murrell and Stewart (1983) study in New South Wales, the authors investigated seven infant botulism cases and two adult cases during 1980-1981. Clinical diagnosis in each case was botulism except where asterisked. The presence of toxin and spores in the faeces from the cases are summarised in Table 15. Cases from home locations 1, 3 and 7 had not eaten honey.

**Table 15: Summary of infant botulism cases in New South Wales, Australia (1980 – 1981)**

<table>
<thead>
<tr>
<th>Home location</th>
<th>Onset of illness</th>
<th>Age (weeks)</th>
<th>Diet</th>
<th>Presence in faeces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey Bridge</td>
<td>10.01.80</td>
<td>19</td>
<td>Breast milk only</td>
<td>800</td>
</tr>
<tr>
<td>Cobar</td>
<td>30.03.81</td>
<td>22</td>
<td>Weaned 2 weeks, honey on dummy</td>
<td>100-1280</td>
</tr>
<tr>
<td>Deniliquin</td>
<td>1.05.81</td>
<td>6</td>
<td>Breast milk, just weaned</td>
<td>+</td>
</tr>
<tr>
<td>Cranebrook</td>
<td>05.08.81</td>
<td>26</td>
<td>Cow’s milk &amp; solids</td>
<td>2</td>
</tr>
<tr>
<td>Kempsey*</td>
<td>06.11.81</td>
<td>52</td>
<td>Not given</td>
<td>+§</td>
</tr>
<tr>
<td>Home location</td>
<td>Onset of illness</td>
<td>Age (weeks)</td>
<td>Diet</td>
<td>Presence in faeces</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>-------------</td>
<td>------------</td>
<td>-------------------</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>Hornsby Heights, Sydney**</td>
<td>06.06.80</td>
<td>182</td>
<td>Solids</td>
<td>+§</td>
</tr>
<tr>
<td>7. Roseville, Sydney</td>
<td>13.12.81</td>
<td>4</td>
<td>Infant formula</td>
<td>≥10 A, 6, A</td>
</tr>
<tr>
<td>Fairfield West, Sydney**</td>
<td>02.03.81</td>
<td>51 years</td>
<td>Normal</td>
<td>+§</td>
</tr>
<tr>
<td>Stanmore, Sydney**</td>
<td>01.08.81</td>
<td>70 years</td>
<td>Normal</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

* Suspected botulism
** Inconclusive botulism
§ Not neutralised by type A-F antisera
# Three faecal samples positive over a period of 46 days

Faecal and serum samples from patients and samples of soil, water, vacuum-cleaner dust were analysed (11 infant formulae and one honey sample from the patients’ environment were also analysed). The results of the environmental sampling found that the presence of *C. botulinum* in dust and water in the home was important in the occurrence of infant botulism, at least in Australia. Dust was thought to be the most likely source of contamination in water, although the viability of vegetative bacteria and their spores in rainwater tanks was not known. In two of the type B infant botulism cases, the bacterium was isolated from soil around the dwelling in one case and from the tank rainwater in another. One type A case (from Monkey Bridge) had the bacteria present in soil, vacuum-cleaner dust and tank rainwater. The authors noted that drought and dusty conditions prevailed more than usual during the investigation period. As far as food sampling was concerned, there did not appear to be extensive sampling with 11 samples of infant formulae (covering 9 brands) and one honey sample tested. None of the food samples contained *C. botulinum* spores.

The authors also noted that out of 13 samples of vacuum-cleaner dust from Sydney suburbs, type A was found in one sample only (0.24 spores/g). This house was newly constructed in an area being extensively developed with considerable earthworks being carried out.

The overall conclusions from this study were that the case’s home location and frequency of *C. botulinum* isolates from environmental samples suggested that infants were at considerably greater risk in rural areas compared to larger cities. The authors advocated the periodic cleaning and chlorination of rainwater tanks used as a drinking source, especially where an infant was in their first six months of life.

Unpublished research cited in Fernández *et al.*, (1999), proposed an association between the regional incidence of infant botulism in the province of Mendoza, Argentina and the prevalence of *C. botulinum* in the soil. Their observations suggest that soil and environmental dust were more important sources of infection than honey. Between 1982 to 1997, Mendoza experienced 46 cases (32%) of Argentina’s infant botulism cases, all type A. None of the samples of honey ingested by the infants in Mendoza yielded any spores.
8 CONCLUSIONS

8.1 Description of Risks to New Zealand Consumers

8.1.1 Risks associated with honey

The risk to New Zealand infants (or adults with altered “at risk” gastrointestinal status) from C. botulinum spores in honey appears to be extremely low. The absence of diagnosed cases of infant botulism (from any source), and the lack of conclusive evidence for toxigenic Group I Type A or B species of C. botulinum from the New Zealand environment support this conclusion. However, there are a number of difficulties in using this information to properly assess the risk.

Potentially there may be a very small number of undiagnosed infant botulism cases among the approximately 50 SIDS cases reported annually in New Zealand, or the illness may be attributed to other causes (see Section 4.1). The environmental isolates of C. botulinum examined by AgResearch were obtained from the South Island only. There was also evidence for the presence of (partial) toxin genes in DNA extracted directly from these environmental samples, although whether this truly indicates the presence of viable bacteria is unclear.

The presence or absence of toxin producing C. botulinum in the New Zealand environment needs to be clarified. Environmental contamination, either directly or indirectly via bees, is the source of the spores in honey, and the types found in honey reflect those found in the environment, but there are insufficient data at present to adequately assess this issue.

Honey imports are tightly controlled (although not specifically for C. botulinum contamination), and apparently only small amounts are imported. This situation may change with the issue on 11 July 2006 of the Import Health Standard allowing the importation into New Zealand of specified bee products from Australia.

The analysis of infant botulism cases in New South Wales (Murrell and Stewart, 1983) indicates the presence of C. botulinum in soils and dust in that environment. C. botulinum spores have been found in Australian honey imported into Finland (Nevas et al., 2002). The risk assessment for C. botulinum in South Australia (Sumner, 2002) prompted a recommendation that honey should not be fed to infants under 12 months of age. The regulation changes in 2006 allowing importation of honey from Australia suggest that a reconsideration of New Zealand recommendations for feeding of honey to infants would be worthwhile.

8.1.2 Risks associated with other foods

Honey is the only laboratory confirmed dietary source of C. botulinum spores overseas. Corn syrup and formula milk has been suggested as possible sources but have not been linked to cases. Corn syrup is not widely available in New Zealand. Overall, there appears to be an extremely low risk of C. botulinum spores being present in other foods fed to infants in New Zealand.
8.1.3 Quantitative risk assessment

A quantitative risk assessment would not be feasible on this food/hazard combination; it would be more useful to examine the data gaps identified below.

8.2 Commentary on Risk Management Options

The New Zealand recommendation that honey is not fed to infants under six months of age is different to that in many other developed countries, where 12 months is the recommended age limit. The New Zealand recommendation appears to be based on a 1981 reference which asserts that 98% of cases occur in infants under 6 months of age. More recent information suggest that the percentage is lower than that, perhaps 90%. A reconsideration of the New Zealand recommendation may be appropriate.

8.3 Data gaps

The data gaps identified in this Risk Profile are:

- Prevalence and types of *C. botulinum* in the New Zealand environment;
- Prevalence of *C. botulinum* spores in honey in New Zealand; and
- Consumption of honey by infants in New Zealand.
9 REFERENCES


Betts GD. (1996) Code of Practice for the manufacture of vacuum and modified atmosphere packaged chilled foods with particular regard to the risks of botulism. Campden and Chorleywood Food Research Association, Gloucestershire, UK.


http://www.fao.org/docrep/w0076e/w0076e30.htm


Meyer KF, Dubovsky BJ. (1922). The occurrence of the spores of *B. Botulinus* in Belgium, Denmark, England, The Netherlands and Switzerland. VI. Journal of Infectious Diseases; 31: 600-609.


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Schocken-Itturino RP, Carneiro MC, Kato E, Sorbara JOB, Rossi OD, Gerbasi LER. (1999). Study of the presence of the spore of *Clostridium botulinum* in honey in Brazil. FEMS Immunology and Medical Microbiology


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APPENDIX 1: VETERINARY CERTIFICATE

I, .........................................................., being an Official Veterinarian or Official Certifying Officer authorised by AQIS certify to after due enquiry with respect to the honey bee products identified in this Zoosanitary Certificate, that:

1 FOR ALL BEE PRODUCTS:

1.1 The bee products have been subject to one of following risk management measures for European Foulbrood (Melissococcus pluton) and Nosemosis (Nosema ceranae):

EITHER 1.1.1 the bee products originate from apiaries in Western Australia, and neither Melissococcus pluton nor Nosema ceranae have not been isolated or reported from bees in the state of origin;

OR 1.1.2 the bee products originate in apiaries in an Australian state or territory other than Western Australia and have been heated to one of the following minimum core temperatures for the corresponding period of time:

50ºC for 54 hours; OR  
55ºC for 22 hours, 41 minutes; OR  
60ºC for 10 hours; OR  
65ºC for 4 hours, 7 minutes; OR  
70ºC for 1 hour, 48 minutes; OR  
75ºC for 48 minutes; OR  
80ºC for 22 minutes; OR  
85ºC for 11 minutes; OR  
90ºC for 6 minutes;

OR 1.1.3 the bee products have been irradiated with at least 15 kGy. (delete options as appropriate)

1.2 The bee products have been subject to one of the following risk management measures for American foulbrood (Paenibacillus larvae larvae):

EITHER 1.2.1 originate from Australian hives that have been inspected within the previous 12 months by an Apiary Inspector approved by the state Department of Agriculture and found not to be clinically infected or suspected to be clinically infected with Paenibacillus larvae larvae;

OR 1.2.2 have been subjected to the test specified as Culture of P. l. l. from bulk honey for detection of American foulbrood in Appendix 2 of the Australia and New Zealand Standard Diagnostic Procedures – Honey Bee Diseases (2003) in an official laboratory, and found to have a Paenibacillus larvae larvae spore count of less than 500,000 per litre;

OR 1.2.3 subject to heat treatment where the core temperature has reached at least 120ºC for a minimum of 24 hours;

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OR 1.2.4 irradiated with at least 10 kGy.
(delete options not used)

2 FOR BULK EXTRACTED HONEY:
Additional origin of product or treatment requirement

2.1 The honey has been subject to one of the following risk management measures for small hive beetle (*Aethina tumida*):

EITHER 2.1.1 the honey originates from the Australian Capital Territory, Northern Territories, South Australia, Tasmania, or Western Australia and *Aethina tumida* has not been reported in this state or territory of origin;

OR 2.1.2 the honey has been removed from the original drum, heated to at least 50°C for a minimum of 24 hours, repackaged into a new bulk honey container, the outside of which has been cleaned and inspected to ensure that it is clean and free of honey.

(delete option as appropriate)

3. FOR RAW BEESWAX OR RAW PROPOLIS:
Additional treatment requirement
3.1 The raw beeswax or raw propolis has been subject to the following risk management measures for hitchhiker organisms:

3.1.1 freezing at –18°C or below for at least 7 days.

(delete clause if product is not raw beeswax or raw propolis).

Signature of Official Veterinarian or Certifying Officer Date

Name and address of office:
N.B Official stamp of the Australian government must be applied to all pages of zoosanitary certification.