Ministry for Primary Industries
Manatū Ahu Matua

Risk profile:

New Zealand Government

Growing and Protecting New Zealand

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SUMMARY

The purpose of a Risk Profile is to provide 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.

The food/hazard combination addressed by this Risk Profile is *Clostridium botulinum* in ready-to-eat (RTE) smoked fish and shellfish in sealed packaging.

An update to the 2006 Risk Profile on this topic (Gilbert *et al.*, 2006) was required to include:

- more recent scientific knowledge on *C. botulinum* in fish and shellfish and non-seafood ingredients for RTE fish and shellfish products; and,
- more detailed information on the types of products and hurdles that may restrict *C. botulinum* growth in current New Zealand smoked fish production and storage systems.

This update was written with specific reference to the Codex Draft Standard for Smoked Fish Appendix on *C. botulinum* controls (Codex, 2011).

Although it has been postulated that at 3% or more, New Zealand seawater has too high a salt content for *C. botulinum* Type E, there have been reports of salinity below 3% around the coast, as would logically occur at river mouths etc. In addition, New Zealand salmon are at least partially grown in fresh water, and shellfish may occur in brackish water.

The processes used by New Zealand manufacturers of smoked fish and shellfish in sealed packaging would not control the germination and growth of *C. botulinum* Type E spores. Salt concentrations are too low, and seafood is predominantly vacuum packed. The suggested shelf life for New Zealand product of five weeks (Cathy Webb, Seafood Standards Council, personal communication) is at the upper end of the range of shelf lives reported in the literature. For example, a shelf life of 20 days at 4°C was determined for vacuum-packed cold-smoked salmon, as assessed by a range of microbial (total aerobic and anaerobic counts, *Lactobacillus* spp.), chemical (total volatile bases, trimethylamine, biogenic amines) indicators and sensory assessment (Dondero *et al.*, 2004). However, shelf lives longer than five weeks have been reported, with shelf life being dependent on storage conditions and the initial microbial quality of the fish.

Hot smoking time and temperature conditions will reduce the number of spores but not eliminate them, while cold smoking would not be expected to have a significant effect on numbers. Controls on growth through cold chain management (i.e. ≤3°C) may not be completely maintained (Peck and Stringer, 2005). The influence of other conditions on growth of the pathogen at temperatures >3°C is complicated and results from the summation of the inhibitory effects of a number of hurdles. There is a lack of data on the effects of some hurdles such as the effect of smoke or smoke components on *C. botulinum*. While some predictive models are available which take into account the effects of various hurdles, the literature which has evaluated them tends to conclude that they are unreliable.

Of the non-seafood ingredients considered in this Risk Profile, surveys of spore prevalence are few, and results are mixed. Overall, we consider that there are insufficient data on which to base an assessment of this specific issue.

No data on the prevalence of *C. butyricum* and *C. baratii* in fish or shellfish have been located. The minimum temperature allowing growth of *C. butyricum* in food is reported to be 12°C (Anniballi *et al.*, 2002), considerably higher than that allowing growth of *C. botulinum* Type E.

Based on the information in this Risk Profile, we consider that the risk from *C. botulinum* in smoked fish in sealed packaging in New Zealand is very low. This is based on the following:

- Illness due to foodborne botulism has not been reported in New Zealand since 1985, and this case involved proteolytic *C. botulinum* Type A;
- A survey of 501 marine sediment samples in New Zealand did not identify any toxigenic extracts, or detect DNA from *C. botulinum* Type E;
- Overseas surveys of sediment and local fish show a correlation in prevalence at a regional level (for example in the Great Lakes and Pacific Coast of the USA);
- A situation similar to New Zealand applies in Australia (absence of reported illnesses of Type E, and lack of isolations of *C. botulinum* Type E from sediments);
- The single Southern Hemisphere survey of fish for *C. botulinum* (in Indonesia) identified several isolates, but only one was Type E.

Data gaps identified in this Risk Profile are:

- Few data are available concerning the presence of type B, E and F spores in ingredients such as sugar and spices that are used in the manufacture of smoked fish and shellfish products, and none were located specifically for New Zealand.
- Few data are available describing the physicochemical parameters (hurdles such as pH, water activity, water phase salt concentration) affecting potential growth of non-proteolytic *C. botulinum* (or other hazards) in New Zealand smoked fish and shellfish products.
- We were unable to locate data in the literature which systematically assesses the affects of some specific hurdles such as the presence of smoke (as phenol) and naturally occurring lactic acid on the growth kinetics of non-proteolytic *C. botulinum* in smoked fish and shellfish.
- There is no reliable, validated predictive model for this hazard.

1   STATEMENT OF PURPOSE

The purpose of a Risk Profile is to provide information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles are part of the Risk Management Framework (RMF)[1] approach taken by the Ministry of Agriculture and Forestry (MAF). The Framework consists of a four step process, as shown in Figure 1.
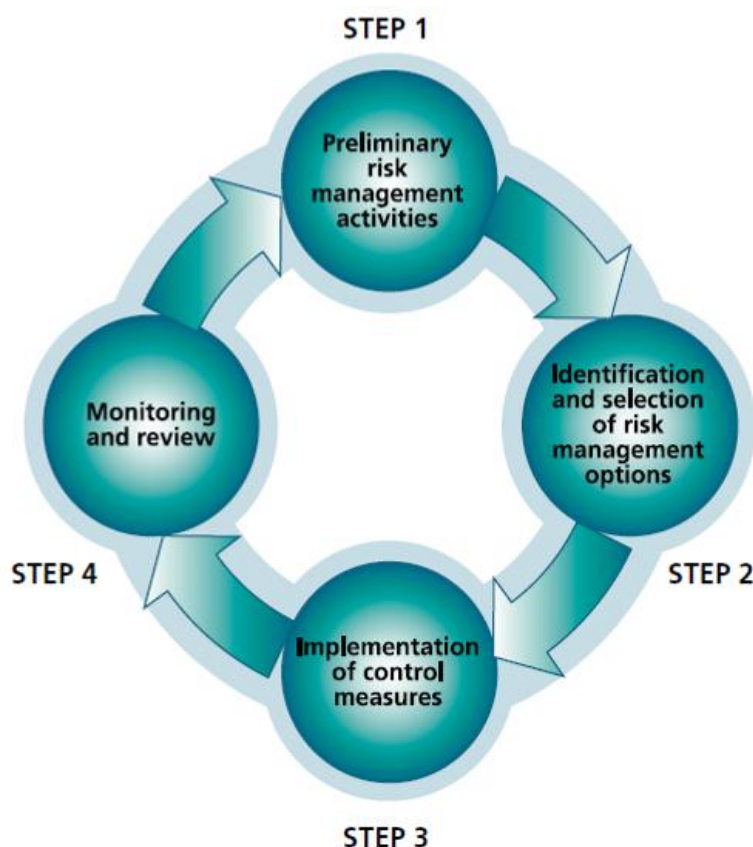
Figure 1:   The four steps of the Risk Management Framework

This initial step in the RMF, Preliminary Risk Management Activities, includes a number of tasks:

- Identification of food safety issues
- Risk profiling
- Establishing broad risk management goals
- Deciding on the need for a risk assessment
- If needed, setting risk assessment policy and commissioning of the risk assessment
- Considering the results of the risk assessment
- Ranking and prioritisation of the food safety issue for risk management action.

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[1] http://www.foodsafety.govt.nz/elibrary/industry/RMF_full_document_-_11604_NZFSA_Risk_Management_Framework_3.1.pdf

Risk profiling may be used directly by risk managers to guide identification and selection of risk management options, for example where:

- Rapid action is needed;
- There is sufficient scientific information for action;
- Embarking on a risk assessment is impractical.

1.1   Food/hazard combination and risk management questions

The food/hazard combination addressed by this Risk Profile is *Clostridium botulinum* in ready-to-eat (RTE) smoked fish and shellfish in sealed packaging.

An update to the 2006 Risk Profile on this topic (Gilbert *et al.*, 2006) was required to include:

- more recent scientific knowledge on *C. botulinum* in fish and shellfish and non-seafood ingredients for RTE fish and shellfish products; and,
- more detailed information on the types of products and hurdles that may restrict *C. botulinum* growth in current New Zealand smoked fish production and storage systems.

This update was written with specific reference to the Codex Draft Standard for Smoked Fish Appendix on *C. botulinum* controls (Codex, 2011).

2   HAZARD AND FOOD

2.1   The Pathogen

Cells of *C. botulinum* are straight to slightly curved, Gram positive rods. They form heat-resistant endospores (Szabo and Gibson, 2003). The organism can produce the most potent biological neurotoxins known. *C. botulinum* may cause either intoxication by pre-formed toxin or infection and toxin production *in vivo* (sometimes called toxico-infection). Ingestion of the pre-formed toxin produced by germinated spores in food is an intoxication, and is the subject of this Risk Profile.

Seven recognised antigenically distinct protein toxins, designated as types A, B, C, D, E, F and G, can be produced by *C. botulinum* (Dolly and Aoki, 2006). *C. botulinum* type G has been renamed *C. argentinense*. Most isolates produce one toxin, although some can produce multiple toxins (Szabo and Gibson, 2003).

Four of the toxin types (A, B, E and F) cause botulism in humans, although incidents involving Type F toxin are rare. The toxin type most commonly associated with marine environments (and therefore seafood) is toxin Type E, and Type E-producing *C. botulinum* is considered an aquatic organism (Huss, 1980).

Other species of the genus, *C. butyricum* and *C. baratii*, may also produce toxins (Types E and F respectively) and have caused human botulism (Fu and Wang, 2008; Szabo and Gibson, 2003)[2].

The species is sub-divided according to 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 toxin of types A, B and F, and their proteolytic activity generally causes spoilage of food and offensive odours that render the food unacceptable. Group II includes isolates producing toxin types B, E and F. Proteolytic spoilage does not occur so that foods can appear acceptable with no unpleasant accompanying odours.

Growth and toxin production by *C. botulinum* in foods is influenced by several factors (some of which can be regarded as "hurdles'), including initial levels of spores, temperature, salt (water phase)[3], acidity (pH), and atmosphere (oxygen). The risk posed by any toxin production is complicated by spoilage activity of other microbial flora. The advantage of the protection afforded by using an oxygen-containing atmosphere, and the fact that *C. botulinum* grows anaerobically, is offset by the oxygen removal resulting from the metabolism of the natural aerobic flora.

This Risk Profile is primarily concerned with *C. botulinum* type II organisms. Their minimum growth temperature is 3°C, the optimum 35-40°C and the maximum 40-45°C. Growth may occur down to a pH of 5.0, and a water phase salt concentration of 5%.

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[2] The literature sometimes refers to neurotoxins from *C. botulinum* and *C. butyricum* as BoNT and BuNT respectively. In this Risk Profile we have used the generic term "toxin".

[3] Water Phase Salt is a calculation using percent moisture, and percent salt. Water Phase Salt = (%salt x 100)/ (% salt + % moisture). The potential for microbial growth decreases as Water Phase Salt increases.

Further details describing the taxonomy and characteristics of the organism are presented in section 7.1 of Appendix 1.

2.2   The Food

2.2.1   Definitions

For the purposes of this Risk Profile, the specific food is ready-to-eat smoked seafood in sealed packaging.

The food as defined in this Risk Profile (Appendix 1 section 7.3) includes smoked finfish, shellfish and crustacea. Ready–to-eat products are any foods which are normally eaten in their raw state or any food handled, processed, mixed, cooked, or otherwise prepared into a form which are normally eaten without any further bactericidal steps (adapted from Codex Principles and Guidelines for the Conduct of Microbiological Risk Management). Normal cooking will destroy vegetative cells and toxins but not necessarily spores which require, for example, 90°C for 10 minutes to achieve a 6D reduction for Type II *C. botulinum* spores (Appendix 1, section 7.1.3). The focus is on product prepared without a sporicidal processing step, such as smoked products, and which are stored under conditions where the bacterium may grow e.g. low oxygen or modified atmosphere packaging.

Placing smoked seafood into sealed packaging such as vacuum packaging reduces the amount of oxygen present, so prolonging shelf life by inhibiting the growth of aerobic spoilage bacteria (FDA, 2001). However, the creation of a low redox potential combined with a process which does not destroy spores has the potential, if not properly controlled, to allow the germination of spores and subsequent neurotoxin production.

2.2.2   Summary of manufacture/production of foods

The Codex Committee for Fish and Fishery Products has developed a draft standard for smoked fish, smoke-flavoured fish, and smoke dried fish (Codex, 2011). The proposed draft standard was prepared by Denmark and is currently at Step 7 of the Codex Procedure.

The draft proposes some process definitions, for example salting and smoking. Salting of the fish is defined as dry salting, brining by immersion, or brining by injection.

Draft definitions of smoking as described by Codex are:

- Smoking is a process of treating fish by exposing it to smoke from smouldering wood or plant material. This process is usually characterised by an integrated combination of salting, drying, heating and smoking steps in a smoking chamber.
- Hot smoking is a process in which fish is smoked at an appropriate combination of temperature and time sufficient to cause complete coagulation of the proteins in the fish flesh. Hot smoking is generally sufficient to kill parasites, to destroy non-sporulated bacterial pathogens and to injure spores of human health concern.

- Cold smoking is a process of treating fish with smoke using a time/temperature combination that will not cause significant coagulation of the proteins in the fish flesh but that will cause some reduction of the water activity.

Smoke-flavoured fish is prepared from fish that has been treated with smoke flavours. Such a product will not have undergone a process as described in the points immediately above but will have a smoked flavour. Smoke flavours are defined as "either smoke condensates or artificial flavour blends prepared by mixing chemically-defined substances in known amounts or any combination of both (smoke-preparations)", and smoke flavouring "a process in which fish or fish preparations are treated with smoke flavour. The smoke flavour can be applied by any technology".

The draft standard also states that the formation of *C. botulinum* toxin can be controlled through an application of science based options involving packaging type, storage temperature, and water activity e.g. the use of salt in the water phase.

Packaging can be defined in terms of its oxygen transmission rate (OTR). Oxygen-permeable packaging provides an OTR of 10,000 cm^3 m^{-2} 24 h^{-1} (for example 1.5 mm polyethylene) and could provide sufficient exchange of oxygen to allow aerobic spoilage organisms to grow (and therefore spoilage may take place before any neurotoxin is produced). An oxygen-impermeable package can be defined as having an OTR of less than 100 cm^3 m^{-2} 24 h^{-1}, for example 2 mm polyester (FDA, 2001).

For the purposes of this Risk Profile, vacuum-packaged (VP) and Modified Atmosphere Packaged (MAP) product will be considered as being packaged under oxygen-impermeable conditions. The New Zealand seafood industry reports that most of its product is vacuum packaged (Webb, C. personal. communication).

2.2.3 The Food Supply in New Zealand: Ready-to-eat smoked seafood in sealed packaging

Internet searches find numerous companies in New Zealand manufacturing both hot and cold smoked seafood, most commonly salmon, although the companies may not be involved in primary production or harvesting. Information from the Seafood Standards Council indicates that, in New Zealand, hot and cold smoked seafood types include salmon, mussels, and eel (Cathy Webb, Seafood Standards Council, personal communication, March 2011; Appendix 1, section 7.4.5). The same information states that the salt concentrations used in these products range from 1 - 3.5%, and vacuum packaging is predominantly used by the New Zealand seafood industry. Based on the processing steps provided, the other ingredients considered for this Risk Profile were salt, sugar, herbs and spices.

The New Zealand Salmon Farmers Association[4] reported a harvest of 7,450 tonnes of farmed salmon in 2004, with approximately 50% of production being exported, although only a portion will be smoked and sealed. Farmed salmon production is increasing steadily and by 2007 the estimated harvest was 8,570 tonnes[5]. Farmed salmon in New Zealand are principally "king" salmon (also known as "chinook" salmon) and one company, New Zealand King

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[4] http://www.salmon.org.nz/aboutsalmon.shtml
[5] http://www.seafoodindustry.co.nz/salmon

Salmon, reports current (2011) annual production of 8,600 tonnes, being 70% of New Zealand production[6]. This suggests current New Zealand annual production of farmed salmon is approximately 12,000 tonnes.

Smoked seafood exports from New Zealand are modest compared to the export of fresh or frozen product. According to data from the Seafood Industry Council website[7] "smoked, salted or in brine" finfish made up 209 tonnes out of a total of 207,632 tonnes exported in 2009. This suggests that smoked salmon comprises a small proportion of total salmon exports. There was no equivalent category for shellfish, but "processed" shellfish comprised 1,875 tonnes out of a total of 76,088 tonnes in 2009. Data from Statistics New Zealand give a slightly lower figure for smoked fish exports in 2009 (195 tonnes), with a decrease in the 2010 year (164 tonnes)[8]. The main destination countries for New Zealand exports of smoked fish in 2010 were Japan (40%) and Australia (36%).

A small study on New Zealand hot and cold salmon included measurement of water phase salt and a semi-quantitative assessment of smoke as phenol (Nortje *et al.*, 2001). Only nine samples were tested; four were hot smoked and five cold smoked. The water phase salt concentration varied from 0.8% to 2.4 % with most (five) in the 1-2% range. One sample had a phenol concentration of <10 ppm and the maximum was around 40 ppm. Most (six) were around 10 ppm. In a more recent survey a single smoked tuna sample had a water phase salt (WPS) concentration of 4.7%, while one of smoked dogfish was 2.8% (unpublished data). The industry reports a 2.5-3% WPS concentration for hot and cold-smoked salmon (Appendix 1, section 7.4.5).

2.2.4 Imported smoked seafood

During 2010, 156 tonnes of smoked fish was imported into New Zealand (www.stats.govt.nz). The majority of imported smoked fish was from China (74%), followed by Australia (13%), Thailand (8%) and the Philippines (5%). Imports from China are predominantly smoked hoki, while those from Australia are smoked salmon. The species of smoked fish imported from Thailand and the Philippines is not specified under the tariff code (HS) system.

There appears to be no particular trend in the quantity of smoked fish imported into New Zealand in recent years, with imports increasing from 2008 (73 tonnes) to 2009 (187 tonnes), but decreasing again in 2010 (156 tonnes).

While smoked shellfish are almost certainly imported into New Zealand, the tariff code (HS) system does not allow identification of these items.

2.2.5 Behaviour of the pathogen in foods

The likelihood of toxin production in fish and shellfish is determined by many factors such as, for example, the initial spore load, temperature, duration of incubation, atmosphere, salt

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[6] http://www.kingsalmon.co.nz/Company/
[7] http://www.seafoodindustry.co.nz/f403,20467/20467_Exports_of_Seafood_Produce_for_2008_and_2009.pdf
[8] http://www.stats.govt.nz/

concentration, or presence of other antimicrobials like phenol from smoke (section 7.4, Appendix 1). Extrinsic and intrinsic factors need to be considered as hurdles, each contributing to the inability of a product to support growth and toxin production by the pathogen.

C. botulinum Type E toxin may be produced in vacuum packaged fish and shellfish within periods where the foods are still considered acceptable, though this is dependent on storage temperature. Sensory studies have evaluated the shelf life of cold smoked salmon (2% salt) under vacuum packaging (Dondero *et al.*, 2004). Shelf life decreased from 26 days at 0°C, to 7 days at 8°C, which is shorter than the 35 days allowed by the New Zealand industry.

Full consideration of the factors involved in toxin production is made in section 7.4.6. In summary the incubation temperature is important. Data from the literature were used to produce Figure 2 which shows that as the incubation temperature reduces the time to toxin production increases. The line shown in the figure is an exponential curve fitted to the data.

Figure 2.   Influence of temperature on time to toxin production for *C. botulinum* Type E in various anaerobically incubated seafoods.
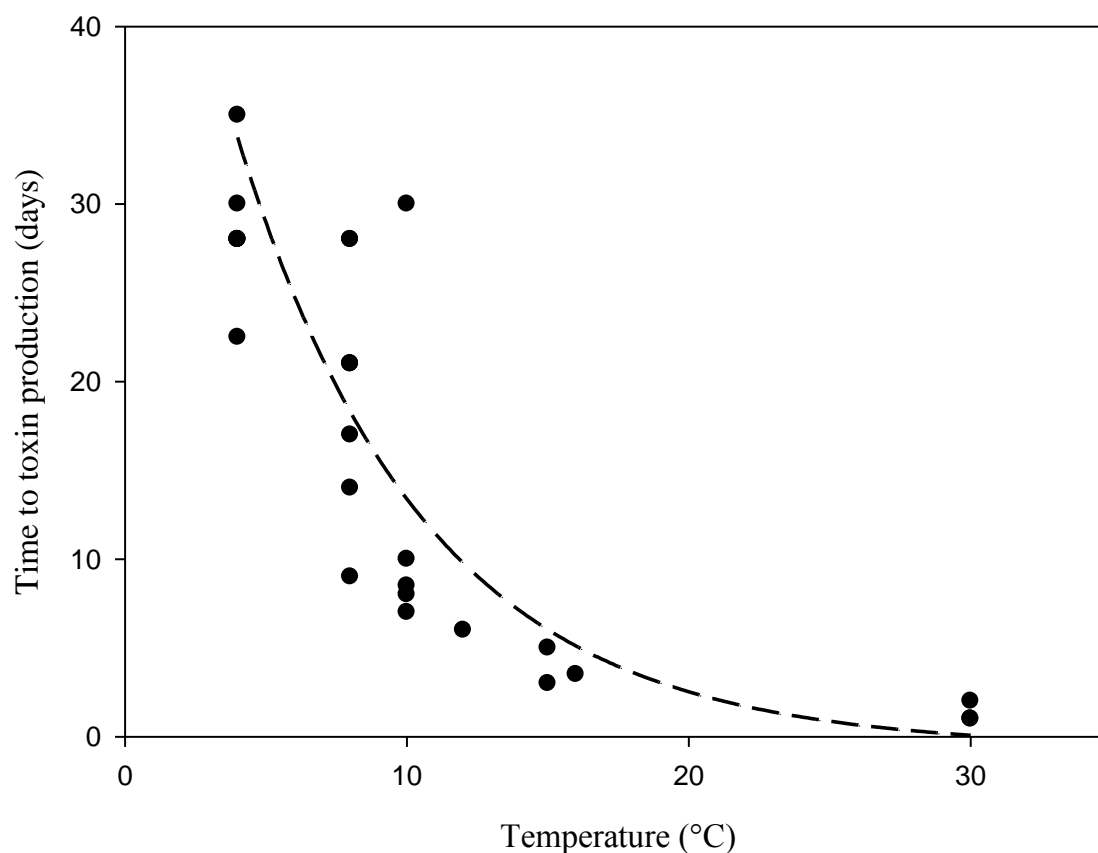
A salt concentration of at least 3% is required to retard toxin production, although this effect is dependent on the initial spore concentration, as demonstrated by experiments with vacuum packed hot smoked salmon stored at 25°C (Pelroy *et al.*, 1982). Vacuum-packaged cold

smoked rainbow trout (3.4% salt, water phase) supported Type E toxin production after four weeks at 4 and 8°C (Hyytiä *et al.*, 1997). In vacuum-packaged hot smoked whitefish stored at 27°C and at 2.3% water phase salt toxin was detected after 7 days, while at 4.4% it was delayed until day 42, and 6.2% salt prevented toxin throughout the entire experiment, up to day 83 (Cuppett *et al.*, 1987).

The inhibitory effect of salt at >3% water phase has also been demonstrated in crustacea (Dalgaard and Jørgensen, 2000). In shrimps with a water phase salt concentration of 3.3% toxin was not produced prior to spoilage at 8, 15 or 25°C, but when the salt concentration was 2.3% toxin was produced at the end of shelf life in product incubated at the highest temperature.

A summary of a seafood challenge study with non-proteolytic *C. botulinum* under MAP conditions demonstrated that as storage temperatures rise, the margin of safety decreases between sensory spoilage and the growth and subsequent neurotoxin formation by *C. botulinum.* The conclusion from this analysis was that storage below 4°C is required to prevent toxin development prior to the product spoiling (Szabo and Gibson, 2003), although one exception to this was noted in fresh cod fillets stored at 4°C under 100% CO_2.

In itself, the smoking process does not present a control for *C. botulinum*, although it may be helpful is association with other controls (multiple hurdles). The times and temperatures involved in hot smoking (60-65°C for up to 15 minutes, see Appendix 1) would be expected to cause only a limited reduction in *C. botulinum* spore numbers (D time at 77°C is 2.4 – 4.1 minutes) (ICMSF, 1996). This process would also reduce other microbial flora, reducing competition. Cold smoking is unlikely to have any effect, unless there is an inhibitory effect of the smoke chemicals. Antimicrobial compounds occur in smoke, but their effectiveness against *C. botulinum* spores is unknown (Sikorski and Kolodziejska, 2002).

Further detailed information is given in Appendix 1.

2.3   Exposure Assessment

2.3.1   Pathogen in the food

No prevalence data for *C. botulinum* are available for smoked seafood produced in New Zealand. However, a report by Crop and Food Research (Fletcher *et al.*, 2008) described the testing of 501 samples of sediment collected from selected harbours and inshore coastal areas around New Zealand. Several regions in the upper North Island were selected, as well as the Marlborough Sounds and Otago Harbour in the South Island. Shallow waters with a muddy seafloor were chosen as the most likely environments to find the organism.   Testing was conducted using mouse bioassays of enriched sediment samples, and PCR tests for the presence of toxin genes Type A, B, E or F.

DNA encoding toxin Type A was detected in one of the samples, but none of the samples tested positive by mouse bioassay after enrichment. The authors suggest that the high salinity of New Zealand waters (>3%) has prevented the establishment of *C. botulinum* type E (see Figure 3).

Salinity and temperature data for seawater around four oyster farms in the upper half of the North Island have been reported (McCoubrey, 1996). Salinity ranged from 1.6–3.6%. A survey by Cawthron (Kirs *et al.*, 2010) of oysters from six sites in Northland over the summer of December-April 2008-2009 also recorded salinity measurements, of which 93.1% were above 3.0%, and the maximum recorded was 3.6%.

Figure 3.   Salinity of Pacific Waters (ppt) at 10 metres.

Source: http://www-pord.ucsd.edu/whp_atlas/pacific/maps/salnty/pac10_salnty.jpg
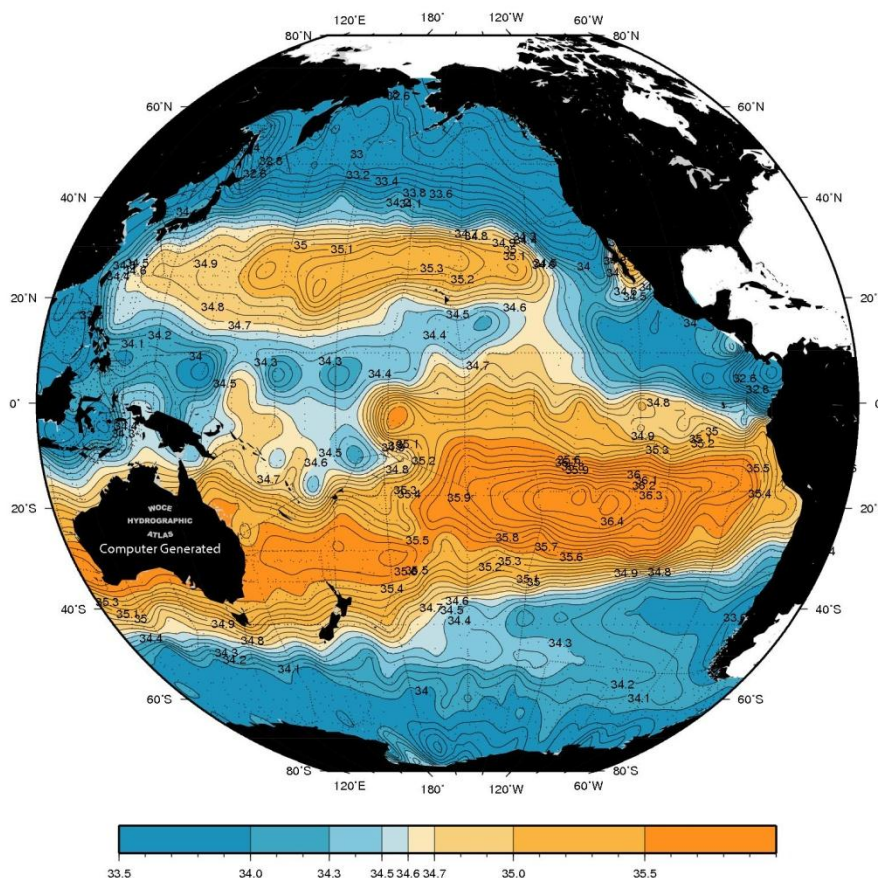
2.3.2   Food consumption

2.3.2.1 Frequency of fish and shellfish consumption

Qualitative Food Frequency Questionnaires (QFFQs) applied as part of the 1997 National Nutrition Survey (adults, 15+ years) (Russell *et al.*, 1999) and the 2002 National Children's

Nutrition Survey (children, 5-15 years) (Ministry of Health, 2003) asked respondents how often they consumed a range of fish and shellfish.

For the child cohort, frequency of consumption information was requested on fish, canned fish, manufactured fish products, and shellfish. Food frequency information was used to estimate that approximately 17% of children consume fish (not including canned or manufactured fish) in any 24 hour period, while 8% are estimated to consume shellfish. However, these estimates may be high as analysis of 24-hour dietary recall records from the same survey revealed that only 15% of respondents reported eating fish in any form during the 24-hour survey period and less than 1% reported consuming crustacea or shellfish. Only three servings reported in the 24-hour dietary recall component of the survey specifically mention smoked fish, representing three different consumers (0.09% of study population). All mention a cooking step in the food descriptor. There were no mentions of smoked shellfish or crustacea.

For the adult cohort, information is more difficult to assess as separate questions were asked about the frequency of consumption of canned fish, battered fish, fried fish, fish consumed raw or prepared by steaming, baking or grilling. Analysis of 24-hour dietary recall records from the same survey revealed approximately 18% of respondents reported eating fish in any form during the 24-hour survey period, 0.9% reported consuming crustacea and 2.4% reported consuming shellfish. Only 30 servings were identified as smoked fish, representing 28 consumers (0.6% of study population). The most common type of smoked fish was smoked salmon (17 servings), followed by smoked fish, type not specified (5 servings), smoked hoki (3 servings) and smoked cod and eel (2 servings each). All servings, with the exception of two servings of lox (smoked salmon) on bagels, include reference to a cooking method in the food descriptor. Two shellfish servings (two different consumers) refer to smoked mussels - 0.04% of study population. No servings of crustacea indicated that the product was smoked.

2.3.2.2 Seafood serving sizes

For children 5-15 years the mean, median and 95th percentile serving sizes are 80, 60 and 210 g respectively for fish, 72, 57 and 148 g for crustacea and 49, 44 and 108 g for shellfish (Cressey *et al.*, 2006). The three servings of smoked fish identified were 22, 44 and 118 g (mean 61 g).

For adults 15+ years the mean, median and 95th percentile serving sizes are 87, 59 and 250 g respectively for fish, 119, 77 and 414 g for crustacea and 92, 64 and 276 g for shellfish (Cressey *et al.*, 2006). The mean serving size for smoked fish servings was 71 g, while the serving sizes identified for smoked mussels were 44 and 122 g.

2.3.3   Evaluation of exposure

2.3.3.1   Number servings and serving sizes

The number of servings of smoked fish and shellfish for the New Zealand population is low, and the serving sizes moderate.

2.3.3.2 *Frequency of contamination*

From the data available it seems unlikely that New Zealand fish used as a raw material are contaminated with *C. botulinum* type B, E or F spores. Insufficient data are available to permit comment on other ingredients which may be used.

2.3.3.3 *Growth rate during storage and most likely storage time*

The organisms concerned could grow given the reported salt concentrations in New Zealand products (2-3.5%) and comparatively long shelf life, particularly if storage temperatures exceed 4°C.

2.3.3.4 *Heat treatment*

Given that these are ready-to-eat foods then no heat treatment of any type may have been applied.

2.3.3.5 *Exposure summary*

Given the lack of cases and the reported lack of spores in 501 sediment samples then exposure is very low/none.

2.4 Overseas context

Surveys of Northern hemisphere marine and freshwater sediments, as summarised in a document from the FDA (Processing Parameters Needed to Control Pathogens in Cold Smoked Fish) have detected *C. botulinum* Type E at prevalences of up to 100% (FDA, 2009). The highest prevalences were found in sediments from Coastal Scandinavia (100%), Lake Washington (91%), Denmark (86%), Lake Michigan (Green Bay) (77%) and the Pacific Coast of the USA (53%).

Two surveys of marine sediments specifically for *C. botulinum* Type E have been conducted in Australia, and are reported in the FSANZ overview ("Primary production and processing standard for seafood. Final Assessment Report Proposal P265"; Food Standards Australia New Zealand, 2005). A survey reported in 1971 failed to isolate *C. botulinum* Type E from 528 samples of soils, marine muds, fish intestines, and potato washings from Tasmania, New South Wales, and Queensland. A later survey reported in 1994 failed to detect the organism in 368 samples from various Australian coastal marine, harbour and estuarine sediments.

Results from overseas surveys of fish and shellfish for *C. botulinum* are given in Table 3 Appendix 1. It is notable that regions where a high prevalence of the organism has been found in sediments also exhibit a high prevalence in fish and shellfish.

3   EVALUATION OF ADVERSE HEALTH EFFECTS

3.1 Disease characteristics

Botulism has five forms: foodborne, infant, adult infectious, wound and inadvertent (WHO, 1999). Food may be a vehicle in the first three forms and the mechanism can be intoxication (ingestion of pre-formed neurotoxin) or toxico-infection (toxin produced during spore germination in the intestine). The latter is associated with infant and adult infectious botulism. Botulism intoxication and toxico-infection are difficult to recognise and relatively rare, so misdiagnosis may occur.

3.1.1   Intoxication

Incubation: Generally 12 to 36 hours, but may be several days.

Symptoms: Range and severity vary. Initial symptoms include nausea, vomiting, and diarrhoea. Neurological symptoms follow, beginning with cranial nerve areas including eye, throat and mouth, and then travelling down the body paralysing motor nerves. Lack of muscle co-ordination, fatigue and respiratory impairment are characteristic. Constipation may develop after onset of neurological symptoms; abdominal pain may be present throughout (Johnson, 2007; Szabo and Gibson, 2003; WHO, 1999).

Condition: Foodborne botulism (confirmed by detection of toxin in patient's serum (Szabo and Gibson, 2003)). Toxins are produced in food by vegetative cells of *C. botulinum*.

Dose: Estimates of type A and B toxins to cause death (in adults) are between 0.1 and 1.0 μg, extrapolated from mouse models (ICMSF, 1996). Toxin types E and F require a dose of approximately 10 μg to cause disease (Bell and Kyriakides, 2000).

At Risk Groups: Everyone.

Long Term Effects: Most cases (up to 80%) require hospitalisation for a 4-5 week period. All botulinal toxins interfere with neurotransmitters, causing a temporary condition which is eventually restored by motor endplate regeneration. Non-fatal effects are not usually long term. Toxin and vegetative cells can be excreted long after recovery (Midura, 1996; Turner *et al.*, 1978). Fatalities are generally caused by respiratory failure and/or obstructed air passages. The mortality rate for foodborne botulism is approximately 10% (ACMSF, 2005).

3.1.2   Toxico-infection

Incubation: 3 to 30 days. Illness can result from a single exposure (WHO, 1999).

Symptoms: First sign is three days or more of constipation followed by lethargy, inability to feed, floppiness and respiratory compromise. Infant botulism is associated with a variety of toxin types and has been reported to account for a variable proportion of sudden infant death

syndrome cases globally[9]. Severity spectrum ranges from asymptomatic (little toxin absorbed) to sudden death.

Condition: Infant and adult infectious botulism are also known as 'intestinal toxaemia botulism' and are confirmed by *C. botulinum* cells or toxins in stools or enema fluids (Szabo and Gibson, 2003). The infant form is also known as floppy baby syndrome. Toxins are produced in the large intestine by germinating spores of *C. botulinum*.

Dose: 10-100 spores in infants (estimate from honey-attributed outbreak) (Arnon *et al.*, 1979; Midura *et al.*, 1979).

At Risk Groups: Adults with radically altered intestinal microflora/major intestinal complications. Infants from 1 week to 12 months old.

Long Term Effects: Infant botulism cases require intensive supportive care. Mortality is approximately 5% (ACMSF, 2005).

Treatment: Infection and toxico-infection are treated similarly by inactivating and removing toxin as early as possible. Drugs cannot reverse the effects of botulinal toxins. Antiserum injections neutralise circulating toxin, and stomach and intestinal contents are cleared. Subsequent treatment is supportive and includes mechanical ventilation to counteract respiratory failure (Johnson, 2007; Szabo and Gibson, 2003; WHO, 1999).

Supplemental information on adverse health effects is given in Appendix 2.

3.2   New Zealand Outbreak Information and Human Health Surveillance

There have been no notifications of human botulism in New Zealand through the surveillance system since records began in 1987 (Esther Lim, ESR, personal communication, 21 February, 2011).

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, a fermented food (Whyte *et al.*, 2001) made from watercress and boiled mussels. The husband of one of the sisters also ate the food but developed no symptoms and another 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 toxin. It appears that the boiling of the mussels rather than steaming may have destroyed inoculating fermentative organisms that may have otherwise rendered the food safe (Hudson *et al.*, 2001). A "very suspicious organism" was cultured later from the food but it 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.

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[9] http://www.foodsafety.govt.nz/elibrary/industry/Risk_Profile_Clostridium-Science_Research.pdf

There have not been any releases of botulinal anti-toxins to treat cases of botulism since the Rotorua cases in 1985 (Aaron McLaughlin, Vaccine Supplies, ESR, personal communication, 21 February, 2011). The antitoxins are owned by the Ministry of Health and administered by ESR. Antitoxins to Types A and B, and to Type E are held at an Auckland hospital.

3.3   Adverse Health Effects Overseas

Botulism caused by non-proteolytic types, when it occurs, is often associated with the consumption of traditional foods containing preserved fish and/or shellfish. For example in Northern Canada and Alaska there have been numerous outbreaks involving home preserved salmon roe, beaver flippers and tails, and whale meat, In Norway the equivalent food is "rakfisk", fish which is canned and allowed to ferment in the can. In Israel and some Islamic countries salted uneviscerated fish is consumed and this has been the cause of outbreaks of botulism; one in Egypt is described as the largest recorded.

However, there are other instances where vacuum-packaged fish or shellfish have been the cause of outbreaks. In general the rates of disease are very low and most of the data are for the number of cases over a period of years rather than an annual incidence. Among these cases, not all are attributable to the non-proteolytic *C. botulinum* types.

Further details are given in Appendix 2.

3.4   Health Burden of Infection with Pathogen

No estimate of the health burden of intoxication with *C. botulinum* in New Zealand has been found (no cases have been reported since 1985). An incidence estimate for the United States has been published (Scallan *et al.*, 2011). This was based on 25 reported cases of foodborne botulism per year, which was scaled up to 55 cases per year to account for underdiagnosis and underreporting.

3.5   Adverse Health Effects Summary

Botulism is a rare but serious intoxication with a high case fatality rate.

4   EVALUATION OF RISK

4.1 Existing Risk Assessments

Standard 4.2.1 of the Australia New Zealand Food Standards Code is the Primary Production and Processing Standard for Seafood which applies only in Australia. As part of the development of this Standard, an assessment report was prepared (Food Standards Australia New Zealand, 2005). In this document, risk assessments from Australia on *C. botulinum* in smoked seafood were discussed. It was concluded that, while foodborne botulism was a severe illness its occurrence was "unlikely". The assessment stated that for ready-to-eat cold smoked fish products *C. botulinum* spores are likely to survive the cold-smoking process, but growth is unlikely except in the case of vacuum or modified atmosphere packed products. Even in the case of such products, the salt concentration (5% or more) is likely to inhibit growth and toxin production. However, this concentration is unlikely to be attained in most RTE smoked seafood products in New Zealand, although it was typically measured in Australian fish in the 1990s (Tom Ross, Pers. Comm.). In other countries similar concentrations have been recorded, for example 13 smoked salmon tested in France but manufactured in a variety of European countries had WPS with a mean of 5.1% and a range from 3.8% to 7.2% (Leroi *et al*. 2001).

For hot smoked products, spores will survive the smoking process, but growth is unlikely except in the reduced oxygen products. Even in the case of such products, salt concentrations (3.5% or more), processing to an internal temperature of greater than 63°C for at least 30 minutes, and proper storage at less than 5°C will inhibit growth and toxin production.

The "medium" relative risk ranking assigned for *C. botulinum* in both these products reflected current risk management including industry inherence to good manufacturing practice, good hygiene practice and appropriate product formulation (e.g. pH, levels of salt, preservatives) (Food Standards Australia New Zealand, 2005). As *C. botulinum* mostly occurs in the gut of fish, the risk was mostly from fish that were not eviscerated before smoking. The hazard identification section also referred to surveys of Australian marine samples which failed to find *C. botulinum* Type E.

The document cites specific risk assessments (Ross and Sanderson, 2000; Sumner, 2001). Ross and Sanderson (2000) considered the risk to consumers from *C. botulinum* in vacuum-packed ready-to-eat fish products was 'relatively low', and would only result from gross temperature abuse. Sumner (2001) concluded that the risk of *C. botulinum* in smoked vacuum-packed fish was "negligible". This was based on the low levels of spores likely to be in products available in the Australian marketplace and the typical salt levels in these products.

For Australia, a semiquantitative seafood safety risk assessment (Sumner and Ross, 2002) assessed a range of hazard/product combinations. The *C. botulinum* score fell into the lowest ranked category and it was reported that no documented cases of foodborne illness had occurred in Australia as a result of *C. botulinum* in vacuum-packaged smoked fish.

The Australian risk assessments cited appear to base their assessments partly on typical salt levels in products available in Australia. Typical salt levels are reported for hot smoked fish

products (3.5% or higher) and ready-to-eat cold smoked fish products (5% or higher) (Tom Ross, Pers. Comm.) Given that salt levels in New Zealand products appear to be much lower (see appendix 7.4.5), then these assessments may not be applicable in this country.

4.2   Estimate of Risk for New Zealand

4.2.1   Risk associated with ready-to-eat smoked fish and shellfish in sealed packaging

Based on the information in this Risk Profile, we consider that the risk from *C. botulinum* in smoked fish in sealed packaging in New Zealand is very low. This is based on the following:

- Illness due to foodborne botulism has not been reported in New Zealand since 1985, and this case involved proteolytic *C. botulinum* Type A;
- A limited survey of marine sediments in New Zealand did not discover any toxigienic extracts, or detect DNA from *C. botulinum* Type E;
- Overseas surveys of the prevalence of *C. botulinum* in sediment and local fish show a correlation at a regional level (for example in the Great Lakes and Pacific Coast of the USA – see Section 2.4 and Table 4 Appendix 1);
- A similar situation to that in New Zealand applies in Australia (absence of reported illnesses beyond infant botulism, and lack of isolations of *C. botulinum* Type E from sediments);
- The single Southern Hemisphere survey of fish for *C. botulinum* (in Indonesia) identified several isolates, but only one was Type E.

Although it has been postulated that at 3% or more (Jillet 1969), New Zealand seawater has too high a salt content for *C. botulinum* Type E (Fletcher *et al.*, 2008), there have been reports of salinity below 3% around the coast. For example, in a survey of oyster farms in Northland, 6.9% of water samples had a salinity of < 3% (Kirs *et al.* 2010). NIWA data collected at Opua wharf show tidal changes in salinity frequently reaching concentrations < 3% because of the effects of the Kawakawa river (Chiswell *et al.,* 2010). In addition, New Zealand salmon are at least partially grown in fresh water, and shellfish may occur in brackish water. Low salinity is considered to be a factor in the ability of the organism to colonise areas such as the Great Lakes and the Baltic Sea.

The processes used by New Zealand manufacturers of smoked fish and shellfish in sealed packaging would not control the germination and growth of *C. botulinum* Type E spores. Salt concentrations are too low, and we believe that oxygen permeable packaging is not being used. The suggested shelf life for New Zealand product of 5 weeks appears to be at the longer end of the range of published shelf life studies. A shelf life of 20 days at 4°C was indicated by a sensory panel (Dondero *et al.*, 2004). When stored at 5°C, sliced cold-smoked salmon was rejected by a taste panel after 21-36 days, with no apparent relationship to the salt content of 4.1-6.1% WPS (Truelstrup *et* al., 1998). Testing of 13 batches of smoked salmon in Europe with a mean 5.1% WPS content measured shelf lives at 5°C ranging from 1 week to > 6 weeks. While the modal shelf life was 5/>5 weeks, seven of the thirteen samples had shelf lives of <5 weeks (Leroi *et al.*, 2001). Other data for European salmon indicate shelf lives ranging from 3-4 weeks to 8.5-9 weeks (Jørgensen *et al.* 2000).

Hot smoking time and temperature conditions will reduce the number of spores but not eliminate them (Appendix 1 section 7.1.3), while cold smoking would not be expected to have a significant effect on numbers. Controls on growth through cold chain management by maintaining a temperature of ≤3°C may not be completely maintained (Peck and Stringer, 2005). There is a lack of data on the effects of smoke itself on *C. botulinum*.

Scientific surveys of spore prevalence in other ingredients considered in this Risk Profile are few and results mixed. Overall, we consider that there are insufficient data on which to base an assessment of this specific issue.

No data on the prevalence of *C. butyricum* and *C. baratii* in fish or shellfish have been located. The minimum temperature allowing growth of *C. butyricum* in food has been reported to be 12°C (Anniballi *et al.*, 2002), considerably higher than that allowing growth of *C. botulinum* Type E.

4.3 Data gaps

The data gaps identified in this Risk Profile are:

- Few data are available concerning the presence of type B, E and F spores in ingredients such as sugar and spices that are used in the manufacture of smoked fish and shellfish products, and none were located specifically for New Zealand.
- Few data are available describing the physicochemical parameters (hurdles such as pH, water activity, water phase salt concentration) affecting potential growth of non-proteolytic *C. botulinum* (or other hazards) in New Zealand smoked fish and shellfish products.
- We were unable to locate data in the literature which systematically assesses the affects of some specific hurdles such as the presence of smoke (as phenol) and naturally occurring lactic acid on the growth kinetics of non-proteolytic *C. botulinum* in smoked fish and shellfish.
- There is no reliable, validated predictive model for this hazard.

5 AVAILABILITY OF CONTROL MEASURES

5.1 Risk Management Strategy

5.1.1 Imported food

Requirements for RTE vacuum packed imported smoked fish are described as an Imported Food Requirement (IFR)[10]. This states that "For smoked vacuum-packed fish products, importers have a responsibility under the Food (Importer General Requirements) Standard 2008 to ensure that imports are not contaminated with *Listeria monocytogenes* or *Clostridium botulinum* Type E. Vacuum packaging provides an anaerobic environment that allows the growth of these bacteria. Good manufacturing practices, particularly during processing, reduce the likelihood of contamination. Addition of salt reduces the likelihood that pathogens will grow."

One of the options given for importers to obtain clearance for product is for sampling and testing on arrival. Criteria to determine whether a sample is safe to be released are:

- Salt content must be greater than 3.4% (aqueous phase basis).
- Aerobic plate count per gram at 35°C where n= 5, c=2, m=50,000 (5 x 10^4), M=500,000 (5 x 10^5)[11].

Imports of smoked vacuum packed fish must also meet any other MAF import requirements for specific fish species.

5.2 Relevant Food Controls

A primary means of controlling growth of non-proteolytic *C. botulinum* is to maintain the temperature of the food at < 3°C. However, the opinion of Peck and Stringer (2005) is that, while this might be possible in some circumstances it is unlikely to be maintained throughout the distribution chain, especially where the consumer is involved in that chain. Their conclusion was that other controls need to be in place. Other relatively simple controls such as pH and salt concentration/a_w may also be used to prevent growth of the organism, either alone or in combination (hurdle technology). They cautioned against the use of oxygen as a preservative factor as the food itself may be sufficiently reduced such that growth can occur even in its presence.

5.3 Options for Risk Management

Should a requirement for risk management be found, options include:

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[10] http://www.foodsafety.govt.nz/elibrary/industry/Imported_Food_Requirements_Fish-Sets_Clearance.pdf

[11] where n= the minimum number sample units which must be examined from a lot of food, c = the maximum allowable number of defective sample units, m = the acceptable microbiological level in a sample unit and M = the level which when exceeded in 1 or more samples would cause the lot to be rejected.

- Continued demonstration of the absence of the organism from NZ growing waters and sediments (marine and freshwater).
- Ensure absence from non-fish ingredients (Appendix 1, section 7.4.5) by GMP.
- Increase time/temperature to achieve 6D kill
- Increase salt concentration to prevent outgrowth (in combination with refrigeration)
- Acidify product to pH <5 with organic acids[12]
- Ensure product achieves an a_w of <0.94
- Package product in oxygen-permeable material (reduces shelf life and may not guarantee safety).

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[12] pH adjustment to pH 5.7 has been achieved in prawns with no adverse affect on quality.

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7 APPENDIX 1: HAZARD AND FOOD

7.1 Pathogen

7.1.1 The organism/toxin

Cells of *C. botulinum* are straight to slightly curved, Gram positive rods. Subterminal heat-resistant endospores are formed 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'.

Seven recognised antigenically distinct neurotoxins, designated as types A, B, C, D, E, F and G can be produced by *C. botulinum*. An additional toxin has been identified, designated C_β, which is an ADP-ribosylating enzyme (Austin, 2001). *C. botulinum* type G has been renamed *C. argentinense*. Most isolates produce one toxin, although some can produce multiple toxins. Four of the types, A, B, E and F, cause botulism in humans although incidents involving Type F toxin are rare, as is Type C botulism. Types C, D and E cause botulism in mammals, birds and fish (World Health Organisation, 2002). The amount of Type A toxin required to cause death in humans varies between 0.1 and 1.0 mg (Szabo and Gibson, 2003).

In another estimate produced by extrapolation from primate studies, the lethal dose for a 70 kg human is estimated to be 0.09 to 0.15 µg delivered intravenously or intramuscularly, 0.70 to 0.90 µg by inhalation, or 70 µg orally (Arnon *et al.*, 2001).

Toxin types E and F require a dose of approximately 10 µg to cause disease (Bell and Kyriakides, 2000).

The toxin type most commonly associated with marine environments (and therefore fish and shellfish) is toxin Type E, and so *C. botulinum* Type E is considered an aquatic organism (Huss, 1980). This toxin type is the primary focus of this risk profile.

Another species of the genus, *C. butyricum*, may also rarely produce toxins (Tsukamoto *et al.*, 2002). Similarities between the toxin produced by two strains of *C. butyricum* and *C. botulinum* neurotoxin Type E were identified and different toxicities were measured for toxin from the two isolates. Ten of eleven isolates tested positive for the Type E gene (Fenicia *et al.*, 2011). However, a survey of *C. butyricum* in English environmental and food samples did not detect isolates containing the toxin gene (Ghoddusi and Sherburn, 2010). The minimum temperature allowing growth of this organism in food has been reported to be 12°C (Anniballi *et al.*, 2002), considerably higher than that allowing growth of *C. botulinum* type E.

Similarly *C. baratii* can produce botulinum toxin type F (Sobel *et al.*, 2009). An unclassified toxigenic isolate, *Clostridium* sp. RKD yielded a PCR product of identical size to that of *C. botulinum* Type F, but a sequence of the amplified product did not match any available in the Basic Local Alignment Sequence Tool database (Dixit *et al.*, 2006).

Groups and types:

The species is sub-divided according to 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 toxin of Types A, B and F, and their proteolytic activity generally causes spoilage of food and offensive odours that render the food unacceptable.

Group II includes isolates producing toxin Types B, E and F. Proteolytic spoilage does not occur so that foods can appear acceptable with no unpleasant accompanying odours.

Illness in animals is usually caused by Group III *C. botulinum* strains. There are no records of disease in humans or animals from Group IV *C. botulinum* (Szabo and Gibson, 2003).

7.1.2   Growth and survival

Growth:

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

	Minimum	Optimum	Maximum
Group I:	10.0°C	35 – 40°C	45 – 50°C
Group II:	3.0°C[13]	25 – 30°C[14]	40 – 45°C

For the proteolytic Group I strains, growth at <10°C has not been reported, and at 15°C it is slow.

Type B spore germination was shown to occur over the temperature range of 1-40°C, but not at 50°C, and the optimum range was 20-25°C (Plowman and Peck, 2002).

At low temperatures, toxin production may take several weeks to occur (Szabo and Gibson, 2003). In the literature, measures of growth are often reported as 'time to detectable toxin' but this does not allow ready comparison between studies as the concentration of the inoculum may differ.

pH: Group I isolates grow slowly down to pH 4.6 whereupon cells generally undergo

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[13] The ICMSF (1996) and Szabo and Gibson (2003) cite 3.3°C as the minimum growth temperature for Group II, but growth and detected toxin production has been reported at 3°C in 5 weeks (Graham *et al*., 1997) . 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 (Peck 2006). Growth at 3.3°C has also been shown for Type B spores, although growth was reported to be slower than for Type E (Eklund *et al*., 1967b) and growth at 3.3°C shown for non-proteolytic Type F (Eklund *et* al., 1967a).

[14] The ICMSF, (1996) cite optimum growth at 28 – 30°C. Austin (2001) cites 18 – 25°C and 25 – 30°C.

sporulation. In some circumstances, germination and growth can take place below an initial pH of 4.6 (Szabo and Gibson, 2003) where, for example, a mould or bacterium raises the pH through metabolic activity. 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).

Spores from Group II grow slowly down to pH 5.0. Spore germination is optimum at pH 5.5-8.0 (Plowman and Peck, 2002). All strains grow and produce toxin down to pH 5.2 (when other conditions are optimal).

Atmosphere: *C. botulinum* is an anaerobe, but it can grow in air if the redox potential (E_h - the overall balance of oxidizing and reducing agents present) is low. For example, a foodborne outbreak of botulism occurred when *C. botulinum* spores were trapped between the skin of baked potatoes and the foil in which they were wrapped (Angulo *et al.*, 1998). It has been suggested that the metabolic activity of high concentrations of spores germinating may alter the E_h to allow growth (Szabo and Gibson, 2003), but Plowman and Peck (2002) reported that germination was similar under aerobic and anaerobic conditions.

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% retarded growth of *C. botulinum*, but toxin production by the Group II organisms in fish (under temperature abuse conditions) still occurred in the presence of 100% carbon dioxide. In broth culture increasing concentrations of CO_2 (from 10 to 70%) resulted in a longer lag phase and generation time (Artin *et al.*, 2008) and approximately twice as much Type E toxin was produced under 70% CO_2 compared to that produced under 10% CO_2. Elsewhere this ratio was reported to be five fold (Lövenklev *et al.*, 2004a). The conclusion of another of another study on the effect of CO_2 of the growth of Types E and B isolates at chill temperatures was that prevention of growth also depended on the concentration of salt and the pH (Gibson *et al.*, 2000). However, the use of a 100% CO_2 atmosphere can have an inhibitory effect under these conditions.

Water activity: The limits for growth of *C. botulinum* are water activity (a_w) values of 0.9353 (NaCl (salt) ≡10%) for Group I and 0.9707 (≡5% salt) for Group II. In contradiction to most of the available information growth has been reported for Type E spores in caviar containing 5% salt (Salmani *et al.*, 2009) but a reference cited as supporting these observations did not find growth in 5% salt (Graham *et al.*, 1997). Interactions with additional factors such as pH can influence the salt concentrations required to permit growth. In cold smoked salmon the hurdle combination of a temperature of ≤5°C in combination with a NaCl concentration of ≥3% is stated to control growth (Huss *et al.*, 1995). Toxins can be produced at a_w values permitting growth.

The effect of salt concentration on non-proteolytic type B spore germination has been described (Webb *et al.*, 2007). The presence of 2% added salt significantly reduced the probability of growth from spores and increased the lag time and variability in the distribution of lag times for individual spores. There was also no correlation between the kinetics of germination and later stages of lag phase under suboptimal conditions. The effect of spore formation occurring in 3% added salt was to increase times to germination and lag phase in subsequent outgrowth in medium with or without added salt. It was concluded that, for the purposes of predictive microbiology, there will be difficulty in predicting outgrowth

kinetics when the prior history is not known. It was also recommended that models need separate consideration of probability of growth and time to growth.

C. botulinum Type E grew and produced toxin in medium containing salt up to 3%, but not 4%, at 30°C after 35 days incubation (Lalitha and Gopakumar, 2007). At 4°C toxicity was achieved after 28 days in the absence of added salt, but required 25 days at 10°C and 6 days at 30°C in the presence of 3% salt. Lowering the pH to 5.5 extended the time required for toxin formation to occur and, in another paper toxin production occurred at pH 5.4 but not at pH 5.2 with incubation at 30°C (Lalitha and Gopakumar, 2005). As the incubation temperature was lowered the pH allowing growth increased such that a pH of 6.5 was required for growth at 10°C, but this increased to 7.0 at 4°C.

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

Toxin production: For non-proteolytic Type B isolates toxin production occurred when bacteria entered the early stationary phase in four of five isolates tested, as measured by *cntB* expression (Lövenklev *et al.*, 2004b). The observation was confirmed by ELISA testing for two isolates. Early stationary phase cultures flushed with air showed no reduction in toxin production, although the viable count reduced, but nitrite at a concentration >15 ppm caused a reduction in toxin gene expression.

For *C. botulinum* Type E, expression was greater (up to 38 fold) at late exponential phase when compared to mid-exponential phase (Sharkey *et al.*, 2005), and gene expression reflected growth rate. Additionally toxin gene expression has been shown at 10°C with gene expression maintained over an eight day period (Chen *et al.*, 2008).

Spore germinants: Spore germination can be influenced by nutrient, non-nutrient, enzymatic and physical stimuli. These germinants activate a germination receptor which triggers the spore to undergo an irreversible reaction progressing towards vegetative growth. With respect to chemical germinants, optimum germination has been demonstrated in the presence of mixtures of amino acids and lactate, namely L-alanine/L-lactate, L-cysteine/L-lactate and L-serine/L-lactate (Plowman and Peck, 2002).

Survival:

Temperature: Reductions in numbers of vegetative cells under frozen storage are poorly understood. All types of spores and toxins are resistant to freezing. Frozen storage does not reduce the activity of preformed botulinum toxin 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).

7.1.3   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.

Spores are more resistant to environmental challenges and control measures than vegetative cells.   Such challenges include freezing, drying, pressure, radiation, ultraviolet light, chemicals, and heat.   Reactivation of spores can be initiated by low pH, a number of chemicals (especially nutrients), and most commonly, sublethal heat (Setlow and Johnson, 1997).

Temperature: At temperatures at or below 3°C, growth of both Group I and Group II *C. botulinum* will not occur. This can be used as a control point for storing products without any additional controlling factors (Betts, 1996).   It is unlikely, however, that such temperatures can be maintained throughout the distribution chain, particularly in products destined for domestic use (Peck and Stringer, 2005). All vegetative cell types of *C. botulinum* are readily killed at pasteurisation temperatures, but destruction of spores requires higher times and temperatures.

Publications 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 (Albert and Mafart, 2005; Mafart *et al.*, 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.   A Monte Carlo simulation and consideration of uncertainty found that, if the uncertainties are similar, then the use of the Weibull model gives a confidence interval as large as, or smaller than, the confidence interval produced form a first-order model (Halder *et al.*, 2007). However, these authors conclude that reducing the uncertainty surrounding input parameters might be useful in itself, and that the cost of moving from a database of first-order estimates to a Weibull model and should be considered objectively.

The 'botulinum cook' was developed for spores of Group I strains. This is a well known food processing procedure for low-acid (pH>4.6) canned foods. The '12D' process, as it is also known, is equivalent to exposure to moist heat at 121°C for 3 minutes. This 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; Peck and Stringer, 2005). $D_{82.2}$ values in phosphate buffer vary between 0.5 and 2.4 min (Peck and Stringer, 2005). In a model meat slurry (pH 6.5) the following time/temperature combinations were required to prevent the outgrowth of a 6 $\log_{10}$ inoculum of spores; 70°C for >2545 min, 75°C for >1793 min, 80°C for >363 min, 85°C for 36 min and 90°C for 10 min. An important caveat to these data is that the presence of lysozyme in foods acts to increase the D time because it assists sub-lethally injured spores to germinate. For example the 90°C for 10 minutes criterion for a 6 D inactivation in the absence of lysozyme has been reported to increase to 65 min at 89.9°C in its presence (Peck and Stringer, 2005). The effect of lysozyme may be less pronounced at

higher temperatures (Reddy *et al.*, 2010) although the data are equivocal (Graham *et al.*, 1996).

Mild processing temperatures used in the production of sous-vide foods have been shown to be insufficient in preventing the outgrowth of nonproteolytic type B spores in ground beef and pork cubes (Lindström *et al.*, 2001). The results also showed growth but not toxin production in one sample thermally processed to achieve a 7.7 D kill (inoculum $\log_{10}$ 5.3 spores, 85°C for 67 min), although the data showed that the process only resulted in a 4.9 D kill. A similar study with a range of sous-vide foods showed that although some of the heat treatments used should have achieved a 6 D kill, only one of the five products was demonstrated to be safe by toxin assay (Hyytia-Trees *et al.*, 2000). The agreement between experimental results and predictions from Pathogen Modeling Program (PMP 7) and Food Micro Model (FMM) was described as "poor" for sous-vide products and a recommendation that, for these products, "models should not be used".

Heating non-proteolytic type B spores at 90°C for 10 minutes was shown not to achieve a 6D kill, although this combination is the "gold standard" for controlling this organism (Stringer and Peck, 1997). This occurred when the recovery medium contained lysozyme.

Combinations of effective heat treatments used as hurdles in combination with other controls have been described in detail by Peck and Stringer (2005).

Samples of picked Dungeness crab (*Cancer magister*) were inoculated with 10^6 or 10^7 (the paper reports two values) spores of a cocktail of three type B isolates, vacuum-packaged and heated at 88.9°C, 90.6°C, 92.2°C and 94.4°C for various periods (Peterson *et al.*, 1997). Data were analysed to give D-values from 12.9 min at 88.9°C to 2.9 min at 94.4°C, and the z-value (15.48°C). This paper states that spores of Type B are more heat resistant than those of Type E, a statement supported elsewhere (Hyytia-Trees *et al.*, 2000).

Experiments using oyster homogenates and spores from five isolates of Type E *C. botulinum* showed variability in the thermal resistance of the spores (Chai and Liang, 1992). D-values (min) obtained ranged from 2 to 8.96 at 73.9°C, 1.28 to 5.28 at 75.0°C, 0.73 to 2.69 at 76.7°C, 0.25 to 1.03 at 79.4°C and 0.08 to 0.43 at 82.2°C. The z-value differed between isolates and by the method used to obtain it with values ranging from 4.2 to 7.1°C. Data for a single Type E isolate in oyster homogenate gave similar results with a D-value of 0.78 at 80°C (Bucknavage *et al.*, 1990). However, biphasic curves were noted and z-values differed markedly depending on the portion of the inactivation curve used. The addition of potassium sorbate and salt appeared to alter D-values but the effect was inconsistent.

The effects of pasteurisation temperature and salt concentration have been studied in surimi (Peterson *et al.*, 2002). Inocula of 10^2 and 10^4 spores g^{-1} were used and heated in the fish to between 80 to 90°C followed by incubation at 10 or 25°C. Spores at the higher concentration survived heating to 80°C for 15 min and were able to produce toxin in the presence of 2.1% salt for Type E and at 2.7% (the highest concentration used) for Type B prior to 120 days of storage at 10°C. Similar results were found when the samples were incubated at 25°C for 15 days. Increasing the temperature of heat processing to 85°C reduced the concentration of salt needed to prevent toxin production; no sample became toxic when incubated at 10°C and only one sample was toxic in the presence of 2.1% salt with incubation at the higher

temperature. When the processing temperature was 90°C no sample became toxic when incubated at 25°C.

Type E spores (10^6) inoculated into the loin muscle of chub (*Leucichthys hoyi*) were not completely destroyed by smoking at an internal temperature of 82.2°C for 30 min (Christiansen *et al.*, 1968). This would not be predicted by the data in Table 2. However, when the brine concentration exceeded 2.75% none of the fish became toxic upon temperature abuse. In this report difference in the moisture level seemed to make little difference to the ability of spores to survive smoking.

A review listing thermal death data for Group II *C. botulinum* in fish and shellfish show a wide range of D-values, D_{80} 1.6-18 min and $D_{82.2}$ 0.08-0.74 min. z-values ranged from 4.2-9.9°C (Silva and Gibbs, 2010). Such wide ranges are not unusual (van Asselt and Zwietering, 2006).

All neurotoxins can be inactivated at time/temperature combinations ranging from 65°C for 1.5 hours to 85°C for 1 minute. The heat resistance of Type E toxin depends on the pH (Gram, 2001) with, for example, the toxin being destroyed after 5 minutes at 60°C in a cooked meat medium of 7.5.

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. Toxins 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 lower temperatures, 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. At higher incubation temperatures salt concentrations <4% did not decrease the proportion of spores capable of outgrowth within 2-3 months at 30°C (Stringer and Peck, 1997). A synergistic effect of thermal treatment and salt concentration was shown

Detailed consideration of the effects of the presence of salt on the growth of *C. botulinum* has recently been made in a review (Taormina, 2010).

Stability in water; In tap water, the toxicity of toxin Types A and B is reduced by 80% at room temperature after 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 growth. Interactions between reduced a_w, pH and temperature are usually used in combination with preservatives to achieve control. A combination of salt concentration (5%) and methyl paraben (0.15%) was shown to inhibit growth at abuse temperature (15°C) better than salt plus 0.3% boric acid and 0.4% borax, or salt on its own in tinned caviar (Salmani *et al.*, 2009).

Nisin added at up to 500 IU g^{-1} was found not to inhibit the growth of non-proteolytic Type B spores minced in beef or pork cubes (Lindström *et al.*, 2001).

Curing salts, particularly sodium nitrite, are used in meat preservation specifically to control *C. botulinum*, and possibly act by making iron unavailable to the pathogen (Grever and Ruiter, 2001). 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). Nitrates and nitrites are not permitted as a preservative in preserved fish by the Australia New Zealand Food Standards Code.

Competitive micro-organisms: The acid produced by lactic acid bacteria such as *Lactobacillus*, *Pediococcus* and *Streptococcus* spp. is inhibitory to the growth of *C. botulinum*, and some of these organisms also produce inhibitory bacteriocins (research cited in Austin, 2001). The ability of strains of different *C. botulinum* toxin types (including Type E) to inhibit one another through the production of bacteriocins and/or bacteriophages has been described (Eklund *et al.*, 2004); the importance of this observation being that when multiple strains are used in challenge studies then their potential interactions should be taken into account.

An inhibitory effect was observed against *C. botulinum* Type E by naturally occurring *Bacillus* spp. in cooked surimi nuggets (Lyver *et al.*, 1998), and isolates of *Paenibacillus polymyxa* and *B. subtilis* have shown activity against *C. botulinum* Types E and B, and *B. cereus* isolates against Type B isolates (Girardin *et al.*, 2002).

Aerobic bacteria may serve to enhance the risk of toxin formation by respiring oxygen (Gram, 2001). However, the same review concludes that relying on the background microbiota is "not an affective or reproducible way of preventing growth" of the pathogen.

Radiation: 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). It was shown that spores surviving irradiation on three species of fish germinated and produced toxin more rapidly then when the spores had not been irradiated (Cann *et al.*, 1965).

Toxins, like all proteinaceous toxins, are not inactivated by the level 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.

Ozone and chlorine dioxide will inactivate spores, as will ethylene oxide, although the mechanism is unknown. 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 when subjected to high pressures. However, under low pressure spore inactivation can be more rapid and complete.   This has led to the suggestion that low pressure could be used to cause the spores to germinate, then further pressure used to inactivate vegetative cells (Patterson, 2005). Throughout the whole pressure range, there is a strong synergy with heat and so a combination of pressure and heat might be an alternative means to reduce spore concentrations. A model for type E spores and the effects of temperature and pressure has been published (Rodriguez *et al.*, 2004).

Human: *C. botulinum* is not a normal part of the healthy human intestinal flora.

Animal: The disease in animals is usually the intoxication form although spores of *C. botulinum* have been found in the intestinal tracts of fishes, birds (Hannett *et al.*, 2011), mammals (Hannett *et al.*, 2011; Myllykoski *et al.*, 2006) and in decomposing carrion. Many predator and scavenger vertebrates feeding from carrion are assumed to have developed immunity by selection. Particularly affected are cattle and birds, and to a lesser extent, horses, sheep, pigs and zoo animals. The main toxins involved in animal cases are types C and D, and to a lesser extent, Types F and G. An outbreak of *C. botulinum* Type C in wild New Zealand waterfowl occurred in 1971. In 1986, the first confirmed case of botulism in a dog in New Zealand (intoxication - type C) occurred in Hamilton (Wallace and McDowell, 1986). Between 1971 and 1991 cases of botulism occurred in waterfowl quite commonly and in dogs occasionally (Gardner, 1992). Toxin type E delivered to the stomachs of live fish was rarely detected in fillets prepared from them, and not at all in fish species caught recreationally in Canada (Yule *et al.*, 2006). However, toxin was shown to accumulate in the non-fillet portions of the fish and the paper cautioned that activities such as filleting could constitute a public health risk if, for example, toxic gut contents spilled on to the edible portion.

Food: Many surveys have identified *C. botulinum* spores in food, especially fish, meats, honey and vegetables/mushrooms.

Environment: *C. botulinum* is found worldwide. Types 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 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 while wetlands soil from Saskatchewan, Canada, yield Type C spores. Despite its ubiquitous nature, the concentration of spores is often low or very low.

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) in the USA (Smith, 1978). The reasons for this are unknown. Type E spores have been detected in sediments from lake Eerie (Hannett *et al.*, 2011) and Type A, B and F organisms in water used for cooling at a cannery (Sachdeva *et al.*, 2010)

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 (World Health Organisation, 2002). Transmission routes for foodborne botulism are predominately through home-preserved foods, particularly traditional native foods.

7.2 *Clostridium botulinum* Detection, Isolation and Typing

The laboratory diagnosis of *C. botulinum* has been the subject of a review (Lindström and Korkeala, 2006).

The organism grows anaerobically and so this poses some technical issues with respect to its detection. Direct plating of food or faeces onto suitable agars rarely yields *C. botulinum* because of the presence of other competitive organisms. Heat treatments (for example 60°C for 10-20 min) to select for spores followed by subculturing from enrichments are the preferred method. Incubation is generally 5 to 10 days at 26 to 35°C. Because of the low concentration normally present most probable number approaches need to be used for enumeration.

Identification of toxin types after enrichment is carried out by mouse bioassay protection tests, which take 48 hours, or ELISA (Enzyme-Linked ImmunoSorbent Assays) assays. Both techniques are detailed in Szabo and Gibson, (2003). A table of detection methods can be found along with type of toxin detectable and associated references in Lindström and Korkeala (2006).

Methods for the detection of toxins in foods have been more recently reviewed (Sharma and Whiting, 2005). Several methods were described including the mouse assay, immunoassays, a chemiluminescent assay (Rivera *et al.*, 2006) and one harnessing the proteolytic nature of the toxins. The review points out the need for rapid methods capable of screening large numbers of samples, and that the currently available rapid methods need proper validation. Subsequently a non-commercially available ELISA was assessed for its ability to detect types A, B, E and F toxins (Sharma *et al.*, 2006) and found to be effective for screening food samples for toxin at 2 ng/ml. However, it was recognised that further validation was required. Another review concluded that "no single assay appears to be capable of replacing the broadly applicable mouse bioassay" (Scarlatos *et al.*, 2005) in the context of detecting pre-formed toxin deliberately added to the food supply as part of a terrorist attack.

Many PCR and genetic probe assays are available (Lindström and Korkeala, 2006). Recently a comparison has been made between a real time PCR assay using the toxin genes and the "gold standard" culture and mouse assay (Fenicia *et al.*, 2011). It was concluded that the method was suitable for rapid typing of toxigenic clostridia (including *C. butyricum* and *C. baratii*) in clinical, food and environmental samples.

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-toxin producing clostridia (Szabo and Gibson, 2003).

Typing methods for Type E isolates include Rapid Amplification of Polymorphic DNA as applied to fish and bird isolates (Hannett *et al.*, 2011) and Pulsed Field Gel Electrophoresis which showed considerable genetic diversity is isolates from Finnish trout farms (Hielm *et al.*, 1998a). In contrast PFGE showed low genetic diversity in German isolates (Hyytiä-Trees *et al.*, 1999). Additional methods include ribotyping, amplified fragment length polymorphism, and repetitive element sequence-based PCR (Lindström and Korkeala, 2006).

The fact that the organism can be a natural inhabitant of unpolluted waters means that testing for faecal indicators is of little use. Samples may be positive for presence of *C. botulinum* yet free of faecal indicators.

7.3   Relevant Characteristics of the Food: RTE Smoked Seafood in Sealed Packaging

Smoking was originally used as a preservation technique, caused by the reduction of water activity in combination with the effect of prior brining or salting. The anti-bacterial qualities in the smoke also served to reduce the number of organisms present (Cutting and Spencer, 1968). With current food preservative technologies, commercial smoking operations are used to impart characteristic organoleptic qualities rather than its original purpose.

7.4   Processing and the Control of *Clostridium botulinum* in Vacuum Packed Seafood

The meat and internal organs of healthy freshly caught fish are virtually sterile, although the skin, gills and alimentary tract can all carry substantial bacterial loads (Sikorski and Kolodziejska, 2002). Fish skin counts may reach 10^5 cfu cm^{-2}, and from 10^3 to 10^9 cfu g^{-1} in gills and intestines. Spoilage is usually microbial although oily fish are susceptible to oxidative rancidity. Obligately anaerobic bacteria are uncommon on the surface of fish but can occur in significant numbers in the intestine (ICMSF, 1998). Because molluscs are commonly associated with inshore environments their microflora can reflect terrestrial influences (ICMSF, 1998).

C. botulinum contamination is most likely to derive from marine sediments, and is usually only present in low concentrations in fish (Table 3). Types E, B and F have been detected in the intestines and occasionally the skin of marine fish.

C. botulinum may also occur in fish farm sediments where the organism may enter the environment via feed and proliferate in any dead fish present (Bell and Kyriakides, 2000). Finfish are not immune from neurotoxin and have themselves died of botulism due to the consumption of other dead finfish; this has been described in farmed juvenile Coho salmon (Eklund *et al.*, 1982).

The evisceration stage is the single most important step to contain *C. botulinum* contamination, but even with the most hygienic methods employed it is not possible to eliminate its presence completely from the raw material (Bell and Kyriakides, 2000).

Annex 2 to the proposed draft standard for smoked fish, smoke-flavoured fish and smoked-dried fish (Codex 2011) provides examples of combinations of product attributes that minimise the likelihood of *C. botulinum* toxin formation. This information is reproduced in Table 1 below. Countries where the fish is to be consumed can be expected to make their

own science-based risk management choices with the aid of this framework. If smoke flavour is imparted by artificial flavour blends then 5% aqueous phase salt is needed to provide complete protection at any temperature between 3°C and 10°C, or 10% aqueous phase salt would be required at any temperature above 10°C. The table does not apply to smoke dried fish with a water activity of 0.75 or below (moisture content < 10%) as growth of all pathogens in inhibited under these conditions such that refrigeration is not required.

The Annex discusses the fact that there are alternative time/temperature parameters that may be used, and that where good enforcement of temperature control and adherence to shelf life is the case the country may use a system that relies on combinations of storage temperatures and shelf lives. Where this is not the case continuous monitoring with, for example, time/temperature integrators on consumer packages can be an adjunct to shelf life monitoring.

Table 1.   Examples of combinations of product attributes that minimise the likelihood of *Clostridium botulinum* toxin formation

Product temperature during storage	Packaging	Aqueous phase salt (NaCl)	Comments
Below 3°C	Any packaging	Not applicable	*C. botulinum* toxin cannot form below 3°C. Temperature monitoring is needed to ensure that the temperature does not exceed 3°C.
>3°C to 5°C	Aerobically packaged	No minimum water activity is needed. Nonetheless, where there is a reasonable possibility of severe time/temperature abuse, the country where the product is being consumed might choose a water phase salt barrier of at least 3% to 3.5% (w/w) as an additional barrier.	When these products are packaged aerobically, 5°C is the maximum recommended storage temperature for the control of pathogens generally and for quality. The aerobic packaging does not necessarily prevent growth and toxin formation of *C. Botulinum*. In air-packaged products, aerobic spoilage organisms provide sensory signs of spoilage before the formation of toxin by *C. botulinum*. In addition in air packaging it is possible for anaerobic micro-environments to exist and toxin may form if the product is subject to severe time/temperature abuse. For that reason, the country where the product is consumed should still require aqueous phase salt as a barrier to growth of non-proteolytic strains of *C. botulinum* if there are concerns about temperature abuse of the product.
Frozen (< or = -18°C)	Any packaging	Not applicable	*C. botulinum* toxin cannot form when product is frozen. In the

>3°C to 5°C	Reduced Oxygen (including vacuum packaging + modified atmosphere packaging)	Aqueous phase salt at minimum level of between 3% & 3.5% (w/w) may be selected by the country where the product is to be consumed.	absence of adequate aqueous phase salt, toxin production can occur after thawing, so labelling information about the need for the consumer to keep the product frozen, to thaw it under refrigeration, and to use it immediately after thawing, is important.
			Aqueous phase salt at minimum level of between 3% and 3.5% (w/w) (aqueous phase salt) in combination with chilling will significantly delay (or prevent) toxin formation. For that reason, the country where the product is consumed may still require the higher aqueous phase salt as a barrier to growth of non-proteolytic strains of *C. botulinum* if there are concerns about temperature abuse of the product.

Peck and Stringer (2005) detail a set of criteria for the UK. Recommended procedures for the control of non-proteolytic *C. botulinum* for in-pack pasteurised products are:

- storage at < 3°C
- storage ≤ 5°C and a shelf-life of ≤ 10 days
- storage 5-10°C and a shelf-life of ≤ 5 days
- storage at chill temperature [15] combined with a heat treatment of 90°C for 10 minutes of equivalent lethality (i.e. 70°C for 1675 min, 75°C for 464 min, 80°C for 129 min, 85°C for 36 min) noting that the European Chilled Food Federation (European Chilled Food Federation, 2006) recommend 80°C for 270 min and 85°C for 52 min.
- storage at chill temperature combined with pH ≤5 throughout the food
- storage at chill temperature combined with a salt concentration of ≥ 3.5% throughout the food
- storage at chill temperature combined with a_w ≤ 0.97 throughout the food
- storage at chill temperature combined with a combination of heat treatment and other preservative factors which can been shown consistently to prevent growth of toxin production by *C. botulinum*

More recently the UK FSA has introduced guidance applicable to vacuum and modified atmosphere packaged chilled foods (Food Standards Agency, 2009). Most of the recommendations shown above are included, but one difference is that products stored between 3 and 8°C should have a maximum 10 day shelf life when other controlling factors are absent. It has been shown that *C. botulinum* can produce toxin in foods stored at 8°C in less than 10 days (Peck *et al.*, 2008) but it was also noted that foods stored correctly have never been associated with an outbreak of botulism. This was attributed to "unquantified controlling factors", possibly comprising raw material quality, damage to spores during heat

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[15] Chill temperature is less than 8°C in the UK

processing, high hygiene during manufacture or a good chill chain.

The potential to use time temperature indicators to reflect conditions where a food may have become toxic has been described (Skinner and Larkin, 1998). As part of this analysis a conservative graph was produced of temperature against time to toxin formation for 1080 datapoints for *C. botulinum* types pathogenic for humans. The equation describing the boundary was:

$$\log LT = 0.65 - 0.0525(T) + 2.74\,(1/T)$$

where *LT* is the lag time for toxin formation (days) and *T* the temperature (°C).

The suggestion of using an indicator of temperature abuse through an aerobic plate count in combination with a test for NaCl concentration has been made in New Zealand in the past as a means of assessing the safety of smoked fish, but the same document also indicated that further consideration of the appropriate concentrations of the plate count needed to be made (Hudson 1997). This was based on data indicating that a WPS of 3.5% in combination with a temperature of <10°C is sufficient to prevent toxin production.

7.4.1   Salting

Salting is used in combination with smoking. As well as providing flavour, the salt in combination with drying achieved during smoking, results in a firmer texture. Traditional products preserved by salting alone have a high salt content (20% or more). A lower salt content requires chilling or freezing to preserve the final product. The main preservative effect of salt is in the reduction of water activity in the product.

7.4.2   Smoking

Cold smoking is carried out in smoking chambers at temperatures between 25 and 30°C for several hours (Bell and Kyriakides, 2000). The fish is not cooked but two distinct processes take place; drying and the penetration of smoke constituents (Cutting and Spencer, 1968). The treatment of cold-smoked products is such that natural spoilage (and therefore competitive) bacteria are not destroyed. Cold smoking is performed under aerobic conditions and so growth of the pathogen will not occur during this processing step (Gram, 2001).

Hot smoking involves placing the seafood in special kilns operating at around 90 – 95°C for several hours during which time the internal temperature of the products reaches 65 – 75°C for approximately 30 minutes. The D values of Group II *C. botulinum* spores are shown in Table 2 (ICMSF, 1996).

Table 2.   D values for *C. botulinum* Group II spores (minutes); effect of temperature on heat resistance

Temperature	D value		
	Type B	**Type E**	**Type F**
77°C	-	2.4 – 4.1	0.2 – 9.5
80°C	-	0.55 – 2.15	-
82.2°C	1.5 – 3.2* (100**)	0.49 – 1.2	0.25 – 42.4

*D value (minutes). Note: D values vary with the medium in which the spores are heated (e.g. buffer or food), the pH and a_w, and with the manner in which spores were produced, and depending on strain.

**10 µg/ml lysozyme in recovery medium

z-values for non proteolytic clostridia were reported as type B 8.6-9.8°C, type E 6.1-8.4°C and type F 9.5-14.8°C (Brown, 2000).

In the field, five commercial hot-smoking processes used by Finnish fish-smoking companies were studied. The fish used were rainbow trout and whitefish media at 75°C to 93°C (Lindström *et al.*, 2003) and lysozyme added during the recovery of the spores. D-values of 255, 98 and 4.2 min were obtained at 75, 85 and 93°C for trout, and 55 and 7.1 min at 81 and 90°C for whitefish. Z-values of 10.4°C and 10.1°C were obtained for trout and whitefish respectively. The reduction in the concentration of non-proteolytic spores calculated from typical Finnish hot smoking processes was less than 3D. The importance of moist heat was demonstrated by enhancing spore inactivation. Current European guidelines recommend a 10^6 pathogen reduction factor (90°C for 10 minutes or equivalent).

Hot smoking will not destroy Group I proteolytic spores; only a botulinum cook equivalent will do this. However, because the minimum temperature for growth of Group I *C. botulinum* is 10°C, the chill chain provides a control. At temperatures above 10°C, the proteolytic nature of the organism would cause spoilage (including pack inflation and blowing). The salt concentration required to inhibit growth of Group I spores (10%) would be unacceptable to the consumer and so refrigeration is an important control step for these spores.

A comprehensive description of the factors influencing the thermal resistance of Group II spores is provided by Lindström *et al*. (2006a) and it is stated that the D- and z-values vary considerably across different types of food, even within fish and shellfish. While this variation may be a result of methodological difference it may also be a result of the presence of lysozyme which assist heat injured spores to germinate. The review emphasises that using average values from the literature to calculate thermal processing times is inappropriate, and that D- and z-values need to be calculated for the specific food that is to undergo the heat treatment. High relative humidity results in greater thermal lability of Group II spores.

Smoke contains antimicrobial compounds including phenols, carboxylic acids and formaldehydes up to 60 mg 100 g^{-1} of tissue (Sikorski and Kolodziejska, 2002).

7.4.3   Sodium nitrite and potassium nitrate

Sodium nitrite has been used in combination with sodium chloride for decades in processed meats as an inhibitor of *C. botulinum.* However its use in fishery products is restricted in many European countries as well as in New Zealand and Australia. The use of sodium nitrite is permitted in the USA. Nitrate alone has little inhibitory effect but where nitrate-reducing bacteria are present, can act as a reservoir for nitrite. Nitrite levels decrease during storage, dependent on temperature and product formulation.

Hyytia *et al.* (1997) have studied the depletion rates for sodium nitrite and potassium nitrate in vacuum-packed cold-smoked rainbow trout over an eight-week period when stored at 4 and 8°C. They also assessed the toxigenesis of non-proteolytic *C. botulinum* over a six-week period at the same temperatures in the presence of each curing salt. The sensorial shelf-lives of products were considerably improved by both curing salts compared to the control using NaCl only. Aerobic plate counts at 4°C were significantly lower in the nitrite and nitrate treated samples then those controls solely using NaCl; the differences at 8°C were smaller. Nitrite depletion was more rapid at 8°C while nitrate depletion was not significantly affected by temperature. Overall the concentrations of nitrite and nitrate used did not completely inhibit toxigenesis of non-proteolytic *C. botulinum* over the six-week storage although numbers of toxic samples were considerably reduced using nitrite and nitrate in the curing process.

7.4.4   Other components of fish

Lactate, which occurs naturally in fish muscle, has been shown to delay growth when present at 2% (Gram, 2001)., but it also has a role as a chemical germinant.

7.4.5   New Zealand production/manufacture

Crop and Food Research have published guidelines for the safe preparation of hot smoked seafood in New Zealand (Fletcher *et al.*, 1998), but the consideration of the thermal processing conditions contained therein is of the thermal inactivation characteristics of *Listeria monocytogenes*, not *C. botulinum*. The thermal processing conditions would not be adequate to achieve a 6D reduction in *C. botulinum* spores. For example a 2 minute 14 second treatment of salmon at 70°C is recommended, but a treatment of 1675 minutes at this temperature is advised in Europe.

The following information was collected and summarised by the New Zealand fishing industry and relates to three specific products; Cold smoked salmon, hot smoked salmon and smoked mussels.

7.4.5.1 Cold Smoked Salmon

1. Water thaw or steam.
2. Fillet salmon
3. Skin salmon

4. Wet or Dry Brine (wet brine time range, 1-5 h, dry brine time 6-8 h)
5. Rack up product
6. Chiller dry (up to 8 h)
7. Cold smoke (approx 15°C, 4-6 h)
8. Prepare (e.g. pinbone, trim fillets, portions, coat with seasonings etc)
9. Vacuum pack whole fillets and portions
10. Freeze or temper cold smoke fillets for slicing to approx -7°C.
11. Slice (if required)
12. Vacuum pack slices
13. Freeze (-18°C) or Chill (0 - + 4°C)
14. Store frozen -18 or chilled +4°C
15. Despatch

Ingredients used

Ingredients vary per processor but include things such as:
Water, salt, sugar, seasonings & herbs, untreated wood-chips

Packaging

Products are vacuumed packed.

Specific processing parameters:

- Water phase salt concentrations
  - Range: 2 – 3.5%. Average of data received 2.3%.

- Water phase nitrate concentrations
  - Nitrate not permitted in smoked fish in NZ

- Water phase lactate/lactic acid concentrations
  - Not used

- Time/temperature parameters used in smoking process
  - Cold Smoked – approx 15°C for 4-6 h
  -
- Any use of oxygen permeable packaging
  - Information not supplied, but vacuum packaging predominates.

- Storage Temperatures and Shelf-life
  - Frozen at -18°C or colder for up to 2 years
  - Chilled at 0-4°C for up to 35 days

7.4.5.2 Hot Smoked Salmon

1. Water thaw or steam.
2. Fillet salmon
3. Wet Brine (wet brine time range, 1-2 h)

4. Rack up product
5. Chiller dry (up to 8 h)
6. Hot smoke (core temp reaching 63°C for 15 min or 64°C for 12 min)
7. Prepare (e.g. pinbone, trim fillets, portions, coat with seasonings etc)
8. Vacuum pack
9. Freeze (-18°C) or Chill (0 - + 4°C)
10. Store frozen -18°C or chilled +4°C
11. Despatch

Ingredients used
  Ingredients vary per processor but include things such as:
  Water, salt, sugar, seasonings & herbs, untreated wood-chips

Packaging
  Products are vacuumed packed.

Specific processing parameters:
- Water phase salt concentrations
  o Range: 2 – 3.5%. Average of data received 2.3%.

- Water phase nitrate concentrations
  o Nitrate not permitted in smoked fish in NZ

- Water phase lactate/lactic acid concentrations
  o Not used

- Time/temperature parameters used in smoking process
  o Hot Smoked – core temp reaching 63°C for 15 min or 64°C for 12 min

- Any use of oxygen permeable packaging
  o Not supplied, but vacuum packaging predominates.
  o

- Storage Temperatures and Shelf-life
  o Frozen at -18°C or colder for up to 2 years
  o Chilled at 0-4°C for up to 35 days

7.4.5.3 Smoked Mussels
1. Thaw if necessary.
2. Remove from containers and wash if necessary
3. Wet Brine (chill overnight, approx 8 h)
4. Rack up product
5. Hot smoke (variety of time/temps used see below)
6. Add flavour/oil (optional)

7. Chill
8. Add sauce (optional)
9. Vacuum pack
10. Freeze (-18°C) or Chill (0 - + 4°C)
11. Store frozen -18°C or chilled +4°C
12. Despatch

Ingredients used

Ingredients vary per processor but include things such as:

Water, salt, acetic acid, sugar, brown sugar, seasonings and herbs, soy sauce, oil, chilli, BBQ sauce, untreated wood-chips

Packaging

Products are vacuum packed.

Specific processing parameters:

- Water phase salt concentrations
  - Range 1-2%, average 1.58%.

- Water phase nitrate concentrations
  - Nitrate not permitted in smoked fish in NZ

- Water phase lactate/lactic acid concentrations
  - Not used

- Time/temperature parameters used in smoking process
  - Hot Smoked, the following ranges are used, core temp reaching
    65 °C for 3 min
    64 °C for 5 min
    63 °C for 8 min
    62 °C for 14 min
    61 °C for 23 min
    60 °C for 60 min

- Any use of oxygen permeable packaging
  - Not supplied, but vacuum packaging predominates.

- Storage Temperatures and Shelf-life
  - Frozen at -18°C or colder for up to 18 months
  - Chilled at 0-4°C for 5 weeks

A small study on New Zealand hot and cold salmon included measurement of water phase salt and a semi-quantitative assessment of smoke as phenol (Nortje *et al.*, 2001). Only nine samples were tested; four were hot smoked and five cold smoked. The water phase salt concentration varied from 0.8% to 2.4 % with most (five) in the 1-2% range. One sample had

a phenol concentration of <10 ppm and the maximum was around 40 ppm. Most (six) were around 10 ppm. In a more recent survey a single smoked tuna sample had a WPS concentration of 4.7%, while one of smoked dogfish was 2.8% (unpublished data). A study of the WPS content of 14 samples of short shelf life fish in Northland produced values between 0.8 and 4.1% (Garnham *et al.* 1996), with a mean of 2.0%. The data are shown below in Figure 3.

A study of the growth of *L. monocytogenes* in smoked salmon was performed by ESR (Wong and Horn 2011) and as part of the work the salt concentration of two batches of fish determined. The water phase salt concentrations were 1.9 and 2.3%.

7.4.6   Behaviour of pathogen on/in food

In challenge studies reported in the literature authentic isolates of toxigenic *C. botulinum* are used. However, in an industrial setting such challenge testing is impractical. To address this, it has been proposed that non-toxigenic variants (surrogates) could be constructed which do not produce toxin, yet behave in an otherwise identical manner to the parent strain (Bradshaw *et al.*, 2010). While the use of *C. sporogenes* has been adopted as a surrogate for Group I *C. botulinum* an analogous surrogate for Group II types is not apparent.

Only a few of the following studies were focused on smoked foods.

Figure 4. Cumulative WPS composition of smoked fish from Northland
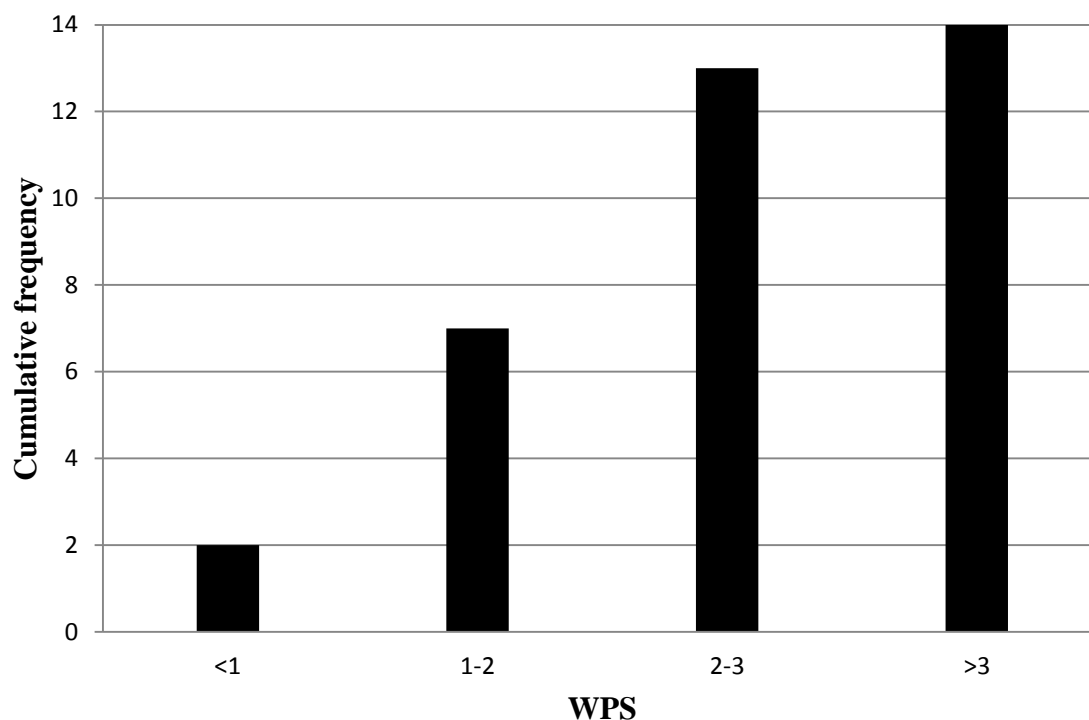
7.4.6.1 Fish

Kipper, herring and haddock became toxic after only a few days when inoculated with a "massive dose" of Type E spores, vacuum-packaged and incubated at 20°C, while considerably longer was needed at 10°C (Cann *et al.*, 1965). At 5°C, herring did not become toxic after 36 days when the inoculum was 10 spores per pack, but toxin was produced within the experimental period at all inocula exceeding this.

An examination of packaging films with different oxygen permeabilities has been conducted with Summer flounder (*Paralichthys dentatus*) fillets. Individual fillets were inoculated with a cocktail of nonproteolytic type B, E and F spores, packaged in film with an oxygen transmission rate (OTR) of 3,000 cm^3 m^{-2}/24 h at 22.8°C, or were vacuum packaged or packaged under 100% CO_2 with a film of OTR 7.8 cm^3 m^{-2} 24 h^{-1} at 21.1°C and stored at 4 and 10°C (Arritt et al., 2007). Samples which were tested for botulinum toxin by the mouse assay were also subject to an aerobic plate count to assess spoilage (a concentration of 7.0 log_{10} CFU g^{-1}) and assessment by a sensory panel for spoilage odours and appearance. For fillets incubated at 4°C packaged under the high OTR film no toxin was produced when the fillets were spoiled (15 days) or even after 35 days storage. At the higher temperature toxin was detected at day 8 but this followed spoilage (day 5). Fillets which were either vacuum or 100% CO_2 packaged in film with the low OTR became toxic at 4°C before spoilage occurred. At 10°C spoilage and toxin production occurred simultaneously, and under 100% CO_2 toxin production occurred before spoilage. It was concluded that the high OTR film could be used to ensure the safety of fish fillets held under refrigeration.

Jack Mackerel inoculated with a cocktail of spores at 20 CFU g^{-1} from 4 Type E isolates, packaged under 100% N_2 in film with an OTR of 8.7 cm^3 m^{-2} 24 h^{-1} and stored at 10°C became toxic by the mouse assay after 7 days of storage (Kimura *et al.*, 2001). The same work demonstrated that Q-PCR could detect an increase in toxin gene prior to the toxin being produced.

Sterile Mullet (*Mugil cephalus*) inoculated with a large concentration ($>10^4$ g^{-1}) of a cocktail of spores from Types A-E was vacuum packaged (OTR not specified) and stored at temperatures ranging from 4 to 30°C (Laltiha and Gopakumar 2001). At 4 and 10°C Type E toxin was detected (inoculum 10^2 spores g^{-1}), after 8 days at 10°C and 28 days at 4°C.

Shelf life and the potential for toxin production by *C. botulinum* Type E in retail type packages of fresh aquacultured salmon fillets stored under a variety of atmospheres, packaging and temperatures were investigated in a study by the US FDA (Reddy *et al.*, 1997). Samples were inoculated with 100 Type E spores per g. At each of the storage temperatures (4, 8 and 16°C) toxin was detected about the same time, or after, the sample was declared spoiled. At 4°C, under 100% air, vacuum and modified atmosphere packaging, toxin was not detected until after the product was considered to be spoiled. However, at 8 and 16°C sensory spoilage occurred at around the same time as the product was considered to be spoiled; no atmosphere performed better that the others. It was concluded that "storage temperature control at 4°C or below is necessary to prevent development of *C. botulinum* type E growth and toxin formation in retail MA -packaged salmon fillets".

Similar work had previously been reported on raw salmon using a cocktail of Types B, E and F spores at various concentrations and conditions of atmosphere, time and temperature (Garcia *et al.*, 1987). Again, no toxin was detected in fillets stored at 4°C for 60 days, but was detected prior to spoilage at both 8 and 12°C. In this case only toxin type B was detected in the fillets. In hot smoked salmon a salt concentration of 3.8% water phase was required to prevent toxin production by 10^2 spores g^{-1} when packaged in oxygen impermeable film and incubated at 25°C (Pelroy *et al.*, 1982), but higher inocula required a greater salt concentration. Processing of the fish to 62.8°C-76.7°C for 30 minutes did not influence toxin production.

Uninoculated Chilean smoked salmon (2.01% salt, whether water phase not specified) was vacuum-packaged (OTR not specified) and stored at temperatures from 0-8°C for 16-46 days with no *C. botulinum* growth detected during storage (Dondero *et al.* 2004). Shelf lives determined by sensory analysis were 26 days at 0°C, 21 days at 2°C, 20 days at 4°C, 10 days at 6°C and 7 days at 8°C.

Vacuum-packaged cold smoked rainbow trout (3.4% salt, water phase) supported type E toxin production when inoculated at 140 and 4140 cfu kg^{-1} after around four weeks at 4 and 8°C (Hyytiä *et al.*, 1997). At higher inocula fish was toxic at 8°C after four weeks incubation, but not when the temperature was 4°C, although the fish was spoiled. Most samples were toxic when incubation was extended to six weeks. Rainbow trout fillets were subject to different treatments and inoculated with a low concentration of type E spores prior to vacuum packaging and incubation (Hyytiä *et al.*, 1999). At 8°C there was a lag time of approximately one week, followed by growth in unprocessed fillets, with toxin production starting after two weeks. At 6°C there was no growth or toxin production in pickled fillets after six weeks incubation, but toxin was detected in the absence of growth after 26 weeks incubation. In cold smoked fillets toxin was detected after three weeks at 8°C and four weeks at 4°C, again in the absence of measured growth.

Combinations of salt and nitrite concentration have been examined for their combined effects in preventing the Type E toxin production when inoculated at 3×10^3 spores g^{-1} in vacuum-packaged hot smoked whitefish stored at 27°C (Cuppett *et al.*, 1987). At 2.3% water phase salt toxin was detected after 7 days, at 4.4% it was delayed until day 42 and 6.2% salt prevented toxin throughout the entire experiment, up to day 83. The addition of nitrite at 57.5 mg kg^{-1} (mean value at day 0) delayed production only in samples containing 4.4% salt by around 20 days.

The effect of headspace gas composition and packaging film OTR has been studied with respect to the growth of *C. botulinum* Type E on MAP fresh trout fillets (Dufresne *et al.*, 2000). The inoculum was applied at 10^2 spores g^{-1} with storage at 12°C. In all cases the fish became toxic within five days, but spoilage preceded toxigenesis. Packaging in film with high OTR (4,000-10,000 cm^3 m^{-2} 24 h^{-1} at 24°C) made no difference. The failure of the exposure to air to inhibit growth was attributed to the growth of spoilage flora consuming oxygen (presumably at a rate faster that diffusion through the film) and producing carbon dioxide. This is less likely to occur in fish that has been hot smoked as the competing microflora will have been reduced during thermal processing.

An extensive seafood challenge study with non-proteolytic *C. botulinum* in MAP has been carried out (Szabo and Gibson, 2003). In summary, all the studies except one demonstrated that as storage temperatures rise, the margin of safety decreases between sensory spoilage and the growth and subsequent neurotoxin formation by *C. botulinum.* The exception was fresh cod fillets which became toxic before sensory rejection, even though the product was stored at 4°C in 100% carbon dioxide. The conclusion from these studies is that storage below 4°C is required to prevent toxin development prior to the product spoiling (Szabo and Gibson, 2003).

Crab meat analog (surimi) is made from whitefish with other ingredients (Peterson *et al.*, 2002). Growth of an inoculum of 10^2 and 10^4 type E spores g^{-1} in pasteurised surimi was followed at 10°C in oxygen permeable (OTR 7,195 cm^3 m^{-2} 24 h^{-1}) and impermeable packaging (OTR not stated). In all cases where toxin was formed the product had spoiled, but the only combination of conditions under which toxin production did not occur was the use of 3% salt with the lower spore concentration. In this case both packaging films gave the same results.

7.4.6.2   Shellfish and crustacea

Sterile shrimp (*Penaeus indicus*) was inoculated with a large concentration ($>10^4$ spores g^{-1}) of a cocktail of spores from Types A-E was vacuum packaged (OTR not specified) and stored at temperatures ranging from 4 to 30°C (Lalitha and Gopakumar, 2001). At 4 and 10°C only Type E toxin was detected (inoculum 10^2 spores g^{-1}), after 10 days at 10°C and 35 days at 4°C. In other work, MAP packaged shrimps (*Penaeus borealis*) were inoculated with 100 spores g^{-1} of a cocktail of Types A, B and E isolates into two batches with different characteristics (Dalgaard and Jørgensen, 2000). In shrimps with a water phase salt concentration of 3.3% toxin was not produced prior to spoilage at 8, 15 or 25°C, but when the salt concentration was 2.3% toxin was produced at the end of shelf life in prawns incubated at the highest temperature.

A study of processed crawfish did not find this bacterium in uninoculated samples (Lyon and Reddmann, 2000). Cooked crawfish were inoculated with 10^3 *C. botulinum* type E spores per g, vacuum packaged in both a high barrier film (OTR 3-6 cm^3 m^{-2} 24 h^{-1} at 4.4°C) and an oxygen-permeable bag (OTR not specified), and stored at 4°C and 10°C for 30 days. *C. botulinum* Type E toxin was not detected in any of the inoculated samples until day 30, when the vacuum packaged samples, but not the samples packaged in stored in the oxygen-permeable bags, at both 4°C and 10°C were toxic. However, the samples under both sets of packaging conditions were considered to be spoiled at day 14.

Inoculated cooked crab meat did not become toxic when incubated in an oxygen impermeable film at 12°C even after 20 days and an inoculum of 10^4 Type E spores 50 g^{-1} (Eklund *et al.*, 2005), while spoilage occurred after 3-6 days. At 25°C the product was spoiled after 24 h incubation while toxin was detected only after 6-13 days at the highest inoculum (10^4 spores). When oxygen permeable packaging was used cooked crab meat again became toxic after the product had spoiled.

Toxin production was shown with a "massive" inoculum of Type E spores on vacuum-packaged scallops at 20°C (Cann *et al.*, 1965).

7.4.7 Effects of other ingredients on *C. botulinum*

C. botulinum Type E has been shown to be sensitive to the presence of the following oils: angelica (one of two preparations), cumin, dill, Japanese mint, lovage, oregano, peppermint, savoury, southernwood, sweet cicely and thyme (Nevas *et al.*, 2004). While the odour and taste of these compounds restricts their use at concentrations inhibitory to bacteria they could be used as part of a hurdle technology approach.

C. botulinum Type B spores and vegetative cells (proteolytic status not stated) have been shown to be inhibited by crude green tea catechins and sub-components at concentrations equal to or greater than 250 μg ml^{-1} in the case of spores and 500 μg ml^{-1} for vegetative cells (Hara-Kudo *et al.*, 2005). Catechins are found in many plant species.

More information is available for dairy products. Carbohydrate-based fat replacer, enzyme-modified blue cheese, sweet whey, modified whey protein and whey protein concentrate did not inhibit the growth and toxin production by types A and C. The addition of soy-based flavor enhancer, enzyme-modified parmesan cheese or enzyme-modified cheddar cheese resulted in the inhibition of toxin production (Glass and Johnson, 2004).

7.4.8 Prevalence of pathogen on/in food in New Zealand

No survey data are available.

7.4.9 Prevalence of pathogen on/in food overseas

Data on the prevalence of *C. botulinum* in seafood overseas have been summarised in Table 3.

Table 3.   Summary of *C. botulinum* prevalence and toxin types in overseas fish and shellfish

Origin	Product	Sample size (g)	% positive samples	MPN per kg	Type(s) identified
Britain (Cann *et al.*, 1966)	Vacuum packaged fish at retail	-	0.8	-	Type E targetted
	Fish from foreign trawlers landed in Britain	-	4.6	Of 6 fishes tested; 62.5 and 5	
Britain (Cann *et al.*, 1975)	Whole fish	Whole fish or viscera incubated	9.4	-	Types B,C,E,F
	Fish intestines		11.1	-	
Caspian Sea	Salted carp	2	63	490	E[1]
	Smoked fish	-	0	<68 *(sic)*	E[2]

Origin	Product	Sample size (g)	% positive samples	MPN per kg	Type(s) identified
Denmark	Smoked salmon	20	2	<1	B[1]
England England North Sea Norwegian Sea	Vacuum packed fish	- - - -	0 <1 0 44	- - - -	-[2] E - E
Europe (France, UK, Portugal, Greece) (Davies et al, 2001)	44 fresh fish flesh and skin	25	0	-	-
Finland (Hielm *et al.*, 1998a)	Farm type 1 Farm type 2 Marine farm	Trout viscera	19 6 14	20.9 15.8 19.5	E
Finland (Ala-Huikku *et al.*, 1977)	Trout intestines; Farm 1 Farm 2	-	10 4	-	E
Finland (PCR) (Hyytiä *et al.*, 1998)	438 Raw fish samples (intestines, surface and whole) 208 fish roe 214 vacuum-packaged retail product 123 air-packed retail product	- - - -	19 overall, 10 – 40 dependent on fish species 8 5 3	- 30-290 30-60	Type E primers used
Finland (PCR) (Merivirta *et al.*, 2006)	67 Fresh water lamprey	20 x 1g	1.5	100	E
France (Carlin *et al.*, 2004)	102 fish and shellfish REPFED ingredients	25 or 50	7.8 (PCR-ELISA)	2-3	A, B
Germany (Bavaria) (Hyytiä-Trees *et al.*, 1999)	Rainbow trout Chub	Viscera Surface Viscera Surface	4 0 50 94	45-85 - 45-1060 45-1060	E

Origin	Product	Sample size (g)	% positive samples	MPN per kg	Type(s) identified
	Whitefish	Viscera	0	-	
		Surface	0	-	
	Brown trout	Viscera	0	-	
		Surface	4	45-320	
	Bream	Viscera	75	45	
		Surface	100	45-1060	
	Brook trout	Viscera	0	0	
		Surface	33	45	
	Arctic char	Viscera	0	0	
		Surface	50	100	
"Global" (Peck *et al*., 2010)	Shrimps/prawns Scallops	200	0 61	- 26 15-20, 1 (type F) 444.	B,F
Iran (Tavakoli *et al*., 2009)	Salted fish	5-10	3.1	-	E, B, A
Iran (Jelodar and Safari, 2006)	Sturgeon caviar	-	0	-	-
India (Lalitha and Gopakumar, 2000)	Wild and farmed shellfish Fish	3-5 3-5	22 22	0-700 0-700 (intestinal content)	C,D,A A,B,C,D
India (Lalitha and Surendran, 2002)	Fresh fish (67) Cured fish (278)	3-5 3-5	19 13	- -	A,B,C,D C,D
Indonesia	Fish	-	3	6	A,B,C,D,F[2]
Indonesia	Freshwater fish Saltwater fish	10g plus viscera	13.5 16.7	-	A,B,C,D,E,F
Iran (Rouhbakhsh-Khaleghdoust, 1975)	Various fish species	6	17.7	-	B, E
Iran	Salted fish	5-10	6.4	-	E (2), B (1), A(1)
Japan, Osaka	Fish and seafood	30	8	3	C,D[1]
Japan (Fujisawa *et al*., 2000)	Bottled seafood products	10	0	-	-
Pacific (NW)	Smoked fish (28 processors)	-	5	9	E
Scandinavian and Atlantic waters	Herring Mackerel/salmon Cod/plaice/eel	Stomach and guts	1-4 0-57 0-43	-	E

Origin	Product	Sample size (g)	% positive samples	MPN per kg	Type(s) identified
(Huss and Pedersen, 1979)	Shellfish Roach Freshwater eel Bream Perch		7-24 0 0 0 0		
Scotland (Burns and Williams, 1975)	Rainbow trout	Whole fish	1.4	-	Non-proteolytic type B
Thailand (Tanasugran, 1979)	Fish	Pooled viscera	0.7	-	D,E
USA, Great Lakes	Eviscerated whitefish chubs	10	12	14	E,C [1]
USA, Great Lakes	Lake fish Superior Erie Huron Michigan Green Bay (Lake Michigan)	viscera	 1 1 4 9 57	-	E
USA (Pace *et al.*, 1967)	Freshly smoked whitefish chubs	10	1.2	-	B,E
USA, New York state	Lake fish	Whole fish	6.3	-	E
USA	Vacuum or plastic packaged	Unshucked oysters Smoked salmon Barbecued cod Smoked herring Kippers Anchovy paste	25 0 0 0 0 0	-	E
USA (domestic and imported) (Heinitz *et al.*, 1998)	201 vacuum-packaged	50-100	0	-	-
USA, Alaska (Houghtby and Kaysner, 1969)	Salmon	Gills Viscera	2.2 0.2	- -	E
USA, Alaska	Salmon	1: heads,	4.9	-	E

Origin	Product	Sample size (g)	% positive samples	MPN per kg	Type(s) identified
(Miller, 1975)		viscera, flesh and roe			
USA Atlantic Ocean	Vacuum-packed frozen flounder	1.5	10	70	E[1]
	Haddock fillets	-	24	170	E[2]
USA	Uncooked: Gills	50	27.8	-	E
	Shell		44.4	-	E
	Cooked: Gills		0	-	
	Shell		0	-	
	Intestines		0	-	
	Picked crab meat		0	-	
USA, California	Dressed rockfish	10	100	2400	A,E[1]
USA, Alaska Alaska Oregon Washington	Salmon	24-36 - - -	100 1 6 8	190 - - -	A[1] E[2] E[2] E[2]
USA, Oregon and Washington (Craig *et al.*, 1968)	Salmon Trout Sole and cod Sturgeon Sole Cod Grouper Perch Clams Oysters Crabs	Gills and viscera 6 individuals -	13.0 23 16.8 11 12.3 22.9 11 0 29.6 37.5 68.2	- - - - - - - - - - -	A,E,F E,B A,B,E E A,E B,E - - A,B,E E B,E
USA, Pacific Northwest (Eklund and Poysky, 1967)	Crabs	-	12-87, depending on location	-	A,B,C and E
USA, Viking Bank	Vacuum-packed fish	-	42	63	E[1]
USA (Paranjpye *et al.*, 2003)	Pollock mince Pollock surimi Uneviscerated pollock	80-100 80-100 Whole fish	0 0.3 0		A, E
USA (Baker *et al.*, 1990)	Variety of fish and shellfish	70	21.7	90-2400 when detected	A, B, E, F

Origin	Product	Sample size (g)	% positive samples	MPN per kg	Type(s) identified
	Fish	100	66.7	-	A, B, E, F

(Austin, 2001[1]: (Dodds, 1993)[2], Hyytiä *et al.,* 1998[3])

A high prevalence of *C. botulinum* spores in Scandinavian/Baltic Sea sediments (Hielm *et al.*, 1998b; Johannsen, 1963) appears to be reflected in the various fish species caught in these regions (Lindstrom *et al.*, 2006a). For example Finnish data show contamination levels of vacuum-packed smoked fishery products were as follows;

	Positive samples	**MPN kg^{-1} (range)**
Cold-smoked rainbow trout	2/64 (3%)	160 (40-290)
Hot-smoked rainbow trout or salmon	2/50 (4%)	30 (30-40)
Hot-smoked whitefish	5/50 (10%)	40 (30-60)

The authors concluded that Type E posed a serious health risk for those consuming fishery products from the Baltic Sea region (Hyytiä *et al.,* 1998). The high level association between the presence of *C. botulinum* in the environment and its epidemiology has been noted (Hauschild, 1993).

In France, a survey was conducted for the presence of *C. botulinum* Types A, B and E in raw food materials destined for refrigerated processed foods of extended durability (REPFEDs) (Carlin *et al.*, 2004). 102 samples of fish and shellfish were collected, PCR testing for botulinal toxin genes found 8 were positive; (3 x A, 2 x B, 3 x A and B). The concentration of spores was 2 – 3 kg^{-1}. There were no samples positive for Type E.

Szabo and Gibson (2003) concluded that Australian vacuum packaged seafood and *C. botulinum* had not been studied extensively and there was little public information available on the occurrence of *C. botulinum* in Australian waters, seafood or in seafood processing environments.

7.4.10 Presence of *C. botulinum* on other ingredients

Some seafood products will contain other ingredients which could serve to introduce spores onto the product. For example some products contain added sugar, salt, pepper or other herbs and spices. However, little information could be located on these other ingredients when considered as likely to occur in fish and shellfish products.

None of a survey of 65 "spices, herbs, and dehydrated mushroom" samples was positive for *C. botulinum* by PCR-ELISA, in a survey of raw materials used for processed foods in France (Carlin *et al*., 2004). Similarly, as part of a survey of ingredients in pasta based meals in the UK, Peck *et al* (2010) tested three chive samples and were unable to detect *C. botulinum* Types B, E or F. However 4 of 100 medicinal herb samples tested in Argentina yielded Type A isolates (Satorres *et al.*, 2000).

Two papers from Japan and one from Costa Rica report on the presence of *C. botulinum* in samples associated with sugar. Of 290 soil samples from sugar cane fields four (1.4%)

yielded *C. botulinum* Type E (Kobayashi *et al.*, 1992). While 9.4% of 53 muscovado (a type of unrefined brown sugar) samples yielded *C. botulinum* type C, none showed the presence of type A, B or E isolates. Type A spores were detected in sugar products used for apiculture, raw sugar, molasses and brown sugar lumps, but not in refined sugar (Nakano *et al.*, 1992). In Cost Rica *C. botulinum* was not isolated from 50 samples of unrefined crystallised cane sugar (Pujo *et al.*, 2008).

7.4.11 Predictive Modelling

Because the concentration of spores in a food is likely to be low there has been interest in the kinetics of the germination and outgrowth of individual spores. Phase contrast microscopy and time to turbidity data showed that there is considerable variability in the times taken for the various stages in non-proteolytic Type B spore outgrowth to occur and that the length of time spent at one stage is not predictive of the others (Stringer *et al.*, 2005). It was concluded that spore germination kinetics cannot be used to predict time to growth or time to toxin production. Heat treatment (80°C for 20 s) reduced the proportion of spores able to grow, extended the lag time and increased the lag variability (Stringer *et al.*, 2009). A later review (Stringer *et al.*, 2010) confirmed the heterogeneous nature of spore outgrowth and also noted that times for germination, outgrowth and first doubling time depend on the history of the spore and prevailing growth conditions. It is important to understand the variability associated with various treatments if the probability of growth from single spores is to be quantified.

Two models are available as web resources that predict the growth of *C. botulinum*; Pathogen Modeling Program v 7.0 (http://ars.usda.gov/Services/docs.htm?docid=11550) which includes a specific time to toxin model for fish, and ComBase (http://www.combase.cc/). Peck *et al.* (2008) comment that ComBase was designed to be the most robust at temperatures <10°C as this is where most of the data were generated. They also state that the models were designed to be worst case and that there is a question as to how well they predict toxin production under commercial conditions.

A polynomial model describing the growth of non-proteolytic *C. botulinum* spores with varying concentrations of CO_2 as influenced by temperature (5-12°C), pH (5.5 and 6.5) and NaCl (0.5 to 2.5%) has been published (Fernandez *et al.*, 2001). A CO_2 concentration of 5% enhanced growth, 50% showed some inhibition and 90% exerted a considerable inhibitory effect. Validation was by comparing predicted and measured values to themselves and to published data.

Another model describes the effects of pH (4.8-7.0), temperature (6-30°C) and sorbic acid concentration (0-2270 mg/l) on the ability of a single non-proteolytic Type B spore to germinate (Lund *et al.*, 1990).

Challenge tests with a variety of seafood products showed PMP 7.0 to be inaccurate and it was suggested that the models needed further development and rigorous validation (Hyytiä *et al.*, 1999).

Models have been produced to describe the effect of heating on the likelihood of spore outgrowth. A predictive model describes the time for growth to occur in a model meat system

containing lysozyme as a function of temperature (70-90°C), duration of heating (0-2,545 min) and incubation temperature (5-25°C) (Fernandez and Peck, 1999). The inoculum was 10^6 spores of Types B, E and F. The model was validated against previous data and produced values "similar to the observations of growth". It was suggested that models produced in the presence of lysozyme should be used to predict growth in foods as the predictions would be fail safe. A similar model had previously been produced in the absence of lysozyme. This model describes the time for growth to occur in a model meat system as a function of temperature (70-80°C), duration of heating (0-2,545 min) and incubation temperature (5-25°C).

8   APPENDIX 2: EVALUATION OF ADVERSE HEALTH EFFECTS

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

- **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,
- **Infant**; ingested spores that survive the acidity of the stomach and germinate, colonise and produce neurotoxin *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 microflora, e.g. conditions such as Crohn's disease. Similar disease mechanism to infants, ingestion of spores and colonisation of the intestines producing toxaemia,
- **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 only foodborne botulism.

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).

8.1   Symptoms

Incubation: The disease is one of ingestion of pre-formed neurotoxin, and the time period from ingestion of the toxin and a response which can range from a few hours to several days. Typically symptom onset is between one and eight days (Horowitz, 2010), but patients suffering from type E intoxication have the shortest incubation periods, which can be less than one day (Woodruff *et al.*, 1992).

Symptoms: Initially: nausea and vomiting within a few hours of consumption (Horowitz, 2010). Type E toxin may also produce diarrhoea. Subsequent symptoms which commonly lead to hospitalisation are fatigue, muscle weakness and difficulty swallowing (Cengiz *et al.*, 2006). Nausea and vomiting occur more often in Type B and E intoxication rather than in Type A, whereas muscle weakness is not often associated with type E (Austin, 2001). The early onset of nausea and vomiting in Type E cases is associated with subsequent lower mortality (ICMSF, 1996) or less severe illness that those with Type A intoxication (Woodruff *et al.*, 1992).   Neurological symptoms follow usually in the cranial nerve areas, symptoms include blurred or double vision, dilated pupils, photophobia, loss of mouth and throat functions. The symptoms descend through the body (Bhutani *et al.*, 2005) and include ptosis, paralysis of motor nerves, fatigue, lack of muscle co-ordination and respiratory impairment. Where the disease is fatal, the main cause is respiratory failure and/or obstructed air passages. Constipation may be present after the onset of neurological symptoms and abdominal pain may be present throughout (Szabo and Gibson, 2003; WHO, 1999). Misdiagnosis is common

and is particularly confused with Guillain-Barre syndrome, for example (Shapiro *et al.*, 1998).

Condition: Foodborne botulism, usually confirmed by toxin detection in the patient's serum (Szabo and Gibson, 2003).

Toxins: Foodborne intoxication is predominately the ingestion of the pre-formed toxin which is absorbed through the gastric and upper intestinal mucosa. The toxin enters the blood and subsequently affects the peripheral nervous system (Szabo and Gibson, 2003). Once the toxin reaches the bloodstream, it circulates the body for between 8 – 10 days (Gelli *et al.*, 2002).

At Risk Groups: All members of the population are susceptible. There are no particular at risk groups identified.

Long Term Effects: Before the effect of anti-sera in 1960, the fatality rate was high. Austin (2001) cites the Chinese situation where the fatality rates were around 50% before anti-serum treatment which resulted in a sharp decline to 8%. The fatality rate quoted in Szabo and Gibson (2003) is currently 5 – 15%. Improved medical care mainly through prompt antitoxin administration and respiratory support are credited with the declining mortality rate (WHO, 1999).

Treatment: There are no drugs reported in the literature that can reverse the effects exerted by botulinal toxins (Szabo and Gibson, 2003). The objective in the early stages of the illness is to inactivate and remove the toxin. Injections with anti-serum neutralise circulating toxin can be effective if administered early. Supplies of bivalent antitoxin (type A and B) and Type E are held in Auckland hospital pharmacy department for New Zealand. Removal of residual toxin is performed by gastric lavage or treatment with emetics if food exposure was recent, or cathartics or enema procedures (WHO, 1999). Subsequent treatment focuses on counteracting respiratory muscle and diaphragm failure by mechanical ventilation (Austin, 2001).

8.2   Dose Response

C. botulinum toxin is among the most toxic of naturally occurring substances known. The minimum toxic dose is uncertain in humans. Estimates have placed the amount of Type A toxin needed to cause death at 30 ng (Peck and Stringer, 2005) or between 0.1 and 1.0 μg. These figures were extrapolated from mouse models (ICMSF, 1996). In monkeys and mice, LD_{50} of neurotoxin was measured at approximately 0.4 ng kg^{-1} when the dose was introduced intravenously (Gill, 1982).

8.3   Adverse Health Effects in New Zealand

8.3.1   Incidence

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

8.4 Adverse Health Effects Overseas

8.4.1 Incidence

Reported cases of botulism in developed countries are rare, as shown in Table 4. Georgia was the country with the highest reported incidence, around ten times that reported for Germany.

Table 4. **Comparison of foodborne botulism incidence between countries**

Country	Period	Rate	No. of cases	Reference
Argentina	1992-2004	-	41	(Rebagliati *et al.*, 2009)
Brazil	1982-2001	-	8 cases/outbreaks	Gelli *et al.*, 2002
Canada*	1995 1996 1997 1998 1999 2000 2001 2002 2003 2004		18 15 19 7 8 4 8 10 6 7	(Health Canada, 2006)
China	1994-2006	-	254 (0.44%) of cases and 32 (62.75%) of deaths in foodborne disease "events"	(Wang *et al.*, 2007)
Denmark	1984 - 89	-	16 cases (11 outbreaks, 12 fatalities) 100% E	Gelli *et al.,* 2002
England and Wales	1999 2000	-	0 0	(World Health Organisation, 2000)
France	1951-1999	-	16 cases of type E and in 1 case with type B too (where the food was ham)	(Boyer *et al.*, 2001)

Country	Period	Rate	No. of cases	Reference
France*	1999	-	32	(World Health Organisation, 2000)
	2000	-	28	
	1978-1989		304 cases (in 175 outbreaks, 2 fatalities) 2% type E	Gelli *et al.,* 2002
France	2007-2009	-	22 identified (45 cases), 2 suspected (2 cases). 1 outbreak type E	(Mazuet *et al.*, 2011)
Georgia	1980-2002	0.3-0.9	879 cases, 58 deaths, 10% (from 20 events) type E	(Varma *et al.*, 2004)
Germany*	1999	0.023	19	(World Health Organisation, 2000)
	2000	0.013	11	
Italy (Lazio)	1996-200	-	8 outbreaks, 12 cases	(Faustini *et al.*, 2003)
Norway	1975-1997	-	Foodborne: 22 cases, 10 outbreaks. Types B and E	(Kuusi *et al.*, 1999)
UK	1922-2005	-	62	(McLauchlin *et al.*, 2006)

Country	Period	Rate	No. of cases	Reference
USA	1899-1995	-	2,444 cases in 1026 outbreaks. 1,040 fatalities	Gelli *et al.,* 2002
	1971-1989	-	597 cases (in 272 outbreaks, 11 fatalities) 17% type E	Gelli *et al.,* 2002
	1990-2000	0.01	160 events, 263 cases, 37% type E (90% of Alaskan cases)	(Sobel *et al.*, 2004)
	2006	-	13 cases, 4 outbreaks, 1 death	(Centers for Disease Control and Prevention, 2009)
	2007	-	12 cases, 4 outbreaks, 1 death	(Centers for Disease Control and Prevention, 2010)
	1973-2006	-	Of all seafood outbreaks: Fish 23%, crustacean 0%, molluscs 0%. Of all seafood cases: Fish 4%, crustacean 0%, molluscs 0%. 61 hospitalisations, 9 deaths.	(Iwamoto *et al.*, 2010)

*Botulism not distinguished by clinical form therefore could include infant, wound etc.

Food-borne botulism cases recorded in 38 countries between 1951 and 1989 have been reviewed (WHO, 1999). This shows that 72% of outbreaks and 48% of cases were reported from Poland where home-preserving is common. The toxin type was determined in 2622 outbreaks during this period, the findings were 34% type A, 52% type B, and 12% Type E. The rarity of type F foodborne botulism was confirmed; only two incidents during this period were assigned to this toxin type.

In Canada, Alaska, Scandinavia and Northern Japan, food-borne botulism outbreaks involving fish, particularly traditional native dishes, have been reported. Most of these outbreaks have been linked to Type E neurotoxin (Austin, 2001). Specific reported foodborne botulism cases associated with seafood are summarised in Table 5.

Table 5.   Reported foodborne botulism cases associated with fish and shellfish overseas

Country	Period	Toxin type	No. of cases	Food type	Reference
Canada	1957	E	3 (all fatal)	Salmon roe	(Dolman *et al.*, 1960)
	1958	Non proteolytic B	1 fatal		
	1959	E	1 fatal		
	2001	E	2, 1 fatal 2		(Dawar *et al.*, 2002)
Egypt	1991	E	91 (hospital) 18 fatal	Uneviscerated salted mullet fish	(Weber *et al.*, 1993)
Finland	2006	E	1	Vacuum-packed smoked whitefish	(Lindstrom *et al.*, 2006b)
France	1999	E	1	Frozen vacuum-packed prawns (?) -	(Boyer *et al.*, 2001)
		E	1		
France	2009	E	3	Vacuum-packed hot smoked white fish	(King *et al.*, 2009)
Germany (imported fish from Finland)	1997	E	2	Hot-smoked Vacuum-packed whitefish	(Korkeala *et al.*, 1998)
Germany	2003	E	3	Salted fish	(Eriksen *et al.*, 2004)
Japan	1951 - 1987	E	479	Fish or fish products	Hauschild, 1993
Norway	2003	Not stated	4	Rakfisk (fermented fish)	(Eriksen *et al.*, 2004)
Russia	2004 2005	? ?	35 (2 fatal) 6	Fish Salted fish	http://www.promedmail.org/pls/apex/f?p=2400:1000
UK	1978	E	4	Tinned Alaskan salmon	(Ball *et al.*, 1979)
UK	1955	A	2	Pickled fish from Mauritius	(McLauchlin *et al.*, 2006)
USA	1990	B	3	Grilled surgeon fish	(Centers for Disease Control, 1991)
USA	1992	E	4	Uneviscerated white fish	(Centers for Disease Control, 1992)
USA	2005	E	5	Uneviscerated white fish	(Sobel *et al.*, 2007)

USA (Alaska)	2002	E	8	Beached whale	(McLaughlin *et al.*, 2004)
USA (Alaska)	2001	E	3	Fermented beaver tail and paws	
USA and Israel	1987	E	8 (1 fatal)	Uneviscerated salt-cured, air-dried fish	(Telzak *et al.*, 1990)
USA	1985	E	2 (both fatal)	Uneviscerated salt-cured, air-dried fish	(Badhey *et al.*, 1986)

Between 2000 and 2010, twelve cases of botulism have been reported to the Nationally Notifiable Diseases Surveillance System in Australia[16]. All but one case was infant botulism. For the infant botulism cases, no sources were identified, although honey was suspected in one case. The remaining case of botulism was reported in 2007, and was suspected to be associated with nachos.

8.4.2   Contributions to outbreaks and incidents

The importance of home food preservation as a risk factor for outbreaks of botulism is shown by the data shown in Table 6 (Hauschild, 1993). Meats are the most common food type, with fish and fruits/vegetables following.

Table 6.   Food vehicles and preparation methods associated with outbreaks of botulism

Country	Number of outbreaks with food identified	Fish (%)	Meats (%)	Fruit/ vegetables (%)	Other* (%)	Home-prepared (%)
Argentina	14	21	29	36	14	79
Belgium	8	12	75	0	62	38
Canada	75	20	72	8	0	96
Czechoslovakia	14	7	72	14	7	100
Germany - East	31	26	52	19	3	73
- West	55	13	78	9	0	100
Poland	1500	12	83	5	0	75
USA	222	17	16	59	9	92

* includes mixed vehicles

In the USA, among the 294 outbreaks during the period 1973 to 1997, 47 (16%) outbreaks were due to fish (Gelli *et al.,* 2002).

[16] http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-annlrpt-nndssar.htm

8.4.3   Case control studies

The largest documented outbreak of type E foodborne botulism in Cairo, Egypt led to a case control study being undertaken (Weber *et al.*, 1993). Forty five patients were interviewed with an investigation subsequently conducted among five families of hospitalised patients. Illness was associated with eating uneviscerated salted mullet (95% CI = 6.6, p < 0.001).

8.4.4   Risk assessment and other activity overseas

Food Standards Australia New Zealand (FSANZ) has prepared a Primary Production and Processing Standard for Seafood, Proposal P265, which applies to Australia only (Food Standards Australia New Zealand, 2005). In this document risk assessments from Australia on *C. botulinum* in smoked seafood were discussed.

The FSANZ report included risk assessments on *C. botulinum* in hot and cold smoked finfish products. The FSANZ conclusion was that while foodborne botulism was a severe illness its occurrence was "unlikely". The assessment stated that for ready-to-eat cold smoked fish products *C. botulinum* spores and vegetative cells are likely to survive the cold-smoking process, but growth is unlikely except in the case of vacuum or modified atmosphere packed products. Even in the case of such products, salt concentrations (5% or more) are likely to inhibit growth and toxin production.

For hot smoked products, the assessment stated that spores will survive the smoking process, but growth is unlikely except in the reduced oxygen packed products. Even in the case of such products, salt concentrations (3.5% or more), processing to an internal temperature of greater than 63°C for at least 30 minutes, and proper storage at less than 5°C will inhibit growth and toxin production.

The "medium" relative risk ranking assigned for *C. botulinum* in both these products reflected current risk management including industry inherence to good manufacturing practice, good hygiene practice and appropriate product formulation (e.g. pH, levels of salt, preservatives) (Food Standards Australia New Zealand, 2005). As *C. botulinum* mostly occurred in the gut of fish, the risk was mostly from fish that were not eviscerated before smoking. The hazard identification section also referred to surveys of Australian marine samples which failed to find *C. botulinum* Type E.

The FSANZ document cited specific risk assessments by Ross and Sanderson (2000) and Sumner (2001). Ross and Sanderson (2000) were commissioned by Safe Food Production, New South Wales (NSW) to carry out a Risk Assessment of selected seafoods in NSW. They considered the risk to consumers from *C. botulinum* in vacuum-packed ready-to-eat fish products was 'relatively low', and would only result from gross temperature abuse.

Sumner (2001) compiled a Seafood Food Safety Risk Assessment for Seafood Services Australia Ltd and concluded that the risk of *C. botulinum* in smoked vacuum-packed fish was "negligible". This was based on the low levels of spores likely to be in products available in the Australian marketplace and the typical salt levels in these products.

For Australia, a semiquantitative seafood safety risk assessment (Sumner and Ross, 2002) assessed a range of hazard/product combinations. Scores for *C. botulinum* in canned fish and vacuum packed smoked fish were 25 and 28 respectively. Risk ratings occurred on a scale of 0-100 (0 = no risk, 100 = everybody eating a meal containing a lethal dose of the hazard every day. Because the scale is logarithmic, an increment of 6 in the ranking relates to a 10-fold increase in absolute risk estimate. The *C. botulinum* risks fell into the lowest ranked category (risk ranking <32) and it was reported that no documented cases of foodborne illness had occurred in Australia as a result of *C. botulinum* in vacuum-packaged smoked fish.