



Report on Botulinum Neurotoxin-Producing Clostridia

Advisory Committee on the Microbiological Safety
of Food

Working Group on Botulinum Neurotoxin-Producing
Clostridia, 2023

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Executive Summary

In 1992 a working group of the UK Advisory Committee on the Microbiological Safety of Food presented a report on Vacuum Packaging and Associated Processes regarding the microbiological safety of chilled foods. The report supported subsequent guidance provided by the UK Food Standards Agency for the safe manufacture of vacuum packed and modified atmosphere packed chilled foods. In 2021 the ACMSF requested that a new subgroup should update and build on the 1992 report as well as considering, in addition to chilled foods, some foods that are intended to be stored at ambient temperatures. The new subgroup agreed a scope that includes the conditions that support growth and/or neurotoxin formation by *C. botulinum*, and other clostridia, as well as identification of limiting conditions that provide control. Other foodborne pathogens that need to be considered separately and some foods including raw beef, pork and lamb were explicitly excluded.

The subgroup considered the taxonomy, detection, epidemiology, occurrence, growth, survival and risks associated with *C. botulinum* and other neurotoxin-forming clostridia. There has been no significant change in the nature of foodborne botulism in recent decades except for the identification of rare cases caused by neurotoxigenic *C. butyricum*, *C. baratii* and *C. sporogenes*. Currently evidence indicates that non-clostridia do not pose a risk in relation to foodborne botulism.

The subgroup has compiled lists of incidents and outbreaks of botulism, reported in the UK and worldwide, and have reviewed published information concerning growth parameters and control factors in relation to proteolytic *C. botulinum*, non-proteolytic *C. botulinum* and the other neurotoxigenic clostridia.

The subgroup concluded that the frequency of occurrence of foodborne botulism is very low (very rare but cannot be excluded) with high severity (severe illness: causing life threatening or substantial sequelae or long-term illness). Uncertainty associated with the assessment of the frequency of occurrence, and with the assessment of severity, of foodborne botulism is low (solid and complete data; strong evidence in multiple sources). The vast majority of reported botulism outbreaks, for chilled or ambient stored foods, are identified with proteolytic *C. botulinum*, and temperature abuse is the single most common cause. In the last 30 years, in the UK and worldwide where a cause can be identified, there is evidence that known controls, combined with the correct storage, would have prevented the reported incidents of foodborne botulism.

The subgroup recommends that foods should continue to be formulated to control *C. botulinum*, and other botulinum neurotoxin-producing clostridia, in accordance with the known factors. With regard to these controls, the subgroup recommends some changes to the FSA guidelines that reflect improved information about using combinations of controls, the z-value used to establish equivalent thermal processes and the variable efficacy associated with some controls such as herbs and spices. Current information does not facilitate revision of the current reference process, heating at 90°C for 10 minutes, but there is strong evidence that this provides a lethality that exceeds the target 6 orders of magnitude reduction in population size

that is widely attributed to the process and the subgroup includes a recommendation that the FSA considers this issue.

Early detection and connection of cases and rapid, effective coordinated responses to very rare incidents are identified as crucial elements for reducing risks from foodborne botulism. The subgroup recommends that the FSA works closely with other agencies to establish clear and validated preparedness in relation to potential major incidents of foodborne botulism in the UK.

Terms of Reference

- Review the risk posed by botulinum neurotoxin-producing clostridia in foods stored at $\leq 8^{\circ}\text{C}$ that support growth or toxin production.
- A preliminary assessment of the risk posed by botulinum neurotoxin-producing clostridia in food designed to be stored at ambient temperature that supports growth or toxin production.
- Where appropriate, consider other risk-related evidence relevant to neurotoxin-producing clostridia during the lifetime of the group.

Scope

Conditions that support growth and/or neurotoxin formation by *C. botulinum* and other clostridia. Where practical this includes the identification of a limiting condition that allows growth and/or neurotoxin formation by *C. botulinum* as well as identification of a limiting condition that provides control. Preliminary consideration of non-clostridia that have gene sequences homologous with the botulinum neurotoxin genes is included.

The following will be excluded from consideration by the committee, as a wealth of evidence is already in existence:

- Foods given a botulinum cook;
- Foods such as honey that are associated with infant botulism/intestinal colonisation;
- Vacuum packaged/modified atmosphere packaged raw beef, pork and lamb (without added ingredients), to the extent that they have already been assessed by a previous ACMSF subgroup;
- *Listeria monocytogenes*, *Bacillus cereus* and other pathogens not specified in the scope or the terms of reference that need to be considered separately.

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Glossary

Anaerobic broth. Anaerobic microbiological broth, a nutrient medium used to grow bacteria in laboratory settings as a substitute for foods.

Decimal reduction time (D-value), D_T . The time required, at a fixed temperature T , for heat to reduce the size of a population by 90% (The logarithm of the D value is frequently used as an alternative expression).

Enzyme-Linked Immunosorbent Assay, ELISA. An investigative procedure to measure antibodies, antigens, proteins and glycoproteins in biological samples.

Mouse Lethal Bioassay, MLB. An animal test conducted to ascertain the presence/absence of botulinum neurotoxins.

pH. A scale of acidity from 0 to 14. More acidic solutions have lower pH and more alkaline solutions have a higher pH; neutrality has pH = 7.0.

Polymerase Chain Reaction, PCR. A laboratory technique for rapidly producing billions of copies of a specific segment of DNA, which can then be studied in greater detail.

Whole Genome Sequencing, WGS. A comprehensive method for analysing entire genomes.

Multi-Locus Sequence Typing, MLST. An unambiguous procedure for characterising isolates of bacterial species using the sequences of internal fragments of (usually) seven house-keeping genes.

Hazard Analysis and Critical Control Points, HACCP. Principles on which a food safety management system should be based; that is a risk assessment for food production covering all aspects from supplier to customer.

Dark/Ghost kitchens. Physical kitchen premises that exist solely for the delivery market to provide food to the public. There is no dine in or collection option for customers.

z-value, z. The difference in temperature of two isothermal heating processes that have decimal reduction times that differ by a factor of 10 (some conventions express z-value in units 'centigrade degrees' to emphasise the significance of temperature difference).

Water activity, a_w . The chemical potential for water molecules in a system that includes water, ionic species and dissolved solids; alternatively called water availability. Pure water has $a_w = 1$.

Redox potential, E_h . Sometimes called the reduction oxidation potential, measured in millivolts (mV), it is the tendency of food to release electrons (low redox $E_h = 100$ to -300 mV) or accept electrons (high redox $E_h = 300 - 500$ mV).

Salt. In this report 'salt' refers specifically to sodium chloride or NaCl.

1 Introduction and Background

In 1992 a working group of the UK Advisory Committee on the Microbiological Safety of Food (ACMSF) presented a report on Vacuum Packaging and Associated Processes with regard to the microbiological safety of chilled foods¹. The report had particular emphasis on the risks associated with psychrotrophic (non-proteolytic) *Clostridium botulinum* in chilled foods. The report was widely acknowledged and supported subsequent guidance provided by the UK Food Standards Agency, and many other organisations, for the safe manufacture of vacuum packed and modified atmosphere packed chilled foods.

FSA guidance published in 2020² recommends “that, in addition to chill temperatures (3 – 8°C) which should be maintained throughout the food chain, the following controlling factors should be used singly or in combination to prevent growth and toxin production by non-proteolytic *C. botulinum* in chilled foods with a shelf-life of more than 10 days;

- a heat treatment of 90°C for 10 minutes or equivalent lethality at the slowest heating point in the food
- a pH of 5.0 or less throughout the food and throughout all components of complex foods
- a minimum salt level of 3.5% in the aqueous phase throughout the food and throughout all components of complex foods
- a water activity (a_w) of 0.97 or less throughout the food and throughout all components of complex foods
- a combination of heat and preservative factors which can be shown consistently to prevent growth and toxin production by non-proteolytic *C. botulinum*”

In 2021 the ACMSF requested that a new subgroup should update and build on the 1992 report. The new subgroup was requested to review new knowledge concerning *C. botulinum* and the formation of botulinum neurotoxin and to consider, in addition to chilled foods, some foods that are intended to be stored at ambient temperatures. The terms of reference for the subgroup, and an indication of restrictions on the scope of the review, are included as a preface to this report.

Since the publication of the 1992 report a wealth of information on *C. botulinum*, botulinum neurotoxin-producing clostridia and on food processing and packaging technology has been published. The types of food now being produced, corresponding production and distribution technology and the way hazards and risks are assessed and managed have evolved. The 1992 report covered risks from *C. botulinum* growth and toxin formation in chilled foods and also addressed other pathogens of concern. In 1992, the ACMSF considered foods stored at temperatures of “10°C and below” but, soon after, regulation stipulated that chilled foods should be stored “at or below 8°C”. For clarity, in this report, chilled storage corresponds to temperatures that do not exceed 8°C.

As in 1992 microbiological food safety is the issue of primary concern for this report. Contributory considerations include:

- Further information has been published on the prevalence and risk associated with non-proteolytic *C. botulinum* in chilled foods and the impact of this information on the risk assessment conducted by the ACMSF in 1992 requires corresponding review.
- The developments in food processing and packaging technology that have occurred since the publication of the 1992 report require consideration from the perspective of microbiological food safety. New packaging techniques and materials have become more widely used for controlled atmosphere packing and there are movements towards longer shelf life to reduce food waste, lower heat treatments to reduce use of energy, reduced preservation with salt or nitrites and alternative food ingredients as part of a move away from meat that merit consideration from a food safety perspective.
- It has become clear that there are foods on the market that are designed to be stored in ambient conditions that could support the growth of, or toxin formation by, botulinum neurotoxin-forming clostridia. A risk assessment for these types of food is required; this will extend the scope of the consideration to cover clostridia that can only grow and produce toxin at temperatures above those considered to be chilled.
- In the 1992 report *C. botulinum* was considered to be the sole species responsible for the production of botulinum neurotoxin. Recent scientific evidence has shown that clostridial species other than *C. botulinum* contain genes responsible for the production of botulinum neurotoxin and some have been associated with outbreaks of botulism. Additionally, some bacteria of non-clostridial genera have been found to include gene sequences that have some similarity with the *C. botulinum* neurotoxin genes. As previous risk assessments in relation to foodborne botulism have focussed almost exclusively on *C. botulinum*, it is important to review the significance of the new findings in relation to the safety of food.

Some background for this consideration is included in further sections of this introduction and details relating to taxonomy, detection, epidemiology, microbiology and risks are included in subsequent chapters. This report concludes with some recommendations in relation to microbiological safety of foods.

1.1 Clostridia

The genus *Clostridium* is composed of a wide range of Gram-positive, spore forming, rod shaped bacteria. They are considered to be anaerobic although the strictness of a requirement for anaerobiosis has been reported to be quite varied between species and strains. The presence of high levels of oxygen does not, in isolation, guarantee food safety with respect to *C. botulinum* or other toxigenic clostridia. Details of factors controlling growth and survival are included in Chapter 5 of this report.

Clostridium species can be found in a wide range of different environments including in foods where some species can cause food spoilage and others food poisoning. Of the species that cause food poisoning, *Clostridium perfringens* and the botulinum neurotoxin-producing clostridia are the most important. *C. perfringens* is responsible

for outbreaks of food poisoning and is often associated with meat and meat products. These organisms can sporulate in the small intestine producing large amounts of *C. perfringens* enterotoxin which causes illness³; this hazard is beyond the scope of this review.

1.2 *C. botulinum*

The illness caused by *C. botulinum* was recognised in the early nineteenth century but it was not until the 1890s that it was attributed to a toxin produced by a bacterium; the bacterium was initially named *Bacillus botulinus*. Later investigations of outbreaks indicated that there were different types of causative organisms, some being proteolytic and others non-proteolytic, and serological studies indicated that different types of neurotoxins could be produced by different strains. Early in the 20th century *B. botulinus* was categorised as belonging to the genus *Clostridium* in a classification that separated the aerobic members of the genus *Bacillus* from the anaerobic members of the genus *Clostridium*.

1.3 The botulinum neurotoxin-forming clostridia

Originally *C. botulinum* was considered the only species able to produce botulinum neurotoxin. However, as microbial species differentiation has improved, it has become clear that the situation is more complex and that the traditional view of the species and its various groups has changed. Details of taxonomy for botulinum neurotoxin-forming clostridia are included in Chapter 2 of this report.

The scope of the 1992 ACMSF report was limited to chilled foods so only the risks associated with non-proteolytic *C. botulinum*, and risks associated with other pathogens that can survive and grow at low temperatures, were considered. Consideration of foods stored at ambient temperatures means that risks associated with proteolytic *C. botulinum* are within the scope of this update. Details of the conditions that support growth and survival of *C. botulinum* are included in Chapter 5 of this report.

1.4 Non-clostridial species

The development of genome sequencing techniques has resulted in the discovery of botulinum neurotoxin-like sequences within the genomes of some non-clostridial species. These have not been associated with botulism. This was first reported following the identification of a botulinum neurotoxin-like gene sequence in *Weissella oryzae*⁴. Further details are included in Chapter 2 of this report.

1.5 Botulinum neurotoxins

Although botulinum neurotoxins are now known to be produced by species other than *C. botulinum*, the broad group of toxins are still referred to as botulinum neurotoxins. *C. botulinum* neurotoxins are a diverse range of proteins represented by at least seven serotypes and more than 40 subtypes⁵. New clostridial strains producing novel toxin variants are still being identified. The toxins are proteins made up of a heavy chain and a light chain. The role of the heavy chain is reported to be binding of the toxin to receptors in the peripheral nerve and translocation of the light chain into the nerve cell cytoplasm. The light chain blocks the release of the

neurotransmitter acetylcholine which leads to flaccid paralysis and botulism⁵. Toxin types A, B, E and occasionally F have predominantly been associated with human foodborne botulism. Toxin types C and D mainly cause disease in animals and have rarely been linked to human foodborne botulism. Toxin types G and X have not been associated with foodborne botulism. Modern techniques have identified a range of hybrid toxins. Details of the taxonomy of botulinum neurotoxins are included in Chapter 2 of this report.

1.6 Botulism - the illness

The botulinum neurotoxins are the most potent naturally occurring toxins. Human foodborne botulism is a severe, and potentially lethal, neuromuscular intoxication potentially caused by the consumption of as little as 50 ng of botulinum neurotoxin. The classic symptoms of botulism include a severe flaccid muscle paralysis. The WHO provides the following description of foodborne botulism⁶: “Early symptoms include marked fatigue, weakness and vertigo, usually followed by blurred vision, dry mouth and difficulty in swallowing and speaking. Vomiting, diarrhoea, constipation and abdominal swelling may also occur. The disease can progress to weakness in the neck and arms, after which the respiratory muscles and muscles of the lower body are affected. There is no fever and no loss of consciousness. Symptoms usually appear within 12 to 36 hours (within a minimum and maximum range of 4 hours to 8 days) after exposure. The disease can be fatal in 5 to 10% of cases”.

Human botulism tends to be categorised according to how the toxin enters the body (exposure) and several types have been identified:

- Foodborne botulism: toxin is formed during growth of the organism in food and when the food is consumed the pre-formed toxin enters the body causing illness.
- Infant botulism: *C. botulinum* spores are consumed in food and then germinate within the gut before cells grow and form toxin *in situ*. This type of botulism is associated with infants under one year old.
- Wound botulism: spores of *C. botulinum* germinate before cells grow and produce toxin in a wound. This type of botulism has, in recent years, been associated with injected drug use.
- Adult infectious botulism: a rare type of botulism with causation similar to infant botulism.
- Iatrogenic botulism: illness is caused by an accidental overdose of therapeutic botulinum neurotoxin.
- Inhalation botulism: illness is caused by inhalation of aerosolised neurotoxin.

1.7 Industrial and domestic practices

In 1992 contemporary food safety often involved extremes of freezing and heating, in industrial settings, for relatively unprocessed foods as well as fermentation, drying and acidification. Processing was generally followed by storage in sealed metal or robust plastic containers. The 1992 ACMSF report was in response to the increasing use of packaging technologies that were designed to improve the quality and extend the shelf life of chilled foods. Packaging technologies for chilled foods have

continued to change over time with development of new packaging materials, use of skin packing, the movement towards recyclable materials and the push for longer shelf life (to increase production efficiency, meet the needs of modern food distribution chains and to reduce food waste). In addition it is clear that, since 1992, for ambient stable foods there have been some developments that do not include traditionally accepted controls, and could theoretically allow germination, growth and toxin formation by *C. botulinum* leading to foodborne botulism; vegetables stored in oil are one example. Reports of botulism indicate that a significant number of incidents originate from the preparation or preservation of food in non-industrial environments. Historically, notably outside the UK, domestic and small scale non-industrial procedures such as home canning, bottling, preservation in oil and home fermentation have all resulted in incidents of foodborne botulism. Details of worldwide botulism incidents are included in Chapter 4 of this report. In a majority of cases the root cause of domestic outbreaks of foodborne botulism is the absence, or incorrect use, of *C. botulinum* control measures.

Drivers and trends in the UK point to an expanding role for home preservation and small scale non-industrial supply of chilled and ambient stored foods that cannot be neglected as a possible emerging source of foodborne botulism. Evolving systems for off-site or ghost supply, for buying and selling food online, and in social media marketplaces have implications for food safety. With respect to foodborne botulism the potential extent of additional risk for UK consumers, where there is little tradition for home preservation etc., is unknown and the facilities for the application of uniform controls, or effective traceability, in non-commercial food supply chains are not apparent. It may be prudent to establish greater awareness of foodborne botulism amongst the growing population of emerging small scale food producers.

2 Taxonomy of Botulinum Neurotoxin-Forming Clostridia and their Neurotoxins

The name *Clostridium botulinum* has been used for bacteria that form botulinum neurotoxin. For many decades it has been recognised that *C. botulinum* is not a distinct species but a collection of diverse clostridia that share the common property of forming botulinum neurotoxin⁷. Genes potentially encoding a botulinum neurotoxin have also recently been detected outside of the genus *Clostridium*.

Since 1992 scientific developments, notably whole genome sequencing (WGS), have provided a better understanding of the taxonomy and diversity of botulinum neurotoxin-forming clostridia and their neurotoxins. Information from WGS is valuable when selecting strains for challenge test experiments and process validation, is a valuable resource for improved pathogen detection and discrimination, is vital for tracing outbreaks and is central to studies of pathogen biology and evolution for *C. botulinum* and its neurotoxin genes.

This new information and understanding indicates it is unlikely that properties of botulinum neurotoxin-forming clostridia and their neurotoxins, or the risk of human foodborne botulism that they present, have changed in recent decades (although changes in food processing may impact on risk).

The diverse collection of clostridia that form botulinum neurotoxin can be separated into six genomically and phenotypically distinct groups (Table 1). These six groups are sufficiently distinct for each to be considered a separate bacterial species. Two of these groups, proteolytic *C. botulinum* (including *C. sporogenes*) and non-proteolytic *C. botulinum*, are strongly associated with human foodborne botulism, with the other groups either weakly associated or not associated. Each group includes highly related strains that do and do not form botulinum neurotoxin. There has been a greater interest in sequencing strains that form botulinum neurotoxin rather than non-toxic strains. Thus, for each group, the fraction of sequenced toxic strains is likely an overestimate of the fraction of toxic strains found in the environment.

WGS has provided details of the taxonomy, population structure and diversity within each group, and evidence of horizontal transfer of botulinum neurotoxin genes between distantly related clostridia. The gene encoding the botulinum neurotoxin is located on the chromosome or a plasmid or other mobile genetic element.

Different naming conventions are currently used for botulinum neurotoxin-forming clostridia, and the present situation is dynamic. In this report the framework in Table 1 is used for clarity. Further details of the association with human foodborne botulism are given in Chapter 4 of this report.

Table 1: Six genomically and phenotypically distinct groups of botulinum neurotoxin-forming clostridia

Name of botulinum neurotoxin-forming clostridia used in this report	Association of botulinum neurotoxin-forming clostridia with human foodborne botulism	Serotypes of botulinum neurotoxin formed
Proteolytic <i>C. botulinum</i> / <i>C. sporogenes</i>	Strong	A, B, F, X
Non-proteolytic <i>C. botulinum</i>	Strong	B, E, F
<i>C. botulinum</i> Group III	Very weak	C, D
<i>C. argentinense</i>	No	G
<i>C. butyricum</i>	Weak	E
<i>C. baratii</i>	Weak	F

2.1 Properties of the botulinum neurotoxins

Eight antigenically distinct botulinum neurotoxin serotypes (A-G, X) have been identified in bacterial strains within six distinct clostridial groups (Table 1). The eight botulinum neurotoxin serotypes are separated into more than 40 botulinum neurotoxin sub-types⁵. Most sub-types have been identified through derivation of their amino acid sequence from the gene sequence and they differ by at least 2.6% in amino acid sequence from any other sub-type. Various hybrid neurotoxins have also been described. Human foodborne botulism is most frequently associated with botulinum neurotoxin serotypes A, B and E. While there is evidence that the botulinum neurotoxin serotypes show minor differences in potency, all botulinum neurotoxins must be considered to represent a major hazard.

2.2 Taxonomy of proteolytic *C. botulinum*/*C. sporogenes* group

The proteolytic *C. botulinum*/*C. sporogenes* group is frequently associated with human foodborne botulism. It comprises highly proteolytic mesophilic bacteria that form spores of high thermal resistance. WGS has separated this group into two major lineages that could be recognised as separate species⁸. Both lineages contain strains responsible for foodborne botulism, and include toxic and non-toxic strains. The first lineage contains strains of proteolytic *C. botulinum*, with most strains forming one or more botulinum neurotoxins of types A, B, and/or F (and in one case of infant botulism type X). These strains are strongly associated with foodborne botulism. The second lineage contains strains of *C. sporogenes*, with 20 out of 104 strains analysed in a recent study forming botulinum neurotoxin type B, and the remaining 84 strains non-toxic. The strains that form botulinum neurotoxin type B were distributed amongst closely related non-toxic strains, rather than clustering together. The toxic strains of *C. sporogenes* have been occasionally associated with foodborne botulism (including one case in the UK).

Recent findings include the recognition that the high thermal resistance strain PA3679 is a non-toxic strain of proteolytic *C. botulinum* rather than a non-toxic strain of *C. sporogenes*, and that some strains that have caused human botulism

(foodborne, infant and wound) and form type B neurotoxin belong to *C. sporogenes* rather than to proteolytic *C. botulinum*⁸

The identification of distinct lineages/clusters within the proteolytic *C. botulinum*/*C. sporogenes* group, and further genotypic and phenotypic characterisation of strains within these lineages/clusters has the potential to contribute to improved risk assessments. For example, strains within the proteolytic *C. botulinum* lineage seem to present a greater human foodborne botulism risk than strains within the *C. sporogenes* lineage, since (i) a greater proportion of strains form botulinum neurotoxin, and (ii) a greater fraction of strains has been associated with foodborne botulism. However, currently nothing prohibits other circumstances (e.g. alternative approaches to food processing) that might favour toxic strains in the *C. sporogenes* lineage.

Detailed information on the genotype and particularly the phenotype of botulinum neurotoxin-forming strains in the *C. sporogenes* lineage is presently limited. It is not known how closely the phenotype of neurotoxin-forming strains of proteolytic *C. botulinum* is followed compared to that of non-toxic strains of *C. sporogenes*. Further clarity on the homogeneity of the proteolytic *C. botulinum*/*C. sporogenes* group will be of great value in considering the human foodborne botulism risk, and in particular whether it may be appropriate to disaggregate their risks. Currently it is pragmatic to consider neurotoxigenic *C. sporogenes* together with proteolytic *C. botulinum*.

2.3 Taxonomy of non-proteolytic *C. botulinum* group

The non-proteolytic *C. botulinum* group is frequently associated with human foodborne botulism. It comprises psychrotrophic, weakly proteolytic bacteria that form spores of moderate thermal resistance. WGS has separated this group into two major lineages⁹. Both lineages contain strains responsible for human foodborne botulism. The first lineage includes most strains that form type E neurotoxin and also some non-toxic strains. These strains are frequently isolated from fish and the arctic/subarctic environment (including marine and freshwater). The second lineage is dominated by strains that form type B neurotoxin and also contains some strains that form type E or type F toxin and non-toxic strains. The strains are often isolated from European terrestrial environments (including pigs) and marine/freshwater environments.

Foodborne botulism outbreaks associated with strains within the type E toxin lineage frequently involve chilled fish products, while outbreaks associated with strains within the type B (dominant) toxin lineage often involve chilled meat products. This may reflect genotypic/phenotypic characteristics and/or the geographical location in which the strains are found. Preliminary data indicate that spores of strains in the type B (dominant) lineage may have higher thermal resistance than spores of strains within the type E toxin lineage¹⁰. The identification of distinct lineages within the non-proteolytic *C. botulinum* group, and the genotypic and phenotypic characterisation of neurotoxin-forming strains within these lineages will be of great value in evaluating the human foodborne botulism risk, and in particular whether it may be appropriate to consider risks from the two groups independently.

2.4 Taxonomy of *C. botulinum* Group III and *C. argentinense*

C. botulinum Group III has been only very weakly associated with human foodborne botulism (details of the associations are included in Chapter 4 of this report) and there is no evidence that it presents a new or increased risk. Strains within *C. botulinum* Group III are frequently associated with botulism in animals and birds. *Clostridium argentinense* has not been associated with human foodborne botulism. WGS has been used to describe strain diversity within these two clostridial groups, and strains possessing and lacking genes encoding botulinum neurotoxin appear closely related.

2.5 Taxonomy of *C. butyricum* group

The majority of strains of *C. butyricum* are non-toxic, but a fraction form botulinum neurotoxin type E and have been associated with human foodborne botulism on a few occasions. The WGS of a limited number of *C. butyricum* strains (67 strains including eleven toxic strains) are available in public databases, and although this dataset is very limited there is an indication that the toxic strains may form discrete lineages rather than being widely distributed amongst non-toxic strains¹¹. A detailed understanding of the homogeneity of the *C. butyricum* group will contribute to evaluating the human foodborne botulism risk presented by neurotoxicogenic strains of *C. butyricum*.

2.6 Taxonomy of *C. baratii* group

The majority of strains of *C. baratii* are non-toxic, but a minority form botulinum neurotoxin type F and have been associated with human foodborne botulism on a few occasions. The WGS of only 16 strains of *C. baratii* (nine non-toxic strains and seven toxic strains) are presently available; including four toxic strains associated with a single outbreak of foodborne botulism. However, initial indications from this very limited dataset suggest that botulinum neurotoxin strains of *C. baratii* could be distributed amongst non-toxic strains¹¹⁻¹³. A thorough understanding of the genotypic and phenotypic homogeneity of the *C. baratii* group will contribute to evaluating the human foodborne botulism risk.

2.7 Non-clostridial strains that contain potential botulinum neurotoxin genes

Non-clostridial bacterial strains have not been associated with human foodborne botulism. Recent data mining of published WGS has led to the discovery of genes in non-clostridial bacteria with partial homology to genes that encode botulinum neurotoxins. These include *Enterococcus faecium* from a cow¹⁴, *Weisella oryzae* from fermented rice¹⁵ and *Chryseobacterium piperi* from sediment¹⁶. The putative neurotoxin sequences differed from those of the neurotoxin serotypes presently described in clostridia. Recombinant DNA methods indicate a potential for biological activity, although it is not yet established whether biologically active botulinum neurotoxins are formed by these bacteria. It is presently unclear whether further non-clostridial strains contain undiscovered potential botulinum neurotoxin-encoding genes, and whether these strains could form botulinum neurotoxin, and potentially cause botulism. As some of these bacteria are from genera that are used as

probiotics in humans and animals, a watching brief should be maintained on any future developments regarding neurotoxin gene carriage and expression.

2.8 Conclusion

The understanding of the taxonomy and diversity of botulinum neurotoxin-forming clostridia and their neurotoxins has improved over the last few decades and this progression is likely to continue. New information indicates that it is unlikely that the properties of botulinum neurotoxin-forming clostridia and their neurotoxins have evolved significantly since 1992. A detailed understanding of the genotypic and phenotypic homogeneity of the various groups of botulinum neurotoxin-forming clostridia will contribute to assessment of the human foodborne botulism risk that is associated with botulinum neurotoxin-forming strains within these groups.

Non-clostridial strains with potential botulinum neurotoxin-encoding genes have not been shown to form botulinum neurotoxin, and have not been associated with foodborne botulism, and based on present evidence should not be considered as food safety hazards. However, it is prudent to maintain a close watch on scientific reports that identify botulinum neurotoxin type gene sequences in non-clostridia and to develop clearly identified protocols for responding, rapidly, to any significant new information.

3 Detection

For neurotoxin-producing clostridia in food the detection process is multifaceted. Detection refers to the detection and enumeration of spores (and/or viable organisms), to the detection and typing of toxin and to the detection and identification of toxin-encoding genes. Detection of neurotoxin in a laboratory setting, following a clinical diagnosis and subsequent treatment of illness, is paramount. In addition, the detection of toxin or spores of *C. botulinum*, in food or environmental samples, is a fundamental part of incident investigations and is a quantitative element contributing to scientific research programmes and risk assessments¹⁷. However, routine monitoring for the presence of spores, cells or toxin in food is not a practical component of food safety.

C. botulinum is a very serious human pathogen and detection involves potentially hazardous materials that require a high level of laboratory biosecurity at all stages. Laboratory work with *C. botulinum* is only pursued at a few specialist locations in the UK (including the UKHSA). Furthermore, because of the serious impacts associated with human botulism, the results of detection always require expert interpretation.

3.1 Healthcare settings

In emergency healthcare settings, a test to detect botulinum neurotoxin in a specimen of serum or faeces etc., immediately follows any clinical diagnosis of human botulism. Detection of toxin is not a requirement prior to the commencement of treatment and timely treatment with antitoxin is known to significantly reduce the severity of outcomes for human botulism cases. In an incident of foodborne botulism in the UK in 2011¹⁸ a clinical diagnosis followed two days after the initial report of symptoms. Neurotoxin was detected in a clinical sample, by Mouse Lethal Bioassay (MLB), approximately four days after the diagnosis and in a sample of food one day later (PCR assay identified spores and vegetative cells of *C. botulinum* in the food sample at approximately the same time as the MLB result).

The *in vivo* MLB is regarded as the gold standard for detection of botulinum neurotoxins in clinical and epidemiological settings. Results from the MLB establish, simultaneously, multiple elements of functionality for neurotoxin, including binding, internalisation and intracellular activity, which together confirm the ability to cause botulism. The MLB is effective for all toxin types and has sensitivity reported as 5-10 pg per ml. The MLB is usually performed with 0.5 ml intraperitoneal injection of an extract from the food or faecal sample; a human oral lethal dose of botulinum neurotoxin is estimated as ~1ng per kg bodyweight. In the clinical setting the MLB is routinely combined with an array of antitoxins (in cross neutralisation reactions) to provide information on toxin type, including complex situations that involve multiple toxin types, and strongly supports the tracking of outbreaks.

Although the MLB is very sensitive, robust and is strongly established, particularly from a public health perspective, it does have several drawbacks; not least a failure to meet the objectives of Reduction, Replacement and Refinement of animal tests (3 Rs) that are supported by many organisations including the European Union and the OECD. A requirement for expensive specialist animal facilities, turnaround times ~4-

5 days, and some issues surrounding the interpretation of negative results add to the ethical concerns and have stimulated exploration of improved alternative rapid and validated technologies for detection of botulinum neurotoxins¹⁹.

3.2 Technological solutions

Since 1992 detection for a range of toxin types and with sensitivity at least equal to that obtained by MLB has been achieved using a range of alternative methods²⁰.

- Modified *in vivo*, and *ex vivo*, methods dominantly employ stem cells or differentiated neurogenic cells to detect toxin activity directly. These methods are used mainly in research settings but do not improve on the slow turnaround time of the MLB and include a level of variability that is inconsistent with the demands of most clinical settings.
- Immunological detection systems, such as ELISA, that use antibodies specific to the botulinum neurotoxin antigens have rapid turnaround times (typically a few hours) and have been combined, effectively, with other techniques, such as mass spectrometry, to achieve good specificity. These methods can be automated, however, they may detect biologically inactive toxin.
- The enzymatic activity of botulinum neurotoxins (such as the ability to cleave the SNAP-25 protein) can be harnessed, often using a combination of specific antibodies, fluorescent dyes or mass spectrometry, to provide automated *in vitro* detection systems. The biological activity is strongly specific to each toxin type so that extension to detect a wide range of known types, and potential new toxins, is not trivial. In clinical settings the MLB is used to confirm biological activity in some scenarios.
- Nucleic acid-based methods^{21,22}, including PCR, real time PCR, MLST and WGS, can be used for the detection and characterisation of *C. botulinum*, botulinum neurotoxin-encoding genes, but not direct detection of toxin. Generally these methods are readily deployed in multiplex platforms but they do not establish biological activity. A large quantity of gene sequence information for *C. botulinum* is stored in easily accessible databases so that nucleic acid-based methods add powerful tools for use in epidemiological, surveillance and research settings.
- Multiplex PCR methods have been extended to include detection of genes that code for neurotoxins in *C. butyricum* and *C. baratii*.

Some of these methods have obtained regulatory approval but currently none satisfy the stringent requirements which are necessary to replace the MLB for the detection of botulinum neurotoxins in clinical settings.

3.3 Epidemiology settings

Epidemiological investigations are an essential response to human botulism outbreaks. Investigations include a search for toxins, or spores, in foods and food environments and prioritise detection methods that minimise false negatives, have rapid turnover and high throughput to maximise the potential for source tracking and risk management. *In vitro* immunoassays that can work with complex food matrices, sometimes used in combination with confirmatory MLB, are cost effective, operate well over a range of toxin concentrations and are used effectively to detect neurotoxin in public health investigations. Multiplex PCR assays have been

developed to provide accessible, rapid and cost-effective tools for detecting and screening unknown isolates from environmental samples for genes encoding botulinum neurotoxins (but not necessarily corresponding to toxin-producing capability). PCR assays and WGS can provide additional phylogenetic information that has particular value in outbreak investigations.

In epidemiological settings qualitative detection of neurotoxins, or *C. botulinum* spores, is usually combined with characterisations of toxin type, or genotype, to facilitate source tracking (linking clinical cases with contaminated foods). Increasingly, the characterisation step uses sophisticated nucleic technology, with an appropriate amplification step, to establish type matches with high precision.

3.4 Detection of spores and cells

There are no selective culture media for *C. botulinum* so that the detection of spores or vegetative cells involves specialist microbiology. Detection of spores usually involves anaerobic incubation of heated food followed by the detection of toxin (or encoding gene) in the enrichment fluid. ELISA methods with specific sets of antibodies, or multiplex PCR techniques with specialised sets of primers, are used to confirm the detection of spores with toxin types A, B, E and F. Enumeration of spores is achieved by statistical analysis of detection in diluted samples. In research settings this method can detect a few spores per kilogram in food materials²³.

Spores, inactivated toxins, toxin antigens or clostridial DNA do not pose foodborne botulism risks directly (infant botulism, adult infectious botulism and wound botulism are exceptions) so that pre-emptive testing of materials for *C. botulinum* has limited value for ensuring food safety. However, reliable detection of toxin, spores or viable cells, is an essential element of challenge tests that are a pillar of assurance during food product developments that involve new formulation or new processes.

C. botulinum and most clostridia reduce sulphite to sulphide under anaerobic conditions and the reaction is easily detected. However, this phenotype is common among many food bacteria, including many food spoilage bacteria and some non-spore forming bacteria, so that detection has minimal relevance for food safety (historically this reaction has been used as an indicator of hygiene for food production; particularly in the dairy sector). Genetic characterisations of sulphite-reducing spore formers, and sulphite-reducing bacteria, indicate the possibilities for the development of a molecular assay, based on sulphite reduction genes, that has increased sensitivity²⁴.

3.5 Research settings

In research settings the detection and isolation of *C. botulinum* in food materials has helped to inform risk assessments, produced detailed maps of phylogenetic relatedness to support epidemiology and built a picture of diversity, novel toxins, genotypes and horizontal gene transfer possibilities that improves understanding, helps development of therapeutics and aids public health preparedness. Many research activities highlight ongoing searches for sequence-specific primers and for high quality, pure, monoclonal antibodies to assist in the development of detection technologies for *C. botulinum*.

In silico detection methods, involving very powerful search techniques across multiple databases, have detected *C. botulinum* neurotoxin sequence homologs in a small number of non-clostridial strains but the biological significance is not, currently, understood.

3.6 Conclusions

Methods for the detection of botulinum neurotoxin in tissues and food materials are well established and provide strong support for clinical, epidemiological and research activities. Technological advances point to emerging test methodologies that maintain sensitivity and can potentially reduce the burden on the use of experimental animals and these developments should be widely supported.

Specialist laboratory techniques effectively detect spores, and viable cells, of *C. botulinum* in food materials and support research activity but do not establish surveillance as a realistic option for improved food safety.

4 Epidemiology

At the time of the 1992 report, foodborne, infant and wound botulism were reported worldwide but foodborne botulism was by far the most common form reported in the UK. In contrast, currently in the UK, wound and infant botulism are more common forms than that associated with food.

There has been no significant change in the disease symptoms reported since 1992²⁵⁻²⁷. It remains the case that foodborne botulism is such a rare disease in the UK that misdiagnosis is a possibility due to the association of symptoms, by General Practitioners, with other more common diseases²⁷⁻²⁹. Misdiagnosis may result in delayed response to cases and outbreaks and may warrant increased awareness amongst the medical profession. The US Centers for Disease Control and Prevention (CDC) has issued clinical guidelines for the diagnosis and treatment of botulism²⁷.

Investigations into many outbreaks of botulism have focused on the determination of the toxin type, rather than on the identification of the organism and its phenotype, and this has led to the loss of valuable data for subsequent risk assessment.

In this chapter, cases and outbreaks of botulism are reviewed firstly with respect to their geography and then with respect to the type of organism and its associations and therefore some repetition of events is evident.

4.1 Botulism – the global picture

Botulism is regularly reported throughout the world and Table 2 summarises the cases of all types of botulism from selected countries where data are available. Foodborne botulism is responsible for most reports in many countries, but it is not universally the most prevalent form of botulism. For example, in the USA, infant botulism is the most common form of botulism with 1862 reported cases between 2001 and 2017, vastly exceeding 326 foodborne cases. In the UK wound botulism causes significantly more cases than infant and foodborne botulism combined but this may partly reflect the nature of clinical investigations and their capability.

4.1.1 UK

The UK has an extremely low number of outbreaks of foodborne botulism with only 10 reported outbreaks involving 13 cases since 1992 (data to 2019⁸ are included in Table 3). There has been no increase in foodborne botulism in the UK in recent decades. There has been a noticeable change in the origin of foods associated with outbreaks; eight of the ten most recent outbreaks involved foods produced or illness acquired abroad. This highlights effectiveness of the safety controls applied by the UK food industry in relation to this organism. Food produced in the home caused the greatest number of outbreaks since 1992 and five of six outbreaks, where details of food storage were known, involved ambient storage. A single outbreak implicating chilled food was caused by temperature abuse. Where the cause or potential cause of an outbreak was identified the evidence indicates that established controls for *C. botulinum*, if applied correctly, would have prevented the incident.

4.1.2 Worldwide

A selection of outbreaks of foodborne botulism that have occurred globally since 1992 are detailed in Table 4³⁰. There has been no significant change in the nature of foodborne botulism in recent decades with the exception of the identification of rare cases caused by neurotoxigenic *C. butyricum* and *C. baratii*. A few outbreak strains originally thought to be proteolytic *C. botulinum* are now recognised as *C. sporogenes* (details are included later in this chapter). The proportion of botulism outbreaks implicating commercial foods is weighted towards chilled foods rather than ambient foods (details are included later in this chapter and in Table 4) and this may constitute a trend in relation to the food types causing outbreaks. However, it is possible that bias may have been introduced by supplementing the literature sources with review papers specifically on chilled foods. 42 of the 90 outbreaks reported involved non-commercially produced foods i.e. home produced, 40 involved commercially produced food and 8 were unclear. Temperature abuse was a contributory factor in 30 of 36 outbreaks where a cause was known or suspected. For commercially produced chilled foods (and one frozen food) where the cause was known or suspected 23 of 24 products were subject to some form of temperature abuse prior to the outbreak; the remaining product was consumed 3 days beyond the “Use By” date. Outbreaks implicating commercially produced products that were destined for ambient storage and where a cause was known or suspected occurred due to a variety of control failures including inadequate sterilisation, post-process contamination, inadequate formulation to prevent growth and toxin production and/or temperature abuse. In the case of both chilled and ambient storage of commercially produced foods, the established controls for *C. botulinum*, if appropriately applied, would have prevented these outbreaks. Novel food technologies are not a feature of reported botulism outbreaks which tend to implicate traditional technologies which have been widely employed for food production over many years e.g. canning.

4.1.2.1 France

France reported 402 outbreaks of human botulism between 1987 – 2016 consisting of 731 cases and 9 deaths³¹. Cooked ham from home-made preparation or from small scale producers was involved in 73.5% of the botulism outbreaks where the food source was identified. These outbreaks were mostly type B botulism; more specifically B4 (and non-proteolytic *C. botulinum*). The other sources of botulism were home-made canned vegetables or fruits (beans, asparagus, eggplant, spinach, pumpkin, chestnut), home-made meat or fish preparations and a small number of industrial foods (fish soup, chicken/beef sausage, chicken/enchiladas, ground meat, olives/dried tomatoes, fresh pasta carbonara). Most of the outbreaks with non-pork meat were type A botulism (and proteolytic *C. botulinum*). Two outbreaks of rare *C. baratii* neurotoxin type F (F7) botulism were observed in 2014 and 2015. Industrial ground meat prepared in a restaurant was identified as the source of one outbreak³². Previous cases of botulism attributed to *C. baratii* were generally associated with intestinal colonisation i.e. infant botulism or due to other predisposing conditions³³. The origin of the other *C. baratii* type F outbreak was not identified. *Clostridium butyricum* was implicated in a case of botulism in a 10-year-old boy with history of Meckel’s diverticulum and chronic constipation, presenting dysarthria, dry mouth, hypotony, respiratory failure, and cardiac arrest in 2011. Stool analyses were

positive for *C. butyricum* neurotoxin E5 by PCR and DNA sequencing up to 2 months after discharge. Botulism by intestinal colonisation with neurotoxicogenic *C. butyricum* from undetermined origin was strongly suspected³⁴.

4.1.2.2 Italy

Italy has one of the highest numbers of foodborne botulism cases in Europe with 1173 suspected foodborne cases between 1986 – 2015, of which 421 were laboratory-confirmed²⁵. Homemade canned foods were implicated in 80.5% (95/118 incidents, involving 143 persons) of confirmed outbreaks including an outbreak caused by restaurant canned green olives. Vegetables canned in oil and in brine/water were associated with 43.2% and 28.8%, respectively, of laboratory-confirmed outbreaks. Other types of food implicated in confirmed outbreaks were home-bottled tuna (7.6%), ham (5.9%), home-bottled meat (5.9%), salami/sausages (4.2%), cheese (2.5%) and tofu and seitan (1.7%). Among vegetables, the most frequent products involved in cases or outbreaks were mushrooms in oil, olives and turnip tops. Regarding fish products, home-canned tuna was the most common food linked to confirmed incidents. Cheese or dairy products were seldom associated with confirmed incidents despite a large outbreak caused by mascarpone cheese³⁵. Although not reported for foodborne botulism alone, 96% (316/330) of the laboratory confirmed incidents were due to toxin produced by proteolytic *C. botulinum* (neurotoxins Type B 79.1%, Type A 9.7%, Type F 0.3%, Type Ab 1.5% and Type Bf 0.6%). Of 36 cases of infant botulism in Italy from 1986 to 2015, *C. butyricum* neurotoxin type E was implicated in three cases. Botulism involving intestinal colonisation with *C. butyricum* neurotoxin type E was also found in two boys having a Meckel's diverticulum³⁶.

4.1.2.3 USA

There were 326 laboratory confirmed foodborne botulism cases in the USA between 2001 and 2017 of which 277 implicated a food or beverage (a food or beverage was a laboratory confirmed source of botulinum neurotoxin in 156 cases)³⁷. 47% of cases implicated food prepared in the home excluding canned foods, 29% home canned foods, 10% commercially canned foods and 6% other commercially prepared foods (no food preparation method was available for 8% of cases). Neurotoxin types A, E and B were responsible for most of the cases (65%, 25% and 7%, respectively). Outbreaks implicating commercial foods included a chilli meal, chilli sauce and nacho cheese sauce. A case of botulism implicating spaghetti with sauce/meat was attributed to *C. baratii* type F³⁸.

4.1.2.4 Turkey

A systematic review of botulism cases in Turkey from 1983 to 2017 identified 91 foodborne cases³⁹. Not all cases or suspected foods were tested for botulinum neurotoxin but 10 of 19 tested were positive for type A toxin (proteolytic *C. botulinum*). The top-ranking food responsible for cases was canned green beans (30% and 28 reports). Other reported foods include strained yoghurt (x10), home-made local cheese (x24), canned purslane (x16), non-specific canned food, canned *ferula communis* or fennel (x4), canned peppers, scrambled eggs with garlic sausages, canned fried mushrooms (x5) and unknown.

4.1.2.5 Iran

A review of botulism cases in Iran in the period 2007 – 2017 identified 252 confirmed cases of foodborne botulism, 743 suspected foodborne cases and 48 fatalities⁴⁰. The most commonly implicated foods, accounting for 34.1% of events, were home-prepared traditional processed fish (smoked fish, salted fish, ham, bacon, blood pudding, mosaic salami and sausage). Other implicated foods were commercially canned fish (28.6%), fish spawn (10.5%), dairy products (10.1%), vegetables and home-prepared legumes (9.7%), cottage cheese (5.9%) and canned fruits (1.1%).

4.1.2.6 Canada

There were 91 laboratory-confirmed outbreaks of foodborne botulism in Canada between 1985 and 2005 (205 cases and 11 deaths). Seventy-five outbreaks were associated with non-proteolytic *C. botulinum* type E; seven outbreaks associated with type A neurotoxin and five outbreaks with type B neurotoxin. The non-proteolytic *C. botulinum* neurotoxin type E outbreaks were attributed to consumption of traditionally prepared marine mammal and fish products by native communities (principally the Inuit of Nunavik in northern Quebec and the First Nations population of the Pacific coast of British Columbia). Two botulism outbreaks were attributed to commercial ready-to-eat meat products (pâté and cooked boneless pork) and three outbreaks to foods served in restaurants (chopped garlic in oil, bottled chanterelle mushrooms and baked potato). All involved type A or type B toxin. A further eight outbreaks were attributed to non-native home-prepared foods⁴¹. A review of foodborne outbreaks in British Columbia in the period 2009 – 2013 identified 3 botulism outbreaks and 1 death⁴². Implicated foods include fruit and vegetables, seafood and sauces/condiments.

4.1.2.7 Poland

Reviews identified that 54 of 109 botulism cases in Poland between 2014 and 2017 were due to home-produced foods and 55 due to commercial foods. Canned foods accounted for the vast majority of cases (84) with canned meat (other than pork) being the most frequently implicated food followed by canned fish, sausages and cured meat, canned pork, canned meat and vegetables and canned mushrooms, fruits and vegetables^{43–46}.

4.1.2.8 China

A review of botulism in China between 2004 and 2020 identified a total of 80 foodborne outbreaks with 386 illnesses and 55 deaths²⁹. The most common foods implicated were home-prepared traditional processed stinky tofu and dried beef, accounting for 51.2% of outbreaks. Contributory factors causing the outbreaks included improper processing and improper storage (77.5% of outbreaks). Initial misdiagnosis of illness occurred in 27.5% of cases. In an overview of type E botulism⁴⁷, 11 outbreaks between 1965 and 2005 implicated soy bean milk, fermented bean curd, raw dried beef, dried mackerel and blood sausage. The bacteria involved were either non-proteolytic *C. botulinum* type E or *C. butyricum* type E.

4.2 Association of clostridia and non-clostridia with foodborne botulism

4.2.1 Proteolytic *C. botulinum* and *C. sporogenes*

The vast majority of botulism cases associated with foods are caused by proteolytic *C. botulinum* neurotoxin types A and B. Rare cases implicating foods have been reported involving toxin type F.

It is widely recognised that proteolytic *C. botulinum* and *C. sporogenes* are closely related species. Recent genomic studies have highlighted that a number of strains of proteolytic *C. botulinum* should actually be classed as strains of *C. sporogenes* and *vice versa*, and that neurotoxigenic and non-neurotoxigenic strains of proteolytic *C. botulinum* and *C. sporogenes* exist⁸ (details of Taxonomy are included in Chapter 2 of this report). Four outbreaks of foodborne botulism have been attributed to *C. sporogenes* type B, including one case in the UK, along with rare cases of infant and wound botulism. As the historical identification of proteolytic *C. botulinum* associated with cases or outbreaks of botulism was based on the neurotoxin type this does not strongly affect the data presented in this chapter; neurotoxigenic *C. sporogenes* would have been incorrectly assigned to proteolytic *C. botulinum*. Moving forward it may be necessary to redefine historical data on botulism.

Proteolytic *C. botulinum* and neurotoxigenic *C. sporogenes* are far more resistant to adverse conditions than other *C. botulinum* groups, requiring stronger heat processes to destroy spores and lower pH and water activity to prevent growth in food. Foodborne outbreaks associated with proteolytic *C. botulinum* have not shown any significant change in pattern, in recent decades, that would indicate an increased risk to foods. The controls that have been in place to manage the survival, population growth and toxin production by proteolytic *C. botulinum* for many years appear robust and do not need modification (details of the Occurrence, Growth and Survival of *C. botulinum* are included in Chapter 5 of this report).

4.2.2 Non-proteolytic *C. botulinum*

Non-proteolytic *C. botulinum* neurotoxin types B and E (and very occasionally toxin type F) are associated with foodborne outbreaks of botulism, but generally to a lesser extent than proteolytic *C. botulinum* neurotoxin types A and B, although this does vary by country.

Non-proteolytic *C. botulinum* is much less resistant to processing used in the food industry than proteolytic *C. botulinum*. The spores are more readily destroyed by heat and growth is more readily controlled by pH or water activity than for proteolytic *C. botulinum*. However, non-proteolytic *C. botulinum* is able to grow at much lower temperatures than proteolytic *C. botulinum*; this includes growth at refrigeration temperatures (details of the Occurrence, Growth and Survival of *C. botulinum* are included in Chapter 5 of this report). This presents a risk in chilled, minimally processed, extended shelf-life foods and this led to the provision of FSA guidelines for manufacture and sale of chilled foods in the UK² (details of the FSA guidelines are included in Chapter 1 of this report).

Despite the risk presented by non-proteolytic *C. botulinum* in chilled foods there have been no reported outbreaks of botulism involving chilled foods from any commercial product where the food has been stored at the recommended chilled temperature and consumed within its designated shelf life. In a review of global botulism outbreaks⁴⁸ caused by chilled foods, in the period 1985 to 2015, 16 of 26 outbreaks identified (Table 5) were caused by proteolytic *C. botulinum*, one by *C. baratii* and four by non-proteolytic *C. botulinum* (all type E in vacuum packed fish). Five outbreaks did not identify the organism and three of these involved toxin type B (it is unknown whether proteolytic *C. botulinum* or non-proteolytic *C. botulinum* were causal). Temperature abuse was identified as the cause or most likely cause in 25 of the 26 outbreaks and consumption of the product beyond its “Use By” date was the reported cause of the remaining outbreak. In a review of botulism outbreaks in the USA between 1994 and 2021 that involved commercially produced foods intended to be stored chilled, 11 events were due to unrefrigerated storage by the consumer at home and the other two were due to unrefrigerated storage by the retailer prior to sale⁴⁹. In three of the events refrigeration instructions were deemed to be inadequate and contributory to the outbreak. In the 10 events where a toxin type was determined, all involved proteolytic *C. botulinum* (nine type A and one type Bf).

The lack of evidence for outbreaks caused by non-proteolytic *C. botulinum* in correctly stored chilled foods in the UK, both before and after the introduction of recommendations following the 1992 report, together with a similar lack of evidence from other countries throughout the world, provides some useful context regarding the magnitude of the risk and consequent need for controls in chilled foods. However, this review has not extensively examined the potential for under-ascertainment of botulism cases and outbreaks in other countries nor the legislative or industrial controls applied to foods globally. Currently it is not possible to use this insight in revising the risk from non-proteolytic *C. botulinum* in vacuum and modified atmosphere packaged chilled foods. Further study of these factors may provide more conclusive evidence that could indicate a lower risk to chilled foods from this organism that might, in turn, merit reduced controls or a focus on foods where the risk is greatest due to the known frequency of contamination and the associations with outbreaks i.e. vacuum packaged fish and seafood.

4.2.3 *C. botulinum* Group III

C. botulinum Group III (toxin types C and D) is often associated with animal botulism but has been implicated in human disease on rare occasions. In a recent review of all nine of the type C and D botulism outbreaks cited in literature, eight implicated foods; one was associated with infant botulism²⁶. Of the food outbreaks five had food vehicles suspected or confirmed as pâté (x2), smoked chicken, diseased chicken and home-made ham. The vast majority (seven) of these outbreaks were reported before 1992 when methods of identification and typing were less well advanced. Sporadic increases in animal botulism especially in farmed animals such as cows, cattle and chicken have raised concern regarding the potential for increased risk of transmission to humans. However, the ACMSF has reviewed such matters on a number of occasions and has considered the risk to humans to be low and supported the adequacy of current controls^{50,51}. There is no evidence that C.

botulinum Group III presents any new or increased risk in relation to human foodborne botulism.

4.3 Other neurotoxin-producing clostridia

4.3.1 *Clostridium butyricum*

Neurotoxicogenic *C. butyricum* type E has been reported in a small number of cases of infant botulism³³, adult intestinal botulism³⁶ and foodborne botulism in India⁵², China⁵³ and Italy⁵⁴. In the Indian incident clinical samples were not analysed and, despite the organism being isolated from a crisp made from gram flour, it was not possible to definitively confirm foodborne botulism. In a Chinese outbreak that occurred in 1994 implicating salted and fermented paste made of soybeans and wax gourds⁵⁵ six cases were clinically diagnosed with neurotoxin type E botulism that was also confirmed in the food although *C. butyricum* type E was only isolated from the food following further studies several years later⁵³. Retrospective analysis has also indicated an association of *C. butyricum* type E with other historical outbreaks of foodborne botulism in China^{47,56}. Type E botulism in China is most commonly associated with fermented grain/beans and raw meat and is frequently reported far from the sea (e.g. in Qinghai-Tibet plateau at an altitude of approximately 4-5 km). Canederli (bread dumplings) were the suspected food in a case of foodborne botulism in Italy in 1999 involving *C. butyricum* neurotoxin type E. Neurotoxicogenic *C. butyricum* type E is a relatively newly identified hazard. No foodborne outbreaks of this type have been reported in the UK. Low numbers of confirmed outbreaks in other countries indicates that the risk presented by this organism has not substantially changed.

4.3.2 *Clostridium baratii*

Clostridium baratii producing botulinum neurotoxin type F has been associated with several foodborne outbreaks of illness although it was first isolated in an infant botulism case in the USA⁵⁷ and has subsequently been associated with a number of further cases of infant botulism^{38,58} and adult intestinal botulism^{59,60}. Foodborne outbreaks have been reported in Spain, implicating individual meat pit pies⁶¹ and in France, where the food source was not identified although the only common item consumed by the two cases was an alcopop (which tested negative for growth and toxin⁶²) that reportedly contained 5% alcohol and had pH = 3.5. An outbreak in France implicated frozen and defrosted ground beef used for the production of spaghetti Bolognese at a restaurant where temperature abuse was again suspected as the underlying cause^{32,63}. Spaghetti and sauce mixture was implicated in a case of botulism caused by *C. baratii* type F in the USA in 2001³⁸. Raw deer meat was associated with an outbreak of foodborne botulism in Thailand in 2006, and a strain of *C. baratii* type F was isolated and its genome sequenced^{64,65}.

Neurotoxicogenic *C. baratii* is a relatively newly identified hazard. No foodborne outbreaks of this type have been reported in the UK. Low numbers of confirmed outbreaks in other countries indicates that the risk presented by this organism has not substantially changed.

4.3.3 *Clostridium argentinense*

Clostridium argentinense was originally isolated from soil samples in Argentina in the 1960s and despite being capable of producing botulinum neurotoxin type G, there are no cases of botulism that have been associated with this bacterium⁶⁵. *C. argentinense* is not covered further in this report.

4.3.4 Other bacteria with potential botulinum neurotoxin genes

The advent of genomics has allowed the identification of potential botulinum neurotoxin genes in a number of bacteria outside of the *Clostridium* genus (more details are included in Chapter 2 of this report). The presence of botulinum neurotoxin genes is of potential concern but none of these bacteria have been shown to be capable of forming botulinum neurotoxin in foods and there are no known cases or outbreaks of botulism associated with bacteria outside the genus *Clostridium*.

4.4 Conclusions

4.4.1 Outbreak identification and clinical diagnosis

Foodborne botulism in the UK is extremely rare but consequently this may result in delayed responses due to unfamiliarity in the diagnosis of cases and delays in reporting.

4.4.2 Organisms responsible for botulism

There has been no significant change in the nature of foodborne botulism in recent decades with the exception of the identification of rare cases caused by neurotoxigenic *C. butyricum*, *C. baratii* and *C. sporogenes*. Other clostridia and non-clostridia have been identified with botulinum neurotoxin genes but have not been implicated in cases of foodborne botulism in the UK or elsewhere.

4.4.3 Foodborne botulism trends

There has been a noticeable change in the nature of foods that are associated with outbreaks of foodborne botulism in the UK. Eight of the ten outbreaks in the last 30 years involved foods produced or illness acquired abroad and the majority of these involved home-production.

Botulism outbreaks implicating commercially produced chilled foods appear more prevalent than in previous decades although bias in ascertainment of evidence may have affected this conclusion. The vast majority of botulism outbreaks, for both chilled or ambient stored foods, are identified with proteolytic *C. botulinum*, i.e. those organisms that do not grow under chilled conditions, and temperature abuse is the single most common cause identified for foodborne outbreaks. In relation to outbreaks in the last 30 years, in the UK and worldwide where a cause can be identified, there is evidence to believe that known controls for the organism, combined with the correct storage of the foods, if applied correctly, would have prevented the incident. Novel food technologies are not a feature of botulism outbreaks and most implicate traditional technologies, such as canning, employed for food production.

4.4.4 Vacuum packaged, extended shelf-life chilled foods

There have been no reported outbreaks of botulism globally in chilled foods from any commercial product where the food has been stored at the recommended chilled temperature and consumed within its designated shelf life. There has not been an extensive review of the potential for under-ascertainment of botulism cases and outbreaks in other countries nor the legislative or industrial controls applied to foods globally. However, further study of these factors may provide evidence that could indicate a lower risk to chilled foods that might, in turn, merit reduced or more focused controls. Nevertheless, the evidence indicates that where chilled foods are associated with outbreaks of botulism this is almost exclusively due to temperature abuse and in most cases is caused by proteolytic *C. botulinum*.

Table 2: Botulism cases in selected countries

Country	Period	Foodborne cases	Infant botulism	Wound botulism (confirmed only)	Adult intestinal botulism	Inhalation	Iatrogenic	Other	Reference
UK	1992 - 2019	13 (A, B, Bf)	16 (A, B, Bf, E – <i>C. butyricum</i>)	112 (A, B, AB)	1 (NT)				Brunt et al., 2020 ⁸
France	1992-2016	574 (A, B, AB, E, F - <i>C. baratii</i>)		1 (B)	1 (E – <i>C. butyricum</i>)	2 (B)			Rasetti-Escargueil et al., 2020 ²⁶
France	2004 - 2016		17 (A, B, AB, Bf)						Rasetti-Escargueil et al., 2020 ²⁶
Italy	1986 - 2015	421 (Not reported)	36 (A, B, Ab, Bf, E – <i>C. butyricum</i>)	6 (B)	3 (A, E – <i>C. butyricum</i>)				Anniballi et al., 2017 ²⁵
USA	2001 - 2017	326 (A, B, E, F)	1862 (A, B, Ab, Ba, Bf, E, F, F - <i>C. baratii</i>)	372 (A, B, AB)	10 (A, F)		7 (A, B)	5 F – <i>C. baratii</i>	Lúquez et al., 2021 ³⁷ ; CDC, 2022 ⁶⁶

Table 3: Summary of botulism cases recorded in the UK since the publication of the 1992 ACMSF Report on Vacuum Packaged Foods and underlying causes (Adapted from Brunt et al., 2020⁸).

Year	Cases	Organism (toxin type) ^a	Toxin subtype	Food	Country of origin	Storage (cause)	Reference
1998	2	Prot (B)	B2	Home produced bottled mushrooms	Italy	Ambient (Inadequate controlling factors)	McLauchlin et al., 2006 ⁶⁷
2003	1	Cspo (B)	B1	Home produced Sausage	Poland	Unknown (Inadequate controlling factors likely)	McLauchlin et al., 2006 ⁶⁷
2004	1	Un (un)	nt ^b	Commercial Hummus	UK	Chilled (Temperature abuse)	McLauchlin et al., 2006 ⁶⁷
2004	1	Prot (A)	nt	Unknown	Georgia	Unknown	McLauchlin et al., 2006 ⁶⁷
2005	1	NP (B)	B4	Home preserved pork	Poland	Ambient (Inadequate controlling factors)	McLauchlin et al., 2006 ⁶⁷
2010	1	Un (B)	nt	Unknown	Algeria	Unknown	Brunt et al., 2020 ⁸
2011	3	Prot (A)	A1	Commercial korma sauce	UK	Ambient (Unknown cause)	Browning et al., 2011 ¹⁸
2012	1	Prot (B)	B2	Commercial olives	Italy	Ambient (Unknown but inadequate controlling factors likely)	Brunt et al., 2020 ⁸
2013	1	Un (un)	nt	Home produced Mushrooms	Poland	Ambient (Inadequate controlling factors)	Brunt et al., 2020 ⁸ ; Brola et al 2013 ⁶⁸
2016	1	Un (B)	nt	Tuna??	Italy	Unknown storage and controlling factors	Brunt et al., 2020 ⁸

^aProt: proteolytic *C. botulinum*, NP: non-proteolytic *C. botulinum*, Cspo: *C. sporogenes*, un, unknown.

^b not tested in the present study.

Table 4: Reported outbreaks of botulism in foods occurring since the publication of the 1992 ACMSF Report on Vacuum Packaged Foods (for UK, see Table 3).

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
1990	United States	3	B	Point	Unclear	Confirmed	Surgeon fish (palani)	-	Kershaw, 1991 ⁶⁹
1991	Egypt	97	E	Point	Commercial	Confirmed	Uneviscerated gray mullet fish (faseikh) – not reported	Temperature abuse during processing ^b	Weber, 1993 ⁷⁰ ; Hibbs, 1996 ⁷¹
1992	United States	4	E	Point	Non-commercial: other	Confirmed	Uneviscerated fish (moloha) – not reported	-	French, 1992 ⁷²
1993	United States	8	A	Intermittent common	Commercial	Confirmed	Cheese sauce - ambient	Contamination and temperature abuse ^b	Townes, 1996 ⁷³ , Newkirk, 2012 ⁷⁴
1993	Italy	7	B	Intermittent common	Commercial	Suspected	Canned eggplant in oil - ambient	Inadequate controlling factors	D'Argenio, 1995 ⁷⁵
1994	United States	30	A	Intermittent common	Commercial	Confirmed	Baked potato dip - ambient	Temperature abuse	Angulo, 1998 ⁷⁶ , Newkirk, 2012 ⁷⁴
1994	United States	1	A	Point	Commercial	Confirmed	Black bean dip - chilled	Temperature abuse - product not refrigerated	Sobel et al. 2004 ⁷⁷
1994	United States	2	A	Point	Commercial	Confirmed	Clam chowder - chilled	Temperature abuse -	Sobel et al. 2004 ⁷⁷

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
								product not refrigerated	
1995	Canada	3	E	Point	Non-commercial: other	Confirmed	Fermented seal meat	-	Proulx, 1997 ⁷⁸
1995	Canada	5	E	Point	Non-commercial: other	Confirmed	Fermented walrus meat	-	Proulx, 1997 ⁷⁸
1995	Canada	2	B	Point	Commercial: other	Confirmed	Country style pâté– chilled	Temperature abuse – product not refrigerated	Leclair et al., 2013 ⁴¹
1996	Italy	8	A	Intermittent common	Commercial	Confirmed	Mascarpone cheese – chilled	Temperature abuse	Aureli, 1996 ⁷⁹ ; Aureli, 2000 ³⁵
1996	India	34	Unknown	Point	Unclear	Suspected	Sevu (gram flour crisp)	-	Chaudhry, 1998 ⁵²
1996*	Spain	2	Unknown	Point	Non-commercial: other	Suspected	Green beans	-	Polo, 1996 ⁸⁰
1997	United States	1	Bf	Point	Commercial	Suspected	Burrito – chilled	Temperature abuse	Sobel et al., 2004 ⁷⁷
1997	Thailand	6	Unknown	Unclear	Non-commercial: home-canned	Suspected	Canned bamboo shoots	-	Swaddiwu dhipong, 2000 ⁸¹
1997	Germany	2	E	Point	Commercial	Confirmed	Hot-smoked whitefish VP – chilled	Unknown – Temperature abuse ^b	Korkeala, 1998 ⁸²
1998	Argentina	9	A	Intermittent common	Non-commercial: other	Suspected	Matambre meat roll	-	Villar, 1999 ⁸³
1998	Thailand	13	A	Point	Non-commercial: home-canned	Confirmed	Canned bamboo shoots	-	Pantukosit, 2007 ⁸⁴ ;

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
									Swaddiwu dhipong, 2000 ⁸¹ ; Wongwatcharapaiboon, 1999 ⁸⁵
1998*	United States	3	B	Point	Non-commercial: other	Confirmed	Peyote	-	Hashimoto, 1998 ⁸⁶
1999	Canada	4	B	Point	Non-commercial: home-canned	Suspected	Canned tomatoes	-	Loutfy, 2003 ⁸⁷
1999*	Turkey	4	Unknown	Point	Non-commercial: home-canned	Suspected	Uncooked canned vegetables	-	Erol, 1999 ⁸⁸
1999	France	1	A	Point	Commercial	Suspected	Fish soup – chilled	Temperature abuse – at home	Carlier et al., 2001 ⁸⁹
2000	France	9	B	Point	Non-commercial: home-canned	Suspected	Canned asparagus	-	Abgueguen, 2003 ⁹⁰
2001	United States	14	E	Point	Non-commercial: other	Suspected	Fermented beaver tail and paw	-	Horn, 2001 ⁹¹
2001	United States	16	A	Intermittent common	Commercial	Confirmed	Frozen chili	Temperature abuse	Kalluri, 2003 ⁹² ; Newkirk, 2012 ⁷⁴
2001	Iran	2	Unknown	Point	Unclear	Suspected	Salted fish	-	Vahdani, 2002 ⁹³
2001	Canada	2	E	Point	Non-commercial: home-canned	Confirmed	Jar of salmon roe	-	Dawar, 2002 ⁹⁴
2001	Canada	2	E	Point	Non-commercial: other	Confirmed	Stink eggs	-	Dawar, 2002 ⁹⁴

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
2001	Canada	1	A	Point	Commercial	Confirmed	Cooked boneless pork product - chilled	Temperature abuse – product not refrigerated	Leclair et al., 2013 ⁴¹
2001	United States	1	F – <i>C. baratii</i>	Point	Non-commercial: other	Confirmed	Spaghetti noodles and meat sauce	-	Harvey et al., 2002 ³⁸
2002	United States	8	E	Intermittent common	Non-commercial: other	Confirmed	Raw beached whale (muktuk)	-	McLaughlin, 2004 ⁹⁵ ; Middaugh, 2003 ⁹⁶ ; Newkirk, 2012 ⁷⁴
2003	United States	1	A	Point	Commercial	Confirmed	Clam chowder	Temperature abuse	CDC, 2003 ⁹⁷
2004	United States	4	A	Point	Non-commercial: other	Suspected	Pruno	-	Vugia, 2009 ⁹⁸
2004	Germany	1	E	Point	Commercial	Suspected	Vacuum packed smoked salmon	Consumed 3d after Use By date	Dressler (2005) ⁹⁹
2004	Italy	28	B	Intermittent common	Commercial	Suspected	Green olives - ambient	Improper preservation	Cawthorne, 2005 ¹⁰⁰
2004*	India	2	Unknown	Point	Non-commercial: other	Suspected	Canned meat, preserved curd	-	Agarwal, 2004 ¹⁰¹
2005	Turkey	10	A	Point	Non-commercial: other	Confirmed	Suzme yoghurt buried in soil	-	Akdeniz, 2007 ¹⁰²
2005	United States	5	E	Intermittent common	Non-commercial: other	Confirmed	Salted fish	-	Sobel, 2007 ¹⁰³
2005*	Turkey	5	Unknown	Point	Non-commercial: home-canned	Suspected	Canned mushrooms	-	Cengiz, 2006 ¹⁰⁴

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
2006	United States, Canada	6	A	Intermittent common	Commercial	Confirmed	Carrot juice - refrigerated	Temperature abuse	Shuler, 2006 ¹⁰⁵ ; Brown, 2010 ¹⁰⁶ ; Sheth, 2008 ¹⁰⁷
2006	Austria	5	Unknown	Point	Non-commercial: other	Suspected	Barbequed pork	-	Meusburger, 2006 ¹⁰⁸ ; Topakian, 2009 ¹⁰⁹
2006	Finland	2	E	Point	Commercial	Suspected	Smoked whitefish - chilled	Temperature abuse ^b	Lindstrom, 2006 ¹¹⁰
2006	United States	2	A	Point	Non-commercial: other	Confirmed	Fermented tofu	-	Meyers, 2007 ¹¹¹
2006	Taiwan	5	B	Unclear	Non-commercial: other	Confirmed	Fermented raw goat meat (cinkrugan)	-	Tseng, 2009 ¹¹²
2006*	Italy	2	Unknown	Point	Non-commercial: other	Suspected	Preserved asparagus	-	Zanon, 2006 ¹¹³
2007	United States	8	A	Intermittent common	Commercial	Confirmed	Canned chili sauce - ambient	Processing failure	Juliao, 2013 ¹¹⁴ ; Newkirk, 2012 ⁷⁴ ; Ginsberg, 2007 ¹¹⁵
2007	China	66	A	Intermittent common	Commercial	Suspected	Sausage – not reported	Temperature abuse	Zhang, 2010 ¹¹⁶
2008	Turkey	8	B	Point	Commercial	Suspected	Unprocessed black olives - ambient	Unknown	Swaan, 2010 ¹¹⁷

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
2008	France	2	A	Point	Commerical	Confirmed	Chicken enchiladas - chilled	Temperature abuse – product not refrigerated and consumed 1 day after Use By date	King & the French Multidisciplinary Outbreak Investigation Team (2008) ¹¹⁸
2008	Uganda	3	A	Point	Non-commercial: other	Suspected	Oil-based condiment	-	Viray, 2014 ¹¹⁹
2008	United States	6	A	Point	Non-commercial: home-canned	Confirmed	Canned carrots and green beans	-	Date, 2011 ¹²⁰
2009	France	3	E	Point	Commercial	Suspected	Vacuum packed hot-smoked whitefish (VP) - chilled	Temperature abuse ^b	King, 2009 ¹²¹
2009	United States	3	A	Point	Non-commercial: home-canned	Confirmed	Canned green beans	-	Date, 2011 ¹²⁰
2009	United States	3	A	Point	Non-commercial: home-canned	Confirmed	Canned asparagus	-	Date, 2011 ¹²⁰
2010	France	5	A	Point	Unclear	Confirmed	Canned green beans or pork chops	-	Oriot, 2011 ¹²²
2011	United States	8	A	Point	Non-commercial: other	Suspected	Pruno	-	Thurston, 2012 ¹²³ ; Williams, 2014 ¹²⁴
2011	United States	1	A	Point	Commercial	Suspected	Potato soup - chilled	Temperature abuse	CDC., 2011 ¹²⁵

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
2011	United States	1	A	Point	Commercial	Suspected	Potato soup - chilled	Temperature abuse	CDC., 2011 ¹²⁵
2011	Spain	5	F – <i>C. baratii</i>	Point	Unclear	Suspected	Indeterminate	-	Lafuente, 2013 ⁶¹
2011	France	9	A	Intermittent common	Commercial	Confirmed	Green olive paste - ambient	Incorrect sterilisation	Pingeon, 2011 ¹²⁶
2011	Finland	2	B	Intermittent common	Commercial	Confirmed	Jar of olives - ambient	Post process contamination	Jalava, 2011 ¹²⁷ ; Forss, 2012 ¹²⁸
2011*	Turkey	4	Unknown	Point	Non-commercial: home-canned	Suspected	Canned red peppers	-	Agacayak, 2011 ¹²⁹
2012	Canada	3	E	Point	Commercial	Confirmed	Salt-cured fish (fesikh) – unknown need paper	Unknown – need access	Walton, 2014 ¹³⁰
2012	United States	8	A	Point	Non-commercial: other	Confirmed	Pruno	-	Briggs, 2013 ¹³¹
2012	Japan	2	A	Point	Commercial	Confirmed	Adzuki batto (bean soup) (VP) – not reported	Temperature abuse ^b	Momose, 2014 ¹³²
2012	United States	2	Unknown	Point	Commercial	Suspected	Broccoli soup - chilled	Temperature abuse	Edmunds et al., 2022 ⁴⁹
2012	Thailand	2	B	Point	Unclear	Confirmed	Fermented crab	-	Wangroongsarb, 2013 ¹³³
2013	China	12	A	Point	Commercial	Confirmed	Smoked ribs (restaurant) – chilled	Temperature abuse ^b	Feng, 2015 ¹³⁴

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
2013	Greenland	5	E	Unclear	Non-commercial: other	Suspected	Eider fowl	-	Hammer, 2015 ¹³⁵
2013	Iran	5	Unknown	Unclear	Non-commercial: home-canned	Suspected	Canned cheese (kupeh)	-	Faridaalae e, 2013 ¹³⁶
2014	France	2	F – <i>C. baratii</i>	Point	Commercial	Suspected	Alcopop?	Unknown	Castor, 2015 ⁶²
2014	Spain	3	Unknown	Point	Non-commercial: other	Confirmed	Stew	-	Buj-Jorda, 2015 ¹³⁷
2014	USA	2	B	Intermittent common	Commercial	Confirmed	Jar of pesto - ambient	Unlicensed premise; inadequate processing	Burke, 2016 ¹³⁸
2015	Slovakia	1	A	Intermittent common	Commercial	Confirmed	Hummus (pouches) Chilled	Temperature abuse ^b	Mad'arova, 2017 ¹³⁹
2015	USA	29	A	Point	Non-commercial: home-canned	Confirmed	Canned potatoes	-	McCarty, 2015 ¹⁴⁰
2015	France	3	F – <i>C. baratii</i>	Point	Non-commercial: other	Suspected	Meat (ground) frozen used in sauce by Restaurant	Temperature abuse	Trehard, 2016 ⁶³ , Mazuet et al., 2017 ¹²
2015*	New Zealand	1	Unknown	Point	Commercial	Suspected	Risotto - Chilled	Temperature abuse	Smyth et al., 2015 ¹⁴¹
2016	United States	1	A	Point	Commercial	Confirmed	Grain and vegetable product - chilled	Temperature abuse	CDC, 2016 ¹⁴²
2017*	Spain	2	Unknown	Point	Commercial	Confirmed	Canned beans - ambient	Not reported	Ametller, 2017 ¹⁴³
2017	USA	2	A	Point	Commercial	Suspected	Prepackaged Pouches of Liquid	Contamination and	Kim, 2019 ¹⁴⁴

Year	Location	Cases	Toxin type	Exposure source	Food vehicle	Laboratory-confirmed or suspected ^a	Implicated/suspected Food – typical storage	Cause	References
							Herbal Tea - Chilled	temperature abuse ^b	
2017	USA	10	A	Intermittent common	Commercial	Confirmed	Nacho cheese sauce (Pouch) - Ambient	Contamination and temperature abuse ^b	Rosen, 2020 ¹⁴⁵
2018	Nigeria	3	Unknown	Point	Non-commercial: other	Suspected	Fish pepper soup	-	Okunromade, 2020 ¹⁴⁶
2018	USA	3	A	Point	Non-commercial: home-canned	Confirmed	Canned peas	-	Bergeron, 2019 ¹⁴⁷
2019	United States	4	A	Point	Commercial	Suspected	Potato product - chilled	Temperature abuse	Edmunds et al., 2022 ⁴⁹
2019	China	4	A, B, E	Point	Commercial	Suspected	Fish and ham (VP) – not reported	Not reported	Min, 2021 ¹⁴⁸
2020	Italy	35	Unknown	Point	Unknown	Suspected	Salad	Unknown	FSN, 2020 ¹⁴⁹
2020*	Portugal	1	Unknown	Point	Unknown	Suspected	Unknown	Unknown	Costa, 2021 ¹⁵⁰
2021	United States	1	A	Point	Commercial	Confirmed	Clam chowder - chilled	Temperature abuse	Edmunds et al., 2022 ⁴⁹

^a Laboratory-confirmed or suspected food

^b Likely cause but not definitive

* Outbreak year not reported, publication year used instead

Table 5: Examples of foodborne botulism outbreaks involving commercial foods intended to be stored chilled (Peck et al., 2020⁴⁸).

Country (year)	Product	Organism (toxin type)	Cases (deaths)	Factors contributing to outbreak	References
Canada (1985)	Garlic-in-oil	Prot (B)	36	No preservatives; temperature abuse	St. Louis et al. (1988) ¹⁵¹
UK (1989)	Hazelnut yoghurt	Prot (B)	27(1)	Toxin added with canned hazelnut conserve to correctly chilled yoghurt	O'Mahony et al. (1990) ¹⁵²
USA (1989)	Chopped garlic-in-oil	Prot (A)	3	Temperature abuse (product not refrigerated)	Morse et al. (1990) ¹⁵³
USA (1990)	Grilled fresh Palani (surgeon fish)	NR (B)	3	Temperature abuse	CDC (1991) ¹⁵⁴
USA (1993)	Canned cheese sauce (restaurant)	Prot (A)	8(1)	Contamination of canned cheese sauce after opening, then temperature abuse (opened tin not refrigerated)	Townes et al. (1996) ⁷³
USA (1994)	Potato dip ("skordalia") and aubergine dip ("meligianoslata") (restaurant)	Prot (A)	30	Toxin added with temperature-abused baked potatoes to correctly chilled yoghurt dishes	Angulo et al. (1998) ⁷⁶
USA (1994)	Clam chowder	Prot (A)	2	Temperature abuse (product not refrigerated)	Sobel et al. (2004) ⁷⁷
USA (1994)	Black bean dip	Prot (A)	1	Temperature abuse (product not refrigerated)	Sobel et al. (2004) ⁷⁷
Canada (1995)	Country-style pâté	NR (B)	2	Temperature abuse (product not refrigerated)	Leclair et al. (2013) ⁴¹
Italy (1996)	Mascarpone cheese	Prot (A)	8(1)	Temperature abuse; pH > 6	Aureli et al. (2000) ³⁵
Germany (1997)	Hot-smoked, vacuum-packed fish	NP (E)	2	Suspected temperature abuse	Korkeala et al. (1998) ⁸²
Argentina (1998)	Meat roll ("matambre")	Prot (A)	9	Insufficient cooking, lack of preservatives, vacuum-packed in heat-shrunk plastic, & inadequate refrigeration	Villar et al. (1999) ⁸³
France (1999)	Fish soup	Prot (A)	1	Temperature abuse at home	Carlier et al. (2001) ⁸⁹

Country (year)	Product	Organism (toxin type)	Cases (deaths)	Factors contributing to outbreak	References
Canada (2001)	Cooked boneless pork product	Prot (A)	1	Temperature abuse (product not refrigerated)	Leclair et al. (2013) ⁴¹
Germany (2004)	Vacuum-packed smoked salmon	NP (E)	1	Consumed 3 days after 'Use By date'	Dressler (2005) ⁹⁹
UK (2004)	Organic hummus	NR	1	Time/temperature abuse	McLauchlin et al. (2006) ⁶⁷
Canada/USA (2006)	Refrigerated carrot juice	Prot (A)	6	Temperature abuse; product pH between 6 and 7	Sheth et al. (2008) ¹⁰⁷
Finland (2006)	Vacuum-packed smoked whitefish	NP (E)	1	Suspected temperature abuse	Lindström et al. (2006) ¹¹⁰
China (2007)	Sausages	Prot (A)	66	Temperature abuse (product not refrigerated)	Zhang et al. (2010) ¹¹⁶
France (2008)	Chicken enchiladas	Prot (A)	2	Time/temperature abuse (product not refrigerated); consumed 1 day after 'Use By date'	King & the French Multidisciplinary Outbreak Investigation Team (2008) ¹¹⁸
France (2009)	Vacuum packed hot-smoked whitefish	NP (E)	3	Suspected temperature abuse during travel and home storage	King et al. (2009) ¹²¹
Italy (2010)	Cream of vegetable soup	NR (B)	1	Temperature abuse (product not refrigerated); long shelf-life	Daminelli et al. (2011) ¹⁵⁵
USA (2011)	Potato soup	Prot (A)	2	Temperature abuse (product not refrigerated)	CDC (2011) ¹²⁵
New Zealand (2015)	Chilled, ready-to-eat risotto	NR	1	Time/temperature abuse (product not refrigerated; consumed several months past best-before date)	Smyth et al. (2015) ¹⁴¹
Slovakia (2015)	Hummus spread	Prot (A)	1	Suspected temperature abuse	Mad'arova et al. (2017) ¹³⁹
France (2015)	Frozen minced beef used in restaurant Bolognese sauce	<i>C. botulinum</i> (F)	3	Time/temperature abuse (sauce prepared ≥24 h in advance, left at room temperature for several hours)	Mazuet et al. (2017) ³²

Prot: proteolytic *C. botulinum*, NP: non-proteolytic *C. botulinum*, NR: not reported

5 Occurrence, Growth and Survival

The occurrence in foods and the physiological responses in food environments for *C. botulinum*, and other clostridia that form botulinum neurotoxin, are summarised below with emphasis on the organisms that have been associated with foodborne botulism.

5.1 Spore loads in foods

Spores of *C. botulinum* and other neurotoxigenic clostridia are highly resistant to physical and chemical stresses such as desiccation, cold temperatures and weak acids and so are widely distributed in the environment. These spores can be found in the intestinal contents of animals, including humans, in soils and in both salt and freshwater sediments. It is considered impossible to be certain that unprocessed food materials do not contain spores of *C. botulinum* or other neurotoxigenic clostridia.

Dedicated surveys, using specialist detection protocols, can estimate the prevalence and the concentration of *C. botulinum* spores in food materials. In ideal conditions the best laboratory techniques, which include a non-specific enrichment step, can achieve a detection limit in the order of a few spores of *C. botulinum* per kg of food. Estimates of spore numbers are always uncertain but surveillance also highlights significant variability associated with *C. botulinum* spore loads in different batches of food materials. Surveys of materials used in the manufacture of chilled foods, in France¹⁵⁶ and in the UK¹⁵⁷, indicate typical spore concentrations for *C. botulinum* centred around 5-10 spores per kg. Recent examination of 74 vegetable products from Finland and Germany¹⁵⁸ highlight load variability and estimated much higher concentrations, up to 1200 cells per kg, for one product.

Information gathered from investigation of foodborne botulism outbreaks indicates some associations between spore types and specific food sources (e.g. spores from non-proteolytic *C. botulinum* type E have been associated with fish from arctic and North American saltwater fisheries and proteolytic *C. botulinum* type A botulism is often associated with failure to correctly apply the botulinum cook during production of canned and bottled foods) but currently the relationships cannot be generalised.

A survey in the UK¹⁵⁹ estimated the prevalence of *C. butyricum* in a wide range of foods as 31% but did not find any neurotoxigenic strains among the isolates. Currently there is no published evidence concerning prevalence of neurotoxigenic *C. sporogenes* or *C. baratii* in food materials. Recent surveillance does not identify significant trends in the occurrence of *C. botulinum* or other neurotoxigenic clostridia but does increase confidence that, for traditional food materials, large spore loads have only small probability. It is not possible to extrapolate information from targeted surveillance studies to account for untested food materials such as alternative protein sources or specialist dehydrated dairy ingredients that might involve a concentration step in their preparation. Current surveillance methodology cannot rule out undetected loads of *C. botulinum*, with a few spores per kg, in food. The costs associated with high quality anaerobic microbiology and the significant variability

observed for spore loads ensures that routine surveillance for *C. botulinum*, and other neurotoxin-forming clostridia, in food materials is impractical and uninformative.

5.2 Germination, growth and toxin production

In considering the response of *C. botulinum* and other neurotoxic clostridia to environmental factors, it is important to recognise that the ability to form spores and toxin introduces additional considerations for control in comparison to vegetative, non-toxicogenic pathogens. To produce toxin and cause illness, the organisms must be in a vegetative state and metabolising. In many foods, particularly those subject to a heat process, the organism may only be present in a sporulated state and must first germinate before it can produce toxin. Hence, there are potentially three 'states' for the organism where control can be applied; germination, growth and toxigenesis.

Historically, it was widely accepted that population growth, represented by an increase in the number of cells of the organism, occurred before toxin was produced. This was the assumption used in the 1992 report where it was stated that "growth studies can indeed be used to indicate the risk of toxin formation". This came from research, particularly experiments performed in microbiological broth, demonstrating that toxin production occurs in late exponential phase as the organism enters stationary phase and sporulates¹⁶⁰. However, published research since 1992 has demonstrated toxin production in food in the absence of measurable increases in the population size¹⁶¹ so that growth may not be a reliable indicator of toxin formation. Rapid die-off of cells after toxin production could also be misinterpreted as an absence of population growth. In 2020, an ACMSF subgroup on non-proteolytic *Clostridium botulinum* and vacuum and modified atmosphere packaged foods¹⁶² recommended that "detection of toxin is a minimum requirement for challenge testing, and that measuring viable counts is of merit in ensuring safety". The merit in measuring viable counts is based on evidence that growth may precede toxin production and therefore when using growth and toxin production to assess the effect of controlling factors, the presence of toxin or the observation of population growth (commonly when the logarithm of the population size increases by 0.5 or more) would demonstrate unsafe conditions. The following sections on controlling factors include studies conducted on growth and/or toxin production and should be considered in light of the limitations described above.

5.3 Minimum growth temperatures

The temperature during storage affects the germination of spores and the growth of vegetative cells of *C. botulinum* and other clostridia. There are also interactions between temperature, pH, and water activity that affect growth and survival of *C. botulinum* and other clostridia. An understanding of these factors and their interaction is essential for food safety with regard to these organisms (interactions between controlling factors are discussed later in this chapter).

5.3.1 Proteolytic *C. botulinum*

Spores of proteolytic *C. botulinum* survive for long periods under normal frozen conditions, typically at -18°C , applied to foods. Proteolytic *C. botulinum* grows between 12°C and 48°C . Optimal growth temperatures (for fastest growth) are

between 35°C and 45°C. Growth at or below 10°C has been shown not to occur under otherwise optimal growth conditions and growth at 15°C is slow¹⁶³.

5.3.2 Non-proteolytic *C. botulinum*

Spores of non-proteolytic *C. botulinum* survive for long periods under normal frozen conditions, typically at -18°C, applied to foods. The temperature range for growth and toxin formation by non-proteolytic *C. botulinum*^{164,165} is 3°C to 37°C, with indications for growth at higher temperature¹⁶⁶, but optimal growth temperatures (for fastest growth) are between 28°C and 30°C. Growth at 8°C under otherwise optimal conditions is slow.

5.3.3 Other clostridia capable of producing botulinum neurotoxin

Spores of other clostridia survive for long periods under normal frozen conditions, typically at -18°C, applied to foods. There is no evidence to distinguish the growth parameters of neurotoxicogenic *C. sporogenes* from those for proteolytic *C. botulinum*. The minimum temperature for growth of non-neurotoxicogenic *C. butyricum* has been reported¹⁶⁷ as 10°C. The minimum temperature for growth of two neurotoxicogenic strains of *C. butyricum* (ATCC 43181 and 43755) in anaerobic broth are reported as 10°C and 11°C respectively. Some of six neurotoxicogenic strains of *C. butyricum* involved in outbreaks of botulism in Italy, when incubated in anaerobic broth at pH = 7.0, grew and produced toxin at 12°C but not at 10°C when observed over 180 days⁵⁴. There is only one published report on the minimum growth temperature of neurotoxicogenic *C. baratii*, using a single isolate from a case of infant botulism^{168,169}, and this determined that growth occurred at 15°C but not at 10°C.

5.4 Thermal resistance of spores

A heating step during processing can kill spores or vegetative cells of *C. botulinum*, and other clostridia, depending on intensity (temperature) and duration.

5.4.1 Proteolytic *C. botulinum*

A meta-analysis of thermal inactivation for spores of proteolytic *C. botulinum*, including data up to 2006, gave mean value $\text{Log}(D_{120\text{C}}) = -0.78$ and a standard deviation of 0.23 for the dataset. Inverting the mean value of the logarithm gives $D_{120\text{C}} \sim 0.17$ minutes but this cannot easily be identified with the arithmetic mean D-value. The analysis of D-values gave $z = 10.2$ centigrade degrees¹⁷⁰. Another meta-analysis of thermal inactivation for spores of proteolytic *C. botulinum*¹⁷¹, including data up to 2014, gave a mean value $D_{121.1\text{C}} = 0.19$ minutes and standard deviation 0.11 minutes. The analysis of D-values gave $z = 11.3$ with standard deviation 0.3 centigrade degrees. This data was obtained in liquid medium at neutral pH and the authors considered the results were valid for temperatures 100°C and above. The meta-analyses broadly encompass the reference D-value, $D_{121.1\text{C}} = 0.21$ minutes, that is generally accepted as appropriate for evaluating thermal reduction in numbers of proteolytic *C. botulinum* spores. It is also generally accepted that equivalent D-values at different temperatures are calculated using $z = 10$ centigrade degrees, which is similar to the z-value in one meta-analysis but at the lower end of z-values identified in the other.

A heat process resulting in 12 orders of magnitude reduction in numbers for proteolytic *C. botulinum* spores is generally considered sufficient for sterilisation of food stored at ambient temperatures over long periods. However, it should be noted that analysis of this criterion, in several studies, has moved majority opinion to conclude that a 10^{-8} - 10^{-9} probability of growth can be compared with the 12 orders of magnitude reduction in spore numbers for proteolytic *C. botulinum* in phosphate buffer that is described in the original study by Esty and Meyer¹⁷² and is an acceptable food safety objective^{162,173}. Clearly, comparison of deterministic lethality with a residual probability for growth includes a wide range of assumptions. Nevertheless the evidence does not suggest change from an established heat process of 3 minutes at 121.1°C ($F_0=3$) to achieve a sterilisation goal for corresponding foods.

5.4.2 Non-proteolytic *C. botulinum*

In 1992 the ACMSF based their recommendations for a heat process that inactivates spores of non-proteolytic *C. botulinum* on a study which found a reference value $D_{90C} = 1.1$ minutes with $z = 9$ centigrade degrees¹⁷⁴. A meta-analysis of thermal inactivation for spores of non-proteolytic *C. botulinum*, including data up to 2006, gave a mean value $\text{Log}(D_{120C}) = -1.47$ with a standard deviation of 0.71 for the dataset. Inverting the mean value of the logarithm gives $D_{120C} \sim 0.034$ minutes but this cannot easily be identified with the arithmetic mean D-value. The analysis gave $z = 18.6$ centigrade degrees¹⁷⁰ but this study uses a reference temperature that is far removed from temperatures that are traditionally used during the processing of chilled foods so that the z-value is difficult to interpret in terms of the safety of minimally processed foods. Another analysis of published D-values, for spores of non-proteolytic *C. botulinum* recovered in the absence of lysozyme¹⁰, found a mean value $\text{Log}(D_{90C}) = -0.24$ and standard deviation for the logarithm $\sigma(\text{Log}(D_{90C})) = 0.42$. The analysis gives mean value $z = 6.7$ with a credible range 4.4 - 10 centigrade degrees.

In 2020 the ACMSF¹⁶² concluded that it had found evidence to recommend an update in the reference z-value that is included in FSA guidance documents. The recommendation indicated that $z = 6.7 - 7.7$ (more specifically $z = 7.0$) centigrade degrees should be used for calculating equivalent D-values at temperatures below 90°C whilst maintaining the reference value of $z = 10$ centigrade degrees for calculating equivalent D-values at temperatures above 90°C. The evidence above supports the 2020 ACMSF assessment that $z = 7$ centigrade degrees and $z = 10$ centigrade degrees should be used to evaluate equivalent heating below and above the reference temperature, 90°C, respectively and that these values should be used for evaluating equivalent thermal processes for heating temperatures in the range 83°C - 100°C.

Having considered current thermal inactivation data, whilst recognising that D-values derived from inactivation studies in food tend to be higher than those derived in laboratory medium and also the history of safety for chilled food heated at 90°C for 10 minutes or equivalent, it is clear that in the absence of other controlling factors the “90 for 10” process delivers at least a 6 orders of magnitude reduction in spore numbers, is robust and establishes confidence for control of non-proteolytic *C.*

botulinum in chilled foods. However, this widely adopted reference process corresponds with a value $D_{90C} = 1.6$ minutes which is longer than many measured values.

The ACMSF subgroup on non-proteolytic *Clostridium botulinum* and vacuum and modified atmosphere packaged foods¹⁶² reviewed the evidence on the effect of lysozyme in reducing the efficacy of thermal destruction and their recommendation “that ... the maximum shelf-life of foods given a heat process of 90°C for ten minutes (or equivalent) should be limited to 42 days, unless it can be shown that lysozyme is absent from the food ... and that expert advice should be sought if a shelf-life in excess of 42 days is desired” remains appropriate. Challenge tests are a practical means for shelf-life extension of foods that contain lysozyme or other lytic enzymes.

5.4.3 Other clostridia capable of producing botulinum neurotoxin

There is no evidence to distinguish the thermal resistance of neurotoxigenic *C. sporogenes* from that associated with proteolytic *C. botulinum*. Neurotoxigenic *C. butyricum* (2 strains isolated from an infant botulism case) is reported to have $D_{76.7C} = 2.3 - 2.5$ minutes¹⁷⁵ in phosphate buffer at pH = 7.0. A non-neurotoxigenic strain had a D-value 1000 times higher under the same conditions. The z-values for the two strains in phosphate buffer at pH = 7.0 were z = 8.2 and z = 9.5 centigrade degrees¹⁷⁶.

There is no published evidence detailing the heat resistance of neurotoxigenic *C. baratii*.

5.5 Effect of pH and acidity on growth and survival

Acids have an inhibitory effect on the germination and growth from spores of *C. botulinum* and other clostridia. Their inhibitory effect depends on the pH of the final food, on the concentration of the acid as well as on physical properties such as dissociation, molecular weight and the number of carboxyl groups¹⁷⁷. The type of acid can influence the inhibitory effect and organic acids are more effective than mineral acids at controlling growth of microbial pathogens including *C. botulinum*. The nature of the growth medium also significantly affects growth from spores and toxin formation^{178,179}. There are interactions between pH, a_w and temperature that can be exploited to prevent the growth and toxin formation of *C. botulinum* and other clostridia (interactions between controlling factors are discussed later in this chapter).

5.5.1 Proteolytic *C. botulinum*

The minimum pH for growth of proteolytic *C. botulinum*¹⁷⁷ lies in the range pH = 4.6 - 4.8 and it is generally accepted that population growth and toxin production in foods are prohibited at or below pH = 4.6 under otherwise optimal conditions¹⁶³. This has long been recognised as the point of demarcation for acid or acidified foods, below which *C. botulinum* is unable to grow¹⁸⁰. Growth observed below pH = 4.6 has involved carefully controlled high protein conditions that are not representative of the food environment. Citric acid and hydrochloric acid tend to be less inhibitory to proteolytic *C. botulinum* than lactic acid which, in turn, is less inhibitory than acetic acid¹⁷⁹.

5.5.2 Non-proteolytic *C. botulinum*

The inhibitory pH for non-proteolytic *C. botulinum* is recognised as pH = 5.0 and this is regarded as a well-established control factor¹⁶³.

5.5.3 Other clostridia capable of producing botulinum neurotoxin

Neurotoxigenic *C. butyricum* (2 strains isolated from an infant botulism case) grew and produced botulinum neurotoxin at pH = 5.2 but not at pH = 5.0, in broth acidified with citric acid¹⁷⁵, following incubation at 30°C. This is significantly less acid resistant than a non-neurotoxigenic strain isolated from blueberries that grew at pH = 4.2 but not at pH = 4.0 in acidified broth following incubation at 30°C. A report on growth of non-neurotoxigenic *C. butyricum* and *C. baratii* showed a pH limit in canned pasteurised mung bean sprouts of pH = 3.7 and pH = 4.0 respectively¹⁶⁷. The lowest limiting pH for growth of two neurotoxigenic strains of *C. butyricum* (ATCC 43181 and 43755) in anaerobic broth was reported as pH = 4.2 using HCl as the acidulant. With other acids the limiting pH was higher; for example pH = 4.7 was limiting using citric acid and pH = 4.8 was limiting with lactic acid¹⁷⁶. The results relating to organic acids indicate that growth of *C. butyricum* is inhibited in environments that are less acidic than those required for inhibition of proteolytic *C. botulinum*. In these experiments the presence of toxin was not determined and therefore it is not possible to conclude whether toxigenesis occurred. Another study showed that some of six neurotoxigenic strains of *C. butyricum* involved in outbreaks of botulism in Italy when incubated at 30°C, grew and produced toxin at pH = 4.8 but not at pH = 4.6 when the acidulant was HCl⁵⁴. Based on current evidence, the safety controls applied to foods in relation to pH and acidity for proteolytic *C. botulinum* appear to be adequate to control the risk from neurotoxigenic *C. butyricum*, although the current data set is based on results from only a few isolated strains, and a single study indicates that neurotoxigenic *C. butyricum* shows stronger acid resistance in the presence of mineral acids.

There is no published evidence on the acid resistance of neurotoxigenic *C. baratii*.

5.6 Effect of water activity on growth and survival

The water activity of foods is affected by several different solutes; sometimes called humectants. Common humectants are sugars, glycerol and salts, particularly sodium chloride, but combinations of humectants are used widely. The lower the water activity the slower the growth of microorganisms but water activity can also have an effect on heat resistance. There are interactions between a_w , pH and temperature that can be exploited to prevent the growth and neurotoxin formation by *C. botulinum* and other clostridia (interactions between controlling factors are discussed later in this chapter).

5.6.1 Proteolytic *C. botulinum*

A table of minimum water activity values allowing growth¹⁷⁷ indicates, for proteolytic *C. botulinum* producing type A and B neurotoxin, $a_w = 0.94$ resulting from use of 9.4% salt and $a_w = 0.93$ with glycerol as solute. According to ICMSF¹⁶³ most strains of proteolytic *C. botulinum* are unable to grow under otherwise optimum conditions if the salt concentration is 10% or above ($a_w = 0.94$) and the lowest water activity allowing growth and toxin formation was observed in turkey frankfurters where use of

salt resulted in $a_w = 0.95$ and time to toxin formation was 30 days at 27°C with pH = 6.10 - 6.39.

5.6.2 Non-proteolytic *C. botulinum*

A table of minimum water activity values allowing growth indicates under otherwise optimum conditions¹⁷⁷, for non-proteolytic *C. botulinum* neurotoxin type E, $a_w = 0.97$ resulting from use of 5% salt and $a_w = 0.94$ with glycerol as the solute. Most strains of non-proteolytic *C. botulinum* are unable to grow in otherwise optimum conditions if the salt concentration is 5% or above ($a_w = 0.97$)¹⁶³. As temperature is reduced the minimum water activity for growth also reduces and in 1992 the ACMSF¹ stated that growth had not been reported for non-proteolytic *C. botulinum* above 3.5% salt at incubation temperatures below 10°C. However, recent tests indicate growth and neurotoxin formation in anaerobic microbiological broth with 4.5% salt at 8°C after 6 weeks, but not at 5.0% salt ($a_w = 0.97$) at 8°C in 13 weeks¹⁸¹. It has been recognised in FSA guidance since 1992 that the safety of chilled foods can be achieved with more than 3.5% salt or water activity $a_w = 0.97$ or less throughout all components of complex foods, and this guidance has not been associated with outbreaks of foodborne botulism in chilled foods.

5.7 Other clostridia capable of producing botulinum neurotoxin

The lowest limiting water activity for growth of two neurotoxigenic strains of *C. butyricum* (ATCC 43181 and 43755) in anaerobic broth is reported as $a_w = 0.96$ using salt or sucrose as the humectant¹⁷⁶.

There is no published evidence on the minimum water activity permitting growth of neurotoxigenic *C. baratii*.

5.8 Effect of nitrite/nitrate on growth and survival of *C. botulinum*

Nitrite has an antimicrobial effect on *C. botulinum*, including the inhibition of outgrowth from spores, that is dependent on concentration, pH and a range of other factors¹⁶⁴. Nitrite is commonly used for control of *C. botulinum* in chilled meats such as bacon and in ambient meats such as canned cured ham and is equally effective in inhibiting non-proteolytic¹⁸² and proteolytic *C. botulinum*¹⁸³. Acidic conditions are needed for this effect. The mechanism of action exerted by nitrite is not well understood but a review of evidence suggests that the mechanism is oxidative via the nitrite-peroxynitrite system which is pH-dependent and non-oxygen-dependent¹⁸⁴. Peroxynitrite is known to be a strong oxidant and nitrating agent that causes direct oxidation or radical-mediated reactions in proteins, DNA and lipids within microbial cells. The chemical pathways involved are complex and modulated by other intrinsic and extrinsic properties of meat which, as well as the pH and redox potential, include salt concentration, heat treatment, storage temperature and addition of ascorbic acid or other reducing agents. The presence of iron in meat can also reduce the effectiveness of nitrite and this is consistent with the proposed bactericidal pathway.

The ingoing amount of nitrite is considered to contribute to the inhibitory effect rather than the residual level of nitrite. For most meat products 50 –150 mg of added nitrite (as sodium nitrite) per kg of meat is deemed to be sufficient to inhibit *C. botulinum*¹⁶⁴.

Nitrate does not appear to provide any anti-botulinal effect on its own but appears to act as a reservoir for nitrite formation primarily through microbiological activity in some traditionally fermented meat products¹⁶⁴ although some conversion can happen through enzymatic reaction in heated cured meat products¹⁸⁵.

Nitrite also has bactericidal effects on other foodborne pathogens¹⁸⁴ so its reduction/removal should only be considered in conjunction with validation of safe shelf life. There are maximum legal limits for nitrite and nitrate in foods which differ around the world and must be adhered to because of the carcinogenic effect of nitrosamines that result from the in-process transformation of nitrites and nitrates.

There are no data on the effect of nitrite on other botulinum neurotoxin-producing clostridia and this may need further investigation to ensure their effective control.

5.9 Effect of gaseous environment and redox potential

The germination, growth and toxin production of *C. botulinum* and other clostridia in foods is affected by the gaseous environment and anaerobic conditions have traditionally been considered a pre-requisite for growth. This is because many clostridia are sensitive to oxygen and associated oxidative stress. Clostridia possess stress response genes and consequently can survive and even grow under low oxygen conditions although this does vary markedly between species, strains and depends on the medium or food in which the organism is present. The presence of high levels of oxygen does not in isolation prevent clostridia from growing or producing toxin as the cells may be protected from the effects of oxidative stress within a local microenvironment. For example *C. botulinum* can grow and produce toxin in foods packaged in air or in air permeable film¹⁸⁶ because the food can protect the organism from the effect of oxygen by preventing oxidation and therefore removing oxidative stress. Microenvironments are notoriously difficult to access for measurements.

As the headspace oxygen content is not a reliable indicator of potential for growth of clostridia, the oxidative state of food, measured by redox potential, has historically been used to indicate the potential to support the growth of *C. botulinum*^{187,188}.

Both gaseous environments and redox potential of foods can drift during the shelf life of the food due to gaseous exchange through the packaging, growth of microorganisms affecting headspace gas and redox potential or reduction/oxidation within the food matrix due to chemical reaction or breakdown. Consequently, whilst it is reasonable to anticipate that foods packaged under vacuum or reduced oxygen may increase the potential for the growth of, and toxin production by, *C. botulinum* and other toxin-producing clostridia, it is not possible to use the gaseous atmosphere or redox potential as a controlling factor due to the inability to accurately define or control conditions necessary to prevent growth or toxin production. Lower redox potential microenvironments are difficult to access for measurement.

5.9.1 Proteolytic *C. botulinum*

Growth and toxin production by proteolytic *C. botulinum* has been reported in crumpets packaged in air¹⁸⁹, in laboratory media with 15% headspace oxygen¹⁹⁰ and in mushrooms packed in a semipermeable plastic film¹⁹¹. Toxin production occurred

in overwrapped mushrooms stored under ambient conditions and it is likely that continued, post-harvest, respiration of the mushrooms during storage decreased the oxygen content making conditions more favourable for growth of, and toxin production by, *C. botulinum*.

5.9.2 Non-proteolytic *C. botulinum*

Non-proteolytic *C. botulinum* neurotoxin type E produced toxin in packaged catfish stored in oxygen permeable packaging and in an oxygen-barrier bag¹⁹². Similar findings were observed in Atlantic salmon¹⁹³.

5.9.3 Other clostridia capable of producing botulinum neurotoxin

Limited data are available on the effect of gaseous environment on other neurotoxigenic clostridia. Continued growth and toxin production was demonstrated¹⁹⁴ in anaerobic microbiological broth by *C. butyricum* type E initially grown under anaerobiosis, and then transferred to air (aerobic conditions), although this was dependent on sufficiently high number of cells, i.e. 10^3 cells per ml, being present initially.

No published evidence is available to determine the impact of gaseous conditions on neurotoxigenic *C. baratii*.

5.10 Other factors

Other factors have potential to control outgrowth and toxin production by *C. botulinum* and other neurotoxigenic clostridia. However, the efficacy is particularly variable. Some of these factors are considered in this section but others including phosphates, citrates, sorbates, sulphites, etc., which are often used in combination with other controls and cannot be evaluated for general use, are not considered further.

5.10.1 Herbs and spices

Herb and spice extracts can reduce, or in some cases inhibit, the germination, growth and toxin production by *C. botulinum* and other neurotoxigenic clostridia but the effect is highly dependent on the herb or spice, the concentration, the organism and the food matrix¹⁹⁵. Oils of clove, thyme, black pepper, pimenta, garlic, onion, origanum and cinnamon have shown variable amounts of inhibitory effects on the growth of *C. botulinum* producing type A, B and E neurotoxins in anaerobic microbiological broth¹⁹⁶. Alcoholic extracts from 33 herbs and spices have a range of minimum inhibitory concentrations for *C. botulinum* (neurotoxin types not reported) in anaerobic microbiological broth¹⁹⁷; these included mace, nutmeg, bayleaf, black and white pepper, paprika, rosemary, cloves, oregano, turmeric and thyme. Some extracts showed little or no inhibition even at high concentration; these included parsley, mustard, garlic, celery, chives, fennel, tarragon, dill, cumin, onion and coriander seed. The inhibitory effect of spices such as oregano, savory and thyme are potentially due to the high content of carvacrol and thymol but this can vary in concentration between different varieties and regions and is dependent on growing conditions¹⁹⁸.

There is no published evidence detailing the effect of herbs and spices on growth and toxin production for neurotoxigenic *C. butyricum* or *C. baratii*.

Whilst it is possible that specific herb and spice extracts may be able to inhibit *C. botulinum* in vitro little is known about the effect in food matrices¹⁹⁹. Extracts that migrate to the oil phase of complex foods will no longer be active in the water phase. Without specific reproducible evidence it is not possible to consider the generic use of herb or spice extracts as a control factor for *C. botulinum* and other neurotoxin-producing clostridia.

5.10.2 Nisin

Nisin is a natural antimicrobial peptide produced by the lactic acid bacterium *Lactococcus lactis* ssp. *lactis*. It has a spectrum of activity against a number of Gram-positive bacteria including many clostridia. Nisin is authorised for use in a number of products throughout the world and, in the EU and the UK, levels are defined in legislation^{200,201}. In the EU (and UK) nisin is approved for use in a number of dairy products including cream, cheese, some dairy desserts and also in egg products at inclusion levels ranging from 3mg/litre (or kg) to 12.5 mg/litre (or kg). Nisin is most effective at acidic pH values in food and it shows less efficacy in meats due to inactivation by glutathione and proteolytic enzymes together with interactions with phospholipids²⁰². High levels are required to prevent growth and toxin production by *C. botulinum* (200-400 mg/kg) but, commercially it is generally used with other controlling factors at much lower levels i.e. 12.5mg/kg in processed cheeses. In this way nisin can replace other factors traditionally used in the control of *C. botulinum* such as sodium chloride.

Processed cheese inoculated with proteolytic *C. botulinum* types A and B and stored at 30°C for 48 weeks did not support toxin production when 12.5mg/kg nisin was used to replace 2.0% sodium chloride²⁰³.

Non-proteolytic *C. botulinum* has been shown to be more sensitive to nisin than proteolytic *C. botulinum*²⁰⁴.

There is no published evidence on the effect of nisin on neurotoxigenic *C. butyricum* and *C. baratii*.

5.10.3 Competitive microflora

The natural microflora of a food product can influence the growth and toxin production of *C. botulinum*. This may result from enhancing the growth potential by creating microenvironments in foods where growth, that would ordinarily be inhibited, could occur. Mould growth in tomato juice was shown to create conditions suitable for growth and toxin production by proteolytic *C. botulinum* types A and B although spoilage was evident^{205,206} (similar conditions could arise from growth of *Bacillus* spp.). Microflora can inhibit growth and toxin production by *C. botulinum* due to competitive effects or through the production of inhibitory compounds including acids and other antimicrobials. This effect has been reported for proteolytic *C. botulinum* types A and B in experimental meals²⁰⁷. For non-proteolytic *C. botulinum* type E the specific inhibitory effects of the microflora were not clear and it was established that spoilage due to the microflora occurred prior to, or at the same time as, neurotoxin

production^{208,209}. Lactic acid bacteria have specific anti-botulinum properties against both proteolytic and non-proteolytic *C. botulinum* due to the production of bacteriocins²¹⁰ and this had led to the development of compounds suitable for use as specific inhibitors such as nisin (the properties of nisin are described earlier in this chapter). The effect of lactic acid bacteria on the growth of non-proteolytic *C. botulinum* is reported as insignificant²¹¹.

The use of defined cultures in the production of some fermented foods, such as yogurt and cheese, where known and measurable inhibitory compounds such as organic acids are produced are recognised methods used to achieve control of *C. botulinum* and other botulinum neurotoxin-producing clostridia.

There is no evidence detailing the effect of competitive microflora on other botulinum toxin-producing clostridia including *C. butyricum* and *C. baratii*.

Reliable control of *C. botulinum* and other neurotoxicogenic clostridia using competitive microflora cannot be achieved and is not recommended outside the use of known cultures creating specific, defined and measurable inhibitory compounds in fermented products.

5.11 Combination of factors

The factors that are known to inhibit the germination and growth from spores of *C. botulinum* can be combined in many ways to ensure safety of food with respect to botulism. Combining multiple controls adds important flexibility to product development processes but also adds complexity to food safety considerations. Combinations of factors may cause growth rates, or inactivation rates, to change, or may modify the limits for which growth can occur, compared to the values established by single factor tests. Combined controls must be evaluated carefully using predictive microbial models and/or dedicated challenge tests. It is important to appreciate that the controlling factors, considered individually or in combination, relate to bacterial population growth whereas food safety is concerned with botulinum neurotoxin formation.

Predictive microbial models have become increasingly sophisticated and some developments, such as Combase²¹² and the Pathogen Modelling Program²¹³ (PMP), have become large versatile online resources with integrated tools to make them easily accessible and user friendly. Advanced modelling includes non-linear (one step) fitting of responses, complex constructions for multidimensional growth boundaries and statistical tools to evaluate confidence in predictions etc. The fundamentals of predictive microbial modelling, including construction, validation and applications have been reviewed in the context of microbial risk assessment²¹⁴.

Predictive models for the survival and growth of *C. botulinum* are particularly challenging. Difficulties associated with *C. botulinum* microbiology mean that the corresponding predictive models often concentrate on population growth (not toxin production) and are sometimes based on experiments that use non-toxicogenic strains as surrogates (often with unnaturally large inocula) so they can be criticised as unrepresentative. Where predictive models are used to consider extending shelf life

beyond 10 days any indication of population growth must be interpreted as also indicating neurotoxin formation and therefore that the scenario is hazardous.

In addition, *C. botulinum* models are often built from data generated in laboratory media that do not closely resemble real foods, and do not include competitive microflora, so can be considered incomplete.

Predictive microbial models are dominantly deterministic whereas it is now accepted that botulism hazards may involve significant stochastic processes surrounding germination or inactivation of individual spores and particularly heat treated spores²¹⁵. There is very little structured information about the effects of environmental factors in relation to the stochastic elements of spore responses so that interpretation of outputs from deterministic models requires specialist *C. botulinum* expertise.

Combase and the PMP include models, parameterised by temperature, pH and a_w /salt, that predict the time to growth from spores for proteolytic and for non-proteolytic *C. botulinum*. These models have become well established, and are regarded as fail-safe in most conditions that correspond with processing and storage of real foods, but they are inflexible and therefore limited in their applications. Specialised models, such as that developed for growth from proteolytic *C. botulinum* spores during dynamic (cooling) temperature regimes in ground beef²¹⁶ or that developed to account for the effects of multiple food preservatives (organic acids) on growth from non-proteolytic *C. botulinum* in minimally processed fish or chicken²¹⁷, extend the range of applications but are less well established and therefore they are not so easy to implement and evaluate. Expert interpretation is essential for predictive models that relate to *C. botulinum* in food and their use in product development, shelf-life optimisation, timely decision making or in progression of HACCP based safety management.

Challenge tests that explore the possibility of toxin production by *C. botulinum* in real foods are an essential element of safety evaluations. *Clostridium botulinum* challenge tests are an integral part of the development cycle for large groups of foods including minimally processed chilled foods and low acid foods stored at ambient temperatures (often in modified atmospheres). Multifactorial predictive microbial models for *C. botulinum* may effectively guide and support the efficient use of challenge tests in safety evaluations and in product developments. Protocols for challenge tests involving *C. botulinum* have recently been considered by the ACMSF¹⁶². The ACMSF advised that all challenge tests involving *C. botulinum* should centre on detection of neurotoxin and that, even in the absence of toxin, any observation of population growth for *C. botulinum* in food should be considered potentially hazardous. Tests centre on detection of botulinum neurotoxin as published reports indicate that population growth may not be a reliable indicator of neurotoxin formation and foodborne botulism is an intoxication caused by the neurotoxin. Evidence indicates that strong population growth (e.g. completed exponential phase) in food is associated with detection of neurotoxin formation, although initial detection of population growth may in some circumstances precede detection of neurotoxin. This may result in some challenge test studies where growth

is detected in the absence of neurotoxin if the study is terminated in the intervening period. The botulinum neurotoxin appears stable in food environments relevant to challenge tests²¹⁸, but may be inactivated by heat or at high pH not found in food¹⁸². Detection of neurotoxin relies on effective extraction (that should be confirmed through the use of appropriate controls) followed by sensitive and specific detection (detection methods are described in Chapter 3 of this report).

Validated models for inactivation of spores, for *C. botulinum*, are almost exclusively linear and account for temperature as a single lethal factor. Additional dependency of thermal inactivation rates on other physico-chemical properties of food, such as pH or fat content, have been observed²¹⁹ but are not routinely included in probabilistic models of thermal death for *C. botulinum*.

There are no published models for growth or inactivation of neurotoxigenic strains of *C. butyricum* or *C. baratii*.

5.12 Novel processes

Traditional food safety technologies, including stringent heat processes and freezing, can negatively affect perceived product quality. Since 1992 there has been a consistent search for more complex preservative technologies that can maintain product safety with respect to botulism whilst enhancing organoleptic properties of food. These include novel thermal and non-thermal technologies.

5.12.1 Thermal technologies

Ohmic heating, in which alternating electrical current is passed through, heats up food²²⁰ and rapidly destroys the cell membrane of bacteria leading to lethal leakage of bacterial cellular contents. This process has the advantage of being much faster than conventional steam heating²²¹. Since ohmic heating works in water continuous systems, and relies on electrical conductivity, foods containing particles with low conductivity, such as oil droplets, require validation to ensure minimum temperatures are achieved throughout the food being heated.

High-frequency heating uses a radio frequency heating system to induce molecular friction and heat to inactivate pathogens^{222,223}. Although applications have been developed for industrial drying and thawing frozen food, the non-uniform heating caused by uneven distribution of electromagnetic fields in foods mean that more in-depth simulations and investigations are required before it can be applied more widely²²⁴. Although effects on clostridial spores are not recorded, inactivation of spores from other spore-forming bacteria such as *Bacillus* spp. is reported and application of this technology in the future would likely utilise existing thermal processing data, such as D and z-values, for clostridial spores.

Power ultrasound/thermosonication uses pressure waves, with frequencies 20-100kHz, to cause cavitation, generation of hydroxyl radicals and bacterial death²²⁵. Currently, applications focus on the use of this technology for washing and decontaminating fresh foods and reducing the microbiological load. The focus of studies investigating use of this technology is on vegetative pathogens, not bacterial spores. Further research is required if this technology is to be applied as an

intervention technique in the same way that thermal processes are used for inactivating clostridial spores in foods that are processed in-line or in-pack.

Microwave thermal sterilisation, a two-stage process, combines microwave heating with thermal sterilisation with water as an initial heating medium and is considered as an option to destroy clostridial spores and cells while preserving desirable food attributes^{226,227}. Using microwaves to heat water that is then used to heat foods in-pack allows for more uniform heating, avoiding the problems caused by non-uniformity of electromagnetic fields and identification of 'cold spots'. This process technology utilises existing thermal processing data, such as D and z-values, for spores of *C. botulinum*. However, the handling of process deviations is more complex than in conventional thermal processes²²⁶.

5.12.2 Non-thermal technologies

High pressure processing (HPP), involving pressures between 100 and 1000MPa, is used widely, especially to inactivate vegetative spoilage organisms and vegetative foodborne pathogens, in meats, fruit juice and seafood. Pressure treatment alone does not achieve substantial reductions in bacterial spores. However, changes in pressure are implicitly and explicitly linked to heating²²⁸ and HPP is frequently used in combination with other controls for bacterial spores such as refrigeration and low pH^{229,230}. For non-proteolytic *C. botulinum* type E spores, there is a report of high pressure (900 and 1200 MPa) delivering a 6 log inactivation at 60°C and 75°C after 10 min²³¹. For a single strain of proteolytic *C. botulinum* type B, however, pressure-mediated protection of spores has been reported²³² and there appears to be significant variation in susceptibility between different strains of proteolytic *C. botulinum*²³¹. Pressure-assisted thermal sterilisation (PATs) has been certified by the US FDA, involving the use of a chamber between 60°C and 90°C that as a result of internal compression heating (using 600 MPa pressure, or higher), leads to in-process temperatures reaching 90°C -130°C very quickly. High pressure treatment times for the inactivation of *C. botulinum* can be shorter than for traditional heating but there is no single safe process²³⁰.

Pulsed light uses high-frequency light pulses, typically 200–1,100 nm²³³, but the efficiency of this technology has been principally confirmed in liquid foods²²¹. Ultraviolet light treatment is commonly used as an antibacterial strategy, particularly for inactivation of vegetative bacteria, but is practically ineffective for inactivating spores²³⁴.

Cold plasma technology generates reactive oxygen and nitrogen species, and UV radiation which can inactivate bacteria, fungi and viruses²³⁵. It is suggested as a potentially important element in multiple hurdle technologies to decontaminate foods and food packaging surfaces^{224,236}. However, the antimicrobial efficacy is diminished by roughness of the product surface and prediction of the plasma reaction chemistry is made difficult by the wide range of moisture concentration in foods²³⁷. The mechanisms by which cold plasma inactivates bacterial spores have not yet been fully elucidated²³⁷. Additional uncertainties related to the parameters and application of cold plasma technology are still outstanding^{238,239,240} and application as an

alternative intervention process for delivering defined log reductions of clostridial spores in foods is not currently possible.

5.12.3 Packaging technologies

Since 1992 developments include smart or active packaging materials that contain additives that can extend the quality or shelf life of foods. Packaging materials may include antimicrobials, antioxidants, light blockers²⁴¹ and oxygen scavengers, carbon dioxide emitters and moisture regulators²⁴². “Intelligent packaging” can monitor and provide information on the current quality, freshness, maturity, time-temperature status and leakage/contamination of products during transport, retail and domestic storage^{242–245}. Changing consumer perceptions regarding renewable and environmental-friendly packaging²⁴⁶ mean there are significant moves away from single use petroleum-based plastic in food packaging to a range of biodegradable natural-based polymers²⁴³. Compared to oil-based plastic packaging materials bio-based films are desirably biodegradable but have low thermal stability and increased water sensitivity. Research is needed to evaluate the safety and possible unintended consequences when novel materials are used in recyclable food packaging^{247,248}. These characteristics are of particular concern in relation to possible *C. botulinum* growth and neurotoxin production on/in biobased packaging materials at refrigeration temperature²⁴⁹.

Some concerns and/or uncertainties remain in relation to the safety and effectiveness of packaging materials of some systems^{250,251,252}. The FDA has recently developed a control strategy for reducing the hazard of *C. botulinum* on seafood packages by using time and temperature integrators²⁵³. EFSA has recommended rigorous tests before approving the use of some active packaging systems.

5.13 Conclusions

There is no evidence to suggest that spore loads in food materials have changed substantially since 1992 but dedicated surveillance has added confidence to the belief that high spore loads have small probability. A small number of observations emphasise the role of variability in *C. botulinum* spore loads for food materials. The occurrence of neurotoxic clostridia other than *C. botulinum* is poorly characterised.

Some control factors that prevent outgrowth from spores or population growth and toxin formation, by *C. botulinum* or other neurotoxin-producing clostridia, are well established and some relevant parameters are collected in Table 6. In particular heating at 90°C for 10 minutes is a robust process to deliver at least a 6 order of magnitude reduction in spore numbers, for non-proteolytic *C. botulinum*. However, this widely adopted reference process corresponds with a value $D_{90C} = 1.6$ minutes which is longer than many measured values. Equivalent lethality of thermal processes can be calculated with $z = 7$ centigrade degrees and $z = 10$ centigrade degrees below and above the reference temperature respectively; some values are indicated in Table 7. Water activity is a particularly complex control factor because the responses of *C. botulinum*, and other neurotoxic clostridia, depend on the solute used as a humectant as well as the value of the available water.

Neurotoxicogenic *C. butyricum* appears equally or even more susceptible to recognised controls than proteolytic *C. botulinum* although stronger acid resistance in the presence of mineral acids is an exception. There is only a small amount of evidence to quantify the physiological responses of neurotoxicogenic clostridia other than *C. botulinum*.

There is some information available on the impact and implications of some novel thermal and non-thermal technologies available to inactivate undesirable bacteria or spores during food processing. However, most of the sources of evidence note the need for further research to confirm consistently safe, robust, effective and commercial valid use of these technologies. For novel thermal processes thorough validation to establish that a minimum temperature is achieved throughout the food is crucial.

While the advantages in using active (and intelligent) packaging of food are clearly increasing, significantly reducing food wastage and limiting environmental impact, some concerns and/or uncertainties remain in relation to safety.

Table 6: Controlling factors for *Clostridium botulinum* and other botulinum neurotoxin-producing clostridia in foods and recommended safe processes^a

	Proteolytic <i>C. botulinum</i>	Non-proteolytic <i>C. botulinum</i>	<i>C. butyricum</i> (neurotoxigenic)	<i>C. baratii</i> (neurotoxigenic)
Minimum growth temperature - Value	10°C – 12°C	3°C	10°C	10°C – 15°C
Minimum growth temperature - Generally Adopted Control	Store foods at less than 10°C	Store foods at less than 3°C	Store foods at less than 10°C	Store foods at less than 10°C
Thermal destruction of spores - Value	D _{121.1°C} = 0.19-0.21 minutes	D _{90°C} = 1.1 minutes	D _{76.7°C} = 2.3 – 2.5 minutes	NR
Thermal destruction of spores - Generally Adopted Control	Heat process low acid ambient foods pH > 4.6 at 121.1°C for 3 minutes or equivalent using z = 10 centigrade degrees	Heat process foods intended to be chilled (8°C or less) with extended life (greater than 10 days) at 90°C for 10 minutes or equivalent ^b	Heat processes for proteolytic and non-proteolytic <i>C. botulinum</i> would deliver safety for neurotoxigenic <i>C. butyricum</i>	NGAC
Inhibitory pH - Value	< 4.6	5.0	< 4.7	NR
Inhibitory pH - Generally Adopted Control	Formulate to pH = 4.6 or less throughout the food ^c	Formulate to pH = 5.0 or less throughout the food ^c	Formulate to pH = 4.6 or less throughout the food ^c	NGAC
Inhibitory water activity (with salt) - Value	< 0.94	< 0.97 (0.97 at 8°C)	< 0.96	NR
Inhibitory water activity (with salt) - Generally Adopted Control	Formulate to a _w = 0.94 or less throughout the food ^d	Formulate to a _w = 0.97 or less (chilled) throughout the food ^d	Formulate to a _w < 0.96 or less throughout the food ^d	NGAC
Inhibitory water activity (with glycerol) - Value	< 0.93	< 0.94	NR	NR

Inhibitory water activity (with glycerol) - Generally Adopted Control	Formulate to $a_w = 0.93$ or less throughout the food	Formulate to $a_w = 0.97$ or less (chilled) throughout the food	NGAC	NGAC
Inhibitory salt concentration (aqueous) - Limit	10%	5% (4.5% at 8°C)	NR	NR
Inhibitory salt concentration (aqueous)- Generally Adopted Control	Formulate to 10% or greater aqueous salt throughout the food.	Formulate chilled food to 3.5% or greater aqueous salt throughout.	NGAC	NGAC

NR: Not reported

NGAC: No generally adopted control

^a The figures detailed in the rows titled 'Value' are derived from the scientific literature (and are discussed further in the text of this report) under otherwise optimal conditions for growth and, on occasion, indicate growth and / or toxin production beyond established norms that are specified in the rows titled 'Generally Adopted Control'.

^b Calculate equivalent thermal processes using $z = 7$ for process temperatures below 90°C and $z = 10$ for process temperatures above 90°C

^c Ensure that precautions are taken to prevent contamination and growth of microorganisms that could elevate pH e.g. moulds, butyric anaerobes, bacilli

^d If using humectants other than sodium chloride or glycerol, lower values of a_w may be necessary

Table 7: Isothermal processes that have equivalent lethality to the reference process, 10 minutes at 90°C, for non-proteolytic C. botulinum

Temperature (°C)	Time (min)
100	1.0
99	1.3
98	1.6
97	2.0
96	2.5
95	3.2
94	4.0
93	5.0
92	6.3
91	7.9
90	10.0
89	13.9
88	19.3
87	26.8
86	37.3
85	51.8
84	72.0
83	100.0

6 Risk Assessment

In the UK the frequency of occurrence of foodborne botulism is very low (very rare but cannot be excluded). However, the severity of botulism is classified as high (severe illness: causing life threatening or substantial sequelae or long-term illness). High severity demands that risk assessments for foodborne botulism should be regularly updated to keep in step with the changing practice of food production and with improved understanding of the biology of *C. botulinum* and other neurotoxicogenic clostridia.

In 2005 EFSA published the opinion of their BIOHAZ panel with regard to *Clostridium* spp. in foodstuffs²⁵⁴. In addition several process risk assessments relating to *C. botulinum* for specific food products or processes have been published in the scientific literature; examples include packaged smoked fish in New Zealand²⁵⁵, canned foie gras in France²⁵⁶ and chilled dairy-based foods in the UK²⁵⁷. Many national authorities, including the UK NHS²⁵⁸, maintain up to date fact sheets promoting awareness of botulism risks.

Other forms of botulism including infant botulism, adult infectious botulism, wound botulism and inhalation botulism involve different risk pathways and each requires risk assessment that is distinct from that for foodborne botulism.

6.1 Hazard identification

Spores of *C. botulinum* occur widely in the environment and cannot reliably be excluded from unprocessed food materials. Vegetative growth of *C. botulinum*, or other neurotoxicogenic clostridia, may produce potent botulinum neurotoxins so that, in the absence of measures which prevent spore germination and population growth in food prior to consumption, the organism and the botulinum neurotoxin are identified as hazards.

Chilled foods establish a limited period of storage at low temperatures (not exceeding 8°C) that with other controlling factors prevents growth and/or toxin formation by non-proteolytic *C. botulinum*. Chilled storage alone is sufficient to prevent growth and toxin formation by proteolytic *C. botulinum*. Chemical conditions within the food often contribute additional control. Failures of the temperature or chemical controls are identified with hazards from *C. botulinum* and the botulinum neurotoxin.

Low acid foods stored at ambient temperatures traditionally experience a severe 'botulinum' cook (heating at 121°C for 3 minutes or equivalent), and also establish a physical barrier to prevent post process contamination, to ensure that the probability of survival and growth of *C. botulinum* is negligible (most of the *C. botulinum* is destroyed by the thermal process and any residual spores from proteolytic *C. botulinum* are assumed to be non-viable over long periods). Improper application or unjustified moderation of the heating process, or failures of the integrity of the physical barrier to post process contamination, are identified with hazards.

In comparison with proteolytic *C. botulinum* and non-proteolytic *C. botulinum* other clostridia have only very rarely been associated with foodborne botulism. Limited

laboratory data indicates that neurotoxigenic *C. butyricum* has heat resistance that is inferior to proteolytic *C. botulinum* and has a minimum temperature for growth in food that is equal to or exceeds that for proteolytic *C. botulinum*. A small amount of data indicates that, in the presence of organic acids, the growth of neurotoxigenic *C. butyricum* is also less acid resistant than growth of proteolytic *C. botulinum*. Consequently, foodborne hazards associated with formation of neurotoxin by *C. butyricum*, and potentially those associated with other clostridia such as neurotoxigenic *C. baratii*, can be aggregated with corresponding hazards that are identified with proteolytic *C. botulinum*. Present limited information about neurotoxigenic *C. sporogenes* suggests that corresponding hazards can also be aggregated with those associated with proteolytic *C. botulinum*. This aggregation potentially contributes additional uncertainty to risk assessment.

6.2 Botulism - hazard characterisation

Foodborne botulism is a serious intoxication, affecting the human nervous system, which follows consumption of even very small amounts of neurotoxin that has been pre-formed in food (details of the disease are included in the Introduction of this report). Symptoms can arise a few hours post exposure but alternatively may be delayed by several days. Rapid clinical diagnoses, prompt administration of suitable anti-toxin and, in severe cases, respirational support are essential to remediate disease. The effects of foodborne botulism may persist for many months or years and can be life changing.

A systematic review of the effects of treatment on the mortality, hospitalisation rate and adverse effects from botulism²⁵⁹ found evidence in relation to infant botulism and other evidence in relation to foodborne botulism. The evidence indicates that the use of trivalent anti-toxin to treat foodborne botulism had significant benefits in terms of mortality and hospitalisation if administered within 24hrs of symptom onset but the effect was reduced by delay (the review also indicates some side effects of treatment such as increased hypersensitivity reactions). In the UK treatment of botulism now involves administration of heptavalent antitoxin.

Botulinum neurotoxins, and their complexes, are relatively stable in foods and are believed to survive gastric passage unchanged. Toxicity tables expressing variability between different botulinum neurotoxins have been reported²⁶⁰. Botulinum neurotoxin type A1 is identified as the most potent human toxin, by intraperitoneal injection in mice, and type A neurotoxin has often been associated with the most severe human illness³⁰. In the USA type F botulism has been most strongly associated with non-infant mortality²⁶¹ and mortality increases with age. However, consistent relationships between the onset or duration of illness and the dose or type of toxin have not been established so that differential diagnoses and treatments are problematic.

Botulinum neurotoxins are heat sensitive. Historic research indicates that, under laboratory conditions in buffer, botulinum neurotoxin types A, B, E and F, are inactivated after heating at 80°C for a few minutes²⁶². However, following concerns relating to bioterrorism, additional research involving neurotoxins type A and B, and their respective protein complexes, indicated that inactivation by more than 99.5% in milk was achieved following high temperature short time pasteurisation at 72°C for

15 seconds²⁶³. The thermal destruction of botulinum neurotoxins in complex food matrices using domestic cooking appliances remains uncertain and cannot be considered as an effective control with respect to foodborne botulism.

6.3 Foodborne botulism – UK exposure

Subsequent to publication of the 1992 ACMSF report there have been very few reports of incidents in which UK consumers were exposed to botulinum neurotoxin in food (Chapter 4 of this report includes details of UK botulism). The list of UK exposures includes incidents that involved chilled foods as well as incidents that involved foods intended to be stored at ambient temperatures so that for both hazards, independently, the frequency of occurrence can be classified as very low. In the UK botulism events are small and sporadic so that an annual rate is useful but volatile and could change rapidly following a large event. In the UK all reported exposures to botulinum neurotoxins in food, which have been identified with a confirmed source, have been associated with proteolytic or non-proteolytic *C. botulinum* (and in one case neurotoxigenic *C. sporogenes*). Other neurotoxigenic clostridia have not been associated with exposures in the UK but, since 1992, *C. butyricum* and *C. baratii* have been confirmed as the cause of food exposures in a small number of incidents in Europe and elsewhere. Clostridia other than *C. botulinum* cannot be excluded as a source of botulinum neurotoxin in food and so the frequency of occurrence, for the corresponding hazard, is also classified as very low. When compared to the accumulated volume of food consumed in the UK, which has capacity to support germination and growth of neurotoxigenic clostridia, the rate of exposure to botulinum neurotoxins per consumption event is exceptionally small.

The small rate of exposure extends over an increasingly wide variety of foods consumed in the UK that are potential vehicles for botulism. Very small exposure of UK consumers with respect to foodborne botulism is largely attributed to good hygienic manufacturing, adherence to recommended controls and systematic implementation of risk management procedures and practices, using HACCP principles, by food business operators.

Quantitative estimates of exposure for *C. botulinum* combine statistical evidence concerning spore loads in food materials with predictive microbiological models for spore inactivation and for germination, growth and toxin formation in food storage conditions. The modelling process can support risk management and product developments but requires specialist expertise within a broader structured approach in order to be included into food safety decision making (the multi-agency Sussle projects are one example²⁶⁴).

Mathematical models for exposure highlight important uncertainties and variabilities in the current understanding of risk for foodborne botulism. In addition to the (reducible) statistical uncertainty that is associated with the values of model parameters there are deeper uncertainties that stem from (i) grouping of food materials into homogeneous classes (ii) the effect of process history on the subsequent behaviour of spores in food (iii) the effect of food chemistry, particularly the presence of lysozyme or other lytic enzymes¹⁰, on the survival and recovery of spores during food storage and processing (iv) the possible unaccounted

dependency between heat resistance and germination of individual spores (v) the precise relationship between cell growth and toxin production for *C. botulinum*. Research that integrates molecular information into quantitative modelling, and centres on the coordination of regulation for sporulation and toxin production pathways of spore-forming bacteria including *C. botulinum*¹⁶⁰, has begun to address some of these issues.

There are no published mathematical models that relate to germination, growth and toxin production for neurotoxigenic *C. butyricum* or *C. baratii* although some modelling approaches have used data collected from non-toxic surrogates for proteolytic *C. botulinum* and non-proteolytic *C. botulinum*.

6.4 Risk characterisation

Outbreaks of foodborne botulism have been reported worldwide for more than a century³⁰; for incidents that involve two or more linked cases the median outbreak size corresponds with three cases and most outbreaks originate from point sources (consumption in one place at one time). The largest reported outbreaks have been associated with commercial food production and with multiple (complex) exposure events. Outbreaks for which there is strong evidence identifying a source are dominantly associated with *C. botulinum* producing neurotoxin types A, B and E. In a list of reported incidents of foodborne botulism (Table 4 in Chapter 4 of this report) failure to control storage temperatures is the most common confirmed cause and failure surrounding home canning of vegetables is also a common pattern; the list of reported incidents includes multiple records associated with smoked fish and type E neurotoxin from non-proteolytic *C. botulinum* but the data do not support the estimation of statistical significance for any of these causes. Other identified causes include poor control of pH and water activity.

The 1992 report identified a particular hazard “specifically ... the consumption of chilled foods in which the growth and toxin production by psychrotrophic strains of *C. botulinum* may have occurred before the food is perceived to be spoiled. The foods most at risk are those in which the spoilage microflora are eliminated or inhibited whilst psychrotrophic *C. botulinum* may survive and grow”. In a list of incidents involving botulism and chilled foods (Table 5 in Chapter 4 of this report), only a single case, involving vacuum packed smoked salmon that was consumed beyond the Use By date, and where temperature abuse was not reported, can possibly be identified with this hazard.

Evidence from outbreaks suggests that some mild cases of botulism may be underreported^{27,30}, either misdiagnosed or unascertained. In the United States⁷⁴ approximately 50% of cases of botulism in adults are diagnosed correctly prior to outbreak recognition. In the UK it is possible that some mild sporadic cases of botulism are undetected but observed incidents of foodborne botulism are all classified as public health emergencies and are reported in detail, at a national level, by an incident management team. Uncertainty associated with the assessment of the frequency of occurrence, and with the assessment of severity, of foodborne botulism is low (solid and complete data; strong evidence in multiple sources).

In the UK foodborne botulism is characterised by infrequent, isolated but serious events in which an individual, or a small group, are exposed to toxin during consumption or preparation of food. A clinical diagnosis of botulism is the trigger for coordinated multi-agency activity involving clinicians, epidemiologists, public health experts and, in the case of foodborne botulism, food manufacturers and food regulators. The response aims to minimise the case severity, rapidly identify additional cases and those at risk, track and trace all the potentially contaminated foods and remove it from the market, and ultimately limit the size of the event. The effectiveness of the response to an incident involving botulism is crucial to the reduction of risk (restriction of the event size for each outbreak and reduction in the number of incidents both contribute to the reduction of risk). Such is the potential severity of foodborne botulism outbreaks that product recalls are initiated immediately if a failure in commercial processing is suspected or known.

In relation to foodborne botulism, an appropriate level of preparedness should include a widely established suspicion and awareness of botulism among clinicians in order to increase the probability of early detection. In addition an effective response requires sufficient accessible medical resources including epidemiological and laboratory support and the incident management team should be able to access exhaustive, real time, traceability systems for the relevant food products. In many countries a steady increase in preparedness, particularly rapid access to supplies of anti-toxin, has contributed to a declining case fatality rate for botulism. The investigation of a widely reported incident of foodborne botulism in Scotland during 2011¹⁸ pointed to significant benefit from multi-agency coordination, time savings obtained by using real time PCR to detect botulinum neurotoxin encoding genes in environmental samples and to some inconsistencies in formal notification processes.

A review of outbreak investigations in the United States⁷⁴ highlights epidemiological linking of the earliest cases as crucial in expediting control over dispersed foodborne botulism incidents. This places emphasis on accurate early diagnosis and on rapid active case discovery. Botulism incidents that lack significant spatial or temporal clustering, such as those that might occur from foods with long shelf life, from foods designed for individual consumption, from foods that involve off site (“dark”) or unidentified production elements, from foods with dispersed or individual distribution patterns or from consumption settings where misdiagnosis may be more likely, may compromise case linkage, delay outbreak recognition, and present the most significant risks.

Since 1992 there have not been any clearly discernible trends in the occurrence of foodborne botulism in the UK. It is noted however, that the majority of incidents since 1992 have been associated with imported, home-produced foods and that there has been an apparent increase in global outbreaks associated with temperature abuse of chilled foods. Treatment of individual cases of disease, and linking cases with food and environments, has improved substantially as part of a move to a dominantly molecular scientific approach.

The successful control and the established decision-making framework for botulism safety in the UK is potentially challenged by emerging priorities for food manufacture.

Reduction in energy use, reduction of waste (United Nations Sustainable Development Goals) and increased awareness of environmental concerns can manifest as reduction of preservatives, reduced packaging, radical new sources for food ingredients (e.g. meat replacements) or extended periods for acceptable use. In turn, the emergence of unexpected patterns of foodborne botulism cannot be dismissed. A move towards smaller scale, even individual, facilities for food production such as the increasing popularity of ghost kitchens and home canning presents concerns²⁶⁵. Home prepared foods, such as herbs and spices or vegetables or mushrooms preserved in oil, and other small scale (unregistered) production, are less likely subject to rigorous controls relating to food safety and may include unpredictable and unrecorded distribution. Botulism risk associated with home and commercial bottling of vegetables in oil has previously been considered by the UK ACMSF²⁶⁶.

In the UK, the control of foodborne botulism is moving into a complex multi-objective decision space that is part of the current development of UK food manufacture, delivery and use.

6.5 Conclusions

6.5.1 Controls

In the UK, established controls designed to prevent foodborne botulism, and the corresponding FSA guidelines for food manufacturers, are based on science.

Accumulated evidence indicates that for chilled foods, commercially manufactured in the UK and correctly stored at temperature not exceeding 8°C and consumed before the Use By date, existing controls act to maintain safety with respect to botulism. In addition, there are examples that suggest that the current FSA guidelines, and particularly the use of “a combination of heat and preservative factors which can be shown consistently to prevent growth and toxin production by non-proteolytic *C. botulinum*” or an updated statement that does not prioritise heating, include sufficient flexibility to support innovation by food business operators that can lead to reduced energy usage, waste reduction and safe shelf-life extensions. However, this may require significant financial investment and may not be accessible to SMEs in the industry.

Evidence continues to show that traditional low acid foods that experience a valid botulinum cook or other valid preservation step are exceptionally safe with respect to botulism. Currently it can be assumed that the controls aimed at prevention of hazards from *C. botulinum*, in foods that are intended to be stored at ambient temperatures, are effective for controlling hazards that could be associated with other neurotoxicogenic clostridia. It is not clear that appropriate controls, or the application of a valid botulinum cook, can be guaranteed for low acid sealed foods produced outside of commercial manufacturing environments.

6.5.2 Incidents

Since 1992 there have been only a small number of reported incidents of foodborne botulism in the UK.

For chilled foods, the reported incidents of botulism are dominated by incorrect home storage of food at temperatures exceeding 8°C. For low-acid foods that are intended to be stored at ambient temperatures in cans or sealed containers, the majority of reported incidents involve an incorrect (non-commercial), or absent, heat treatment or alternative valid preservation step (often for food sourced from outside the UK by individual consumers).

The majority of reported incidents of foodborne botulism identify proteolytic *C. botulinum* as the causative agent, for foods intended to be stored at chilled or ambient temperature, although for some incidents involving type B neurotoxin the associated bacterium is not reported.

Investigations of recent UK incidents point to a system for effective multi-agency response to small, point-source outbreaks of botulism. It is not clear that current levels of preparedness with respect to foodborne botulism extend to include risks associated with small scale production or online distribution of food.

6.5.3 Trends

Since 1992 reported incidents of foodborne botulism in the UK have been small and localised with relatively routine epidemiological linkage of cases. Trends in food manufacture, particularly dispersed food distribution and individual food behaviours, as well as relatively large intervals between reported incidents, mean that a larger more dispersed incident cannot be ruled out and that preparedness is essential.

The science of *C. botulinum* continues to move forward and there are prospects for almost real time, high resolution, *in vitro* detection of botulinum neurotoxins and for improved, better characterisation of the biology and diversity of botulinum neurotoxin-forming clostridia, and mechanistic, understanding of stress response and toxin production pathways that can support next generation risk assessments.

7 Recommendations

(1) Foods should continue to be formulated to control *C. botulinum* and other botulinum neurotoxin-producing clostridia in accordance with the known factors (described in this report). With regard to these controls, actions recommended to the FSA are;

- At the first opportunity replace in their guidelines the current statement “a combination of heat and preservative factors which can be shown consistently to prevent growth and toxin production by non-proteolytic *C. botulinum*” with a modified statement “a combination of controlling factors which can be shown consistently to prevent growth and toxin production by non-proteolytic *C. botulinum*”
- At the first opportunity make explicit in their guidelines that $z = 7$ and $z = 10$ centigrade degrees should be used to evaluate equivalent thermal processes for operating temperatures below and above the 90°C reference temperature respectively
- At the first opportunity make explicit that all studies relating to controlling factors for *C. botulinum* should determine neurotoxin production with potential merit in also monitoring growth
- Emphasise that other controlling factors, including herbs and spices, bacteriocins, phosphates, citrates, sorbates, sulphites, etc., can be highly variable in delivering consistent and reliable control of *C. botulinum*, and other botulinum neurotoxin-producing clostridia, and ensure that food business operators relying on such controls must provide evidence to demonstrate initial efficacy of the factors used for controlling these organisms in their foods together with ongoing control and measurement
- Emphasise that nitrites, in combination with other controlling factors, exert an important anti-*C. botulinum* effect, which is difficult to predict due to the complexity of the chemical pathway leading to inhibition of growth and toxin formation, and therefore provide guidelines that ensure other preservation factors are adjusted if nitrite concentration is to be reduced in, or removed from, foods traditionally containing it where there is a significant history of safety with respect to *C. botulinum*.

(2) The evidence does not facilitate revision of the current reference process, heating at 90°C for 10 minutes or an equivalent, but there is strong evidence that this process provides a lethality that exceeds the target 6 order of magnitude reduction in population size that is widely attributed to the reference process. The subgroup recommends that the FSA should consider any evidence, from food business operators, indicating the value of further investigations that could address this issue.

(3) Early detection of cases and rapid, effective coordinated responses to very rare incidents are identified as crucial elements for reducing risks from foodborne botulism. It is recommended that the FSA work closely with other agencies to establish clear and validated preparedness in relation to potential major incidents of foodborne botulism in the UK; this may involve methods to increase awareness of cases presenting for healthcare, transparent methods for epidemiological linkage of cases, rapid accessibility to sufficient high quality laboratory capacity, capability to

identify botulism from organisms other than *C. botulinum* and established pipelines for monitoring rapid alerts in other locations.

(4) Temperature abuse has been highlighted as the cause of the majority of incidents relating to botulism in chilled foods. It is recommended that the FSA highlight the importance of temperature control in consumer food hygiene campaigns, together with adherence to recommended Use By dates, to reinforce these critical consumer food safety controls.

(5) It is recommended that the FSA works with other organisations to ensure that, whenever possible, the causative organism that is isolated from foods, cases or outbreaks of botulism is fully characterised to determine its phenotype for use in risk assessments.

(6) Evidence includes relatively few incidents involving chilled foods that identify non-proteolytic *C. botulinum* as the causal organism of botulism and no incidents or outbreaks implicating chilled foods where the food has been stored under the recommended chilled conditions and consumed within its stated shelf life have been reported. It is recommended that the FSA considers commissioning a review of controls used in other countries, for non-proteolytic *C. botulinum* in foods, to determine whether a subsequent exposure assessment should be undertaken for vacuum or modified atmosphere packed chilled foods in the UK.

(7) Emerging information confirms that other clostridia, including *C. sporogenes*, *C. butyricum* and *C. baratii* contribute to foodborne botulism, and may present discrete risks, and it is recommended that the FSA routinely include specific consideration of these organisms, in any plans that are developed to maintain the safety of food, with regard to foodborne botulism. Where practical it is recommended that the FSA identify and support research studies conducted to establish the boundaries for toxin production (and possibly also growth where appropriate), for neurotoxicogenic *C. sporogenes*, *C. butyricum* and *C. baratii* under otherwise optimal conditions in foods.

(8) Evidence shows that *C. botulinum* can grow and produce toxin in foods where relatively high levels of oxygen or air are present and, whilst vacuum or modified atmosphere packaged foods are likely to provide more favourable conditions for *C. botulinum*, it is recommended that FSA initiate a dedicated consideration of botulism risks associated with non-vacuum or modified atmosphere packaged foods.

(9) WGS has provided improved understanding of the taxonomy and diversity of botulinum neurotoxin-forming clostridia, and their neurotoxins, and this has substantially improved the ability to track and trace outbreaks of human foodborne botulism. It is recommended that the FSA involve their academic partners and take a lead in the generation and coordination of molecular information relating to *C. botulinum* taxonomy.

(10) It is apparent that increased understanding of the genotypic and phenotypic homogeneity of distinct groups of botulinum neurotoxin-producing clostridia will contribute to improved assessment of human foodborne botulism risks. It is recommended that the FSA use academic partnerships to develop this knowledge.

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