# Report on Botulinum Neurotoxin-Producing Clostridia

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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 ≤ 8°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), DT.** 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, aw.** The chemical potential for water molecules in a system that includes water, ionic species and dissolved solids; alternatively called water availability. Pure water has aw = 1.

**Redox potential, Eh.** Sometimes called the reduction oxidation potential, measured in millivolts (mV), it is the tendency of food to release electrons (low redox Eh = 100 to -300 mV) or accept electrons (high redox Eh = 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 foods[1]. 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 (aw) 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 illness[3]; 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 20$^{th}$ 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 subtypes[5]. 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 botulism[5]. 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, neuroparalytic 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 botulism[6]: “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.