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Risk Assessment of Botulism from Chilled, VP/MAP (Vacuum Packed/Modified Atmosphere Packed) Fresh Meat held at 3°C to 8°C

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Executive summary

The UK Food Standards Agency VP/MAP (Vacuum Packed/Modified Atmosphere Packed) Guidance (FSA, 2017) restricts the shelf-life of VP/MAP foods (including fresh meat) held at 3°C to 8°C to 10 days, unless suitable grounds for a longer shelf-life can be identified. This project has used a risk assessment approach and carried out a challenge test experiment, to establish whether a shelf-life of greater than 10 days can be applied to fresh chilled meat (as it lacks a single known controlling factor). Fresh meat is taken to mean "meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is VP or MAP wrapped".

A search of the literature failed to uncover any cases of botulism associated with fresh chilled VP or MAP meat.

Data provided by industry members of the project consortium indicates that UK industry applies a maximum chilled retail pack shelf life at 3°C to 8°C of up to 23 days for beef, 27 days for lamb, and 18 days for pork. Using a risk assessment approach, it was established that the current industry practice provides a high level of protection with respect to non-proteolytic *Clostridium botulinum*, estimated as >10.8 safety units (decimal number of products (i.e. $>10^{10.8}$) marketed per number causing botulism).

There is no evidence that currently-applied UK shelf lives combined with current production standards are unsafe. If changes are made to industry practice, then these may affect the level of protection.

A new challenge test study demonstrated that samples of beef and lamb inoculated with spores of non-proteolytic *C. botulinum* and incubated at 8°C, did not become toxic to day 50 for beef, day 35 for lamb, or day 25 for pork (i.e. <40 pg type B toxin g^{-1} of meat and <40 pg type E toxin g^{-1} of meat).

The estimation of the level of protection and the results from the new challenge test experiment both support a shelf-life of greater than 10 days for fresh chilled beef, lamb and pork held at 3°C to 8°C, and also support currently-applied UK shelf lives combined with current production standards.

The ability not to be constrained by a 10-day shelf-life, as indicated in present FSA (2017) guidelines, and the freedom to adopt a shelf-life greater than 10 days at 3°C to 8°C for fresh chilled beef, lamb and pork is of significant economic/social/sustainability benefits to producers/processors/retailers. Such freedom removes a technical barrier to trade. There may also be environmental/consumer benefits through lower food wastage.

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1 Background

The UK Food Standards Agency (FSA) revised its Vacuum Packed/Modified Atmosphere Packed (VP/MAP) Guidance in 2017 (FSA, 2017), and this restricts the shelf-life of VP/MAP foods (including fresh meat) held at 3°C to 8°C to 10 days, unless suitable grounds for a longer shelf-life can be identified. Given the severity of botulism, a precautionary approach is understandable, yet fresh meat has a particularly strong safety record globally, despite no other countries providing similar guidance. Short shelf lives result in trade restrictions, increased waste and cost, therefore any restriction should only be applied if there is a clear food safety benefit.

The primary aim of this work is to provide information to assist industry in dealing with queries from enforcers/customers in relation to the FSA guidance. This aim is achieved through the writing of a document that sets out industry practice in relation to fresh meat distributed and/or sold to the final consumer and clearly establishes the level of protection with respect to non-proteolytic *Clostridium botulinum*, since VP or MAP fresh chilled meat lacks a single known controlling factor. Work is required to collect existing information for a risk assessment to assure safety with respect to non-proteolytic *C. botulinum*.

The risk assessment focuses on fresh meat as this is an important issue identified by industry. In this document "fresh meat" is taken to mean "meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is VP or MAP wrapped".

In 2006, the FSA commissioned a project led by Prof. Mike Peck to consider whether the 10 day rule should become the 5 day rule. A report and peer reviewed publication (Peck *et al.,* 2006; Peck *et al*., 2008) were produced, and a presentation was given to the Advisory Committee on Microbiological Safety of Food (ACMSF). This work led to the adoption of the 10 day rule by the FSA. The report:

- Characterised the hazard
- Calculated the number of packs of chilled foods sold in the UK and internationally (VP, MAP and low oxygen),
- Compiled information on temperature control at various parts of the chain,
- Investigated the number of cases of botulism related to those products, and
- Estimated the level of protection provided by current practice ($10^{9.8}$) for cooked chilled foods (i.e. 1 in $>10^{9.8}$ packs are associated with botulism).

Furthermore, in 2011, Stringer *et al.* (2011) carried out a project funded by the UK Agriculture and Horticulture Development Board (AHDB) to evaluate the safe shelf-life of fresh chilled meat with respect to non-proteolytic *C. botulinum.* This included a literature review on growth and neurotoxin by non-proteolytic *C. botulinum* in fresh meat, the use of ComBase to predict the growth of non-proteolytic *C. botulinum* and a challenge test to determine the conditions under which neurotoxin can be formed in fresh meats. This report provided a strong summary of knowledge in 2011, and the principal conclusion was that the literature data available at this time on growth/toxin formation by non-proteolytic *C. botulinum* in fresh meat was limited and often contradictory.

Following the release of the FSA 2017 guidance (FSA, 2017), an industry consortium was formed which commissioned this project. Meat processors and retailers, most of whom are members of the British

Meat Processors Association, and Meat & Livestock Australia (MLA), jointly funded the project through the MLA Donor Company.

2 Project objectives

The aim of this project is to prepare a risk assessment setting out the level of protection, with respect to non-proteolytic *Clostridium botulinum* and foodborne botulism, when employing current industry practice regarding VP/MAP fresh meat for certain meat species distributed and/or sold to the final consumer (that do not contain known controlling factor(s)). This risk assessment includes and takes account of:

(1) hazard characterisation
(2) specified meat species and product types
(3) industry practice
(4) exposure assessment (market/sales data)
(5) exposure assessment (spore loading)
(6) foodborne botulism incidents related to fresh meat
(7) data on growth/neurotoxin formation by *C. botulinum* in fresh meat.

This report incorporates knowledge and expertise with non-proteolytic *C. botulinum* and in microbiological food safety, importantly including previous FSA and AHDB-funded work. Wide dissemination of the findings will assist industry in dealing with queries from enforcers/customers in relation to the FSA 2017 guidance (FSA, 2017).

3 Methodology/Results/Discussion

3.1 Hazard Characterisation

This section is concerned with hazard identification (the first stage of hazard characterisation), and some elements of hazard characterisation. Other aspects of hazard characterisation are summarised in following sections (e.g. exposure assessment (spore loading), foodborne botulism incidents related to fresh meat).

The Gram-positive, anaerobic bacterium *Clostridium botulinum* is a heterogeneous species that comprises four distinct bacterial groups (*C. botulinum* Groups I to IV), all of which form the botulinum neurotoxin (Peck, 2006; Lindström *et al*., 2009; Peck, 2009). The botulinum neurotoxin is the most potent toxin known, with a human lethal dose potentially as low as 30-100 ng. Foodborne botulism is a severe intoxication caused by consumption of food containing neurotoxin formed by *C. botulinum* in food. *C. botulinum* Groups I to IV are sufficiently distinct as to be considered separate species, and two of these Groups (*C. botulinum* Groups I and II) are associated with most foodborne botulism cases. *C. botulinum* Group I (proteolytic *C. botulinum*) is a mesophilic bacterium that forms highly heat resistant spores. The minimum growth temperature is 10°C-12°C. *C. botulinum* Group II (non-proteolytic *C. botulinum*) is a psychrotrophic highly saccharolytic bacterium that forms spores of moderate heat resistance. The minimum growth temperature is 3°C.

Spores formed by non-proteolytic *C. botulinum* are present in the environment and may contaminate chilled fresh meat. In the absence of controlling factors (e.g. competition from cells of other microorganisms), spores of non-proteolytic *C. botulinum* may germinate, leading to cell multiplication and neurotoxin formation during the storage of chilled fresh meat to a level that is detrimental to human health, and therefore represent a hazard.

3.2 Specified meat species and product types

Following discussions with members of the consortium, beef, pork, and lamb were selected for inclusion in this project as these are the three species of fresh meat to which shelf lives of >10 days under chill may be applied, and for which at least 10^9 portions of 250g have been consumed in the UK or internationally over the last 10-15 years.

The following decisions were made with respect to products within the scope of the assessment:

- For beef, given the relatively recent growth in the market and lack of evidence showing non-proteolytic *C. botulinum*-controlling pH and/or Aw values in comparison with wet-aged beef, dry-aged beef would not be included in this project, and
- For pork, since there was no indication from previous work (Jones, 2018) of there being significant differences in risk between two presentations ("rind-on" and "rind-off"), only rind off would be tested. No distinction between the two presentations would be examined in the report, and
- Neither bacon nor other products such as sausages would be included since what is sold is so varied.

See Appendix 1 for definitions used throughout the report.

3.3 Industry Practice

This review describes industry practice for three relevant meat species from carcase to end of fresh meat shelf life, summarising controls in place to minimise potential for contamination from slaughter, and *C. botulinum* toxin formation and growth at various points in the supply chain.

The majority of products produced by the UK meat industry are from pigs, cattle and sheep (BMPA, 2018a). They are usually sold either as full carcases, animal sections or smaller cuts like steaks and chops. Some of the meat produced also goes on for further processing into products such as sausages, bacon and other cured meats, pies and other manufactured foods.

The focus in this project is on major UK producers and retailers, which made up the project consortium, and represent an estimated 75% of the markets of each of fresh beef, pork and lamb in the UK (see section 3.4).

The last major public review of UK industry practice was FSA Project B13006 (Peck *et al*., 2006), which reported that:

- The pattern of VP widespread usage for primals, joints and cured meat worldwide is largely similar to the UK, with particular emphasis on small producers for VP end products
- Shelf lives outside the UK are reported to be similar to those in the UK, i.e. >10 days.

Notwithstanding the publication by FSA of revised guidance in 2017, these two points remain valid.

Note that in the present report:

- Product, not air, temperature is referred to, and
- P=0 (P, the day of production) is used throughout, being in line with the European Reference Laboratory's guidance (EURL, 2014).

3.3.1 Information Sources

The information sources used in this review include:

- Data provided by consortium members – the outcome of review and discussion with the consortium are presented here (and were used to inform the project's challenge test protocol)
- European legislation and guidance, notably in relation to 853/2004 (EC, 2004)
- BMPA Pork Scheme Modules (BMPA, 2018b): 1 (Pig Welfare and Slaughter & Biosecurity) and 2 (Pork BMPA Quality Assured Pork)
- Australian Standard for the Production and Transport of Meat and Meat Products for Human Consumption (AS 4696, 2007).
- Victoria State Government, Australia: Prime Safe - Shelf Life and Labelling Requirements for Meat Products (Prime Safe, 2018).
- CODEX Alimentarius Commission Code of Hygienic Practice for Meat, July 2005 (FAO, 2005), and associated guidance (OIE, 2011)
- FSA Project B13006 Report (Peck *et al*., 2006)

3.3.2 Production Rules and Standards - Legislative, Trade and Commercial Requirements

As a condition of trading, UK retailers and their suppliers require compliance with relevant legislation, industry scheme codes and their complementary own technical standards as a condition of supply.

This is the typical position in the UK market given that sales of fresh beef, pork and lamb through UK retailers accounts for the vast majority of sales in the UK (see section 3.4).

There is no documented evidence of the '10 day rule' being applied in countries other than the UK, particularly in relation to fresh meat.

The primary relevant legislation applicable to the UK is EU Regulation 853/2004 (EC, 2004), which came into force on 01/01/06, having been published on 30/04/04. Although this was new legislation at the time of the Project B13006 (Peck *et al*., 2006), it does not differ significantly from previous UK legislative requirements (FMHR, 1995). Sector-specific codes of practice were codified in 853/2004 (EC, 2004) without other significant changes to times and temperatures, apart from 853/2004 requiring processing as quickly as possible to eliminate microbiological hazards or to reduce them to an acceptable level, followed by being chilled to no more than 4°C, unless the meat is cut while warm.

EU regulations 853/2004 interlinks to a range of other EU legislation (see Appendix 2), and must be applied and interpreted accordingly.

FSA's Meat Industry Guide published in April 2018 (FSA, 2018) provides general guidance on implementation of legislative requirements in relation to meat, and cross-references industry and other documents, including providing a link to the July 2008 edition of its Guidance on the Safety and Shelf-life of Vacuum and Modified Atmosphere Packed Chilled Foods (FSA, 2008), which is no longer available on FSA's website, having been superseded by the 2017 edition (FSA, 2017). The FSA 2017 guidance requires food stored for any period of time at a core temperature above 3°C to be limited to 10 days life if no other hurdles are in place.

The British Meat Processors Association (BMPA) has codes for i) pork and ii) ham and cooked pork products (BMPA, 2018b). To qualify for membership of the Quality Assured Pork Scheme, all members must be certified in the core standards plus any individual modules that apply to the products they produce. The Core Standards are general industry standards to which all abattoirs, processors and manufacturers are expected to adhere, and form part of the Red Tractor scheme.

Numerous assurance schemes are recognised by BMPA and major UK retailers, such as:

- BRC Global Standard (Food Safety, Storage and Distribution)
- Red Tractor (beef and lamb) and
- Quality Meat Scotland

Each follows EU legislated requirements, and add features in relation to quality, welfare and/or provenance, and is audited against. Each scheme differs in various ways, but are not in conflict with legislation.

However, it is particularly notable that Annex III, Chapter II of 853/2004 (EC, 2004) requires FBOs (Food Business Operators) producing minced meat, meat preparations or MSM (Mechanically Separated Meat) to comply with requirements including that when prepared from chilled meat, minced meat must be prepared within no more than 15 days from the slaughter of the animals in the case of boned, vacuum-packed beef and veal.

The application of FSA's 2017 guidance to fresh meat used to produce minced meat therefore appears to be in direct conflict with this longstanding EU legislation.

FSA guidance on minced meat shelf life is referred to in BMPA's Quality Assured Pork Scheme Module 2 (BMPA, 2018b). BMPA's Quality Assured Pork Scheme (Module 2) requires the shelf life of all chilled pork raw material to be compliant with 853/2004 (EC, 2004), noting that pork raw material intended for use in minced meat shall not exceed K+6 days at the point of use and cannot be extended by the use of deep chill, MAP or vacuum packing (VP).

Control of temperature in processing establishments in Australia is governed by the Australian Standard for the Production and Transport of Meat and Meat Products for Human Consumption (AS 4696, 2007). The '10 day rule' is not applied in Australia. In 1992, AQIS (Australian Quarantine & Inspection Service), published a Code of practice for heat-treated refrigerated foods packaged for extended shelf life (i.e. those with a shelf life of more than 5 days) to provide guidance for the Australian industry (AQIS, 1992).

The CODEX Code of Hygienic Practice for Meat (CCHPM) is the primary international guidance for meat hygiene, providing the baseline trading standard under World Trade Organization rules (FAO, 2005). It incorporates a risk-based approach to application of sanitary measures throughout the meat production chain for raw meat, meat preparations and manufactured meat from the time of live animal production up to the point of retail sale, but does not provide inspection measures for specific hazards, which remains the responsibility of the national competent authorities. Its general hygiene provisions are based on the CODEX Code of Practice: General Principles of Food Hygiene (FAO, 1969). HACCP is applied in the specific context of meat hygiene. Whilst the CCHPM lacks technical detail that could be used as CCP critical limits, it states that it should be the responsibility of the establishment operator to produce meat that is safe and suitable in accordance with regulatory meat hygiene requirements. Similarly, the 2008 CODEX publication Animal Food Production (CODEX, 2008), which was updated in 2009 (CODEX, 2009) contains material from CODEX Codes of hygienic practice in relation to meat and other animal products, but lacks critical limits.

The following sections describe the practical application of regulatory and commercial requirements to the production and sale of fresh beef, pork and lamb. See Appendices 3-5 for information on carcase to pack for each species.

<u>Abattoir – Intake and Lairage to Dressing and Quartering</u>

Where possible, the identification of farms supplying animals contaminated with Clostridia can be a useful control mechanism, but it can be difficult to achieve unless there are only a limited number of farms supplying animals (Christeyns, 2018).

Information from primary production should be taken into account so as to tailor meat hygiene requirements to the spectrum and prevalence of hazards in the animal population from which the meat is sourced.

Hygiene at slaughter is a primary control with respect fresh meat, to limit potential for contamination by spores of *C. botulinum* as an environmental organism.

The conditions of holding of animals presented for slaughter should minimise cross-contamination with foodborne pathogens and facilitate efficient slaughter and dressing.

The cleanliness of animals has a major influence on the level of microbiological cross-contamination of the carcass and other edible parts during slaughter and dressing. Animals being clean (free from mud, excrement etc) at the point of slaughter reduces the probability of microbial contamination (including *C. botulinum* spores) being present on the hide or skin, which can then spread to the meat on butchery.

Industry adheres to EU Regulation 853/2004 (EC, 2004), which sets out requirements for slaughter hygiene to production of quarters (Annex III, Chapter IV), including that:

- Animals must be clean
- Stunning, bleeding, skinning, evisceration and other dressing must be carried out without undue delay and in a manner that avoids contaminating the meat
- Contact between the outside of the skin and the carcase must be prevented
- Operators and equipment coming into contact with the outer surface of hides and fleece must not touch the meat
- Heads and feet must be handled so as to avoid contamination of other meat
- The risk of contamination of porcine meat with water for scalding must be minimised, using only approved additives, and followed by thorough rinsing with potable water
- Carcases and offal must not come into contact with floors, walls, or work stands

Standard GMP requirements for personnel, equipment and premises hygiene apply.

Additional specific points providing for control of Clostridia at the abattoir include (Christeyns, 2018):

- Careful attention to hygiene during de-hiding.
- Ensuring air movement is not from the slaughter and lairage towards the cleaner parts of the line.
- Effective cleaning and disinfection using oxidising disinfectants and concentrating on areas in the cutting room where Clostridia could colonise, e.g. points of contact of meat with equipment, refrigeration units and drip trays in chillers.
- Effective disinfection requires, on a regular basis, careful removal of all dirt, dismantling and cleaning machinery and application of chlorine-based biocide e.g. by fogging to access all contaminated places.
- Using only the minimum temperature and time necessary to heat-shrink wrapping film is an additional precaution where this approach is used, since there is evidence that this process may stimulate any *Clostridium* spores present to germinate and subsequently grow and spoil the meat.

<u>Cutting and Boning</u>

Annex III, Chapter V of 853/2004 (EC, 2004) stipulates requirements for cutting plants in which further size reduction of quarters must be carried out. These include:

- The overarching requirement for work on meat to be organised in such a way as to prevent or minimise contamination
- The general requirement during cutting, boning, trimming, slicing, dicing, wrapping and packaging, that the meat is maintained at no more than 3°C (offal) and 7°C (other meat).

Alternative approaches are permitted to this temperature regime under certain circumstances, for example if the cutting room is on the same site as the slaughter premises, meat must be transferred to the cutting room either directly from the slaughter premises or after a waiting period in a chilling or refrigerating room. As soon as the meat is cut, and where appropriate packaged, the meat must be chilled to no more than 3°C (offal) and 7°C (other meat).

The Australian Standard for the Production and Transport of Meat and Meat Products for Human Consumption (AS 4696, 2007) requires carcase, side, quarters or bone-in separated cut surface temperature to be no more than 7°C, and other parts of the carcase to be no more than 5°C 'at the site of microbiological concern'. Alternative approaches are permitted by an 'approved arrangement'.

Module 2 of the BMPA Quality Assured Pork Scheme requires (BMPA, 2018b):

- Section 4.1: "The temperature of chilled pork raw material during storage shall be -2°C to +5°C… The temperature of chilled meat preparations during storage shall be between -2°C to +4°C. For minced meat the temperature will be -2°C to +2°C… Where deep chilled storage typically this would be 0 to -4°C... Validation of shelf life within the deep chill shall take into account worst case scenarios."
- Section 4.2: "Pork raw material shall be processed within kill +4 days. However, if the raw material is held in chill rooms which are not disturbed, and the pork raw material is not intended to be used in a meat preparation, the storage period may be extended by up to three days. Where pork raw material is vacuum packed, gas flushed or deep chilled… additional storage life beyond these limits may be permitted … subject to satisfactory documented shelf life verification (sensory and microbiological evaluation) that shows that the extension of life does not, under worst case conditions, adversely affect product safety or quality.

Specifications for the temperature of fresh meat on receipt for further processing was confirmed by industry to range from 3-5°C. The consortium agreed that 5°C should be used as the representative storage temperature limits for receipt by further processors.

In contrast, the CODEX Code of Hygienic Practice for Meat does not stipulate a temperature, instead (para 69) referring to the cutting room being 'a room or rooms, capable of being temperature-controlled'.

<u>Maturation of Primals</u>

'Maturation' prior to retail packing can be referred to as 'wet maturation' where the meat is not directly exposed to air, but in a sealed no/low oxygen pack and normally VP.

Dry maturation, where meat is directly exposed to air, and is also known as dry-ageing, is outside the scope of this project, but is becoming more widespread in the UK retail fresh beef market.

Maturation temperature profiles provided by project consortium members indicated a general adherence to storage core temperatures of 0-3°C, which would prevent toxin formation by non-proteolytic *C. botulinum* and is considered to not significantly affect competitive microflora.

It is important to note that time spent guaranteed at <3°C is additive to shelf life with respect to non-proteolytic *C. botulinum* as this temperature suppresses toxin formation and vegetative growth. Storage

under these conditions for up to 77 days (New Zealand lamb) is well established, relying on this effect. If storage is guaranteed to be at <3°C then it does not have a temporal limit with respect to non-proteolytic *C. botulinum,* but other factors such as organoleptic quality need to be taken into account.

Maturation times reported range from 0-42 days (beef), 0-21 days (pork) and 0-77 days (lamb), with maturation being at 0-3°C, with some exceptions. See Table 1 for the upper time and product temperature limits reported by consortium members to be applied during maturation.

Table 1: Upper Time/Temperature Limits for Beef, Pork and Lamb Maturation Reported by Project Consortium Members

	"Maturation" time prior to retail packing (days)		Max product storage temp range (°C)	Packing type	Comments
MEAT	**Min**	**Max**			
Beef	1	42	0°C to 5°C	VP	Deviation upwards from 3°C is not prolonged, e.g. hours. Note that beef from Australia or New Zealand would be in the order of 60+ days, as with NZ lamb, and at lower temperature.
Pork	0	21	0°C to 5°C -2°C to +4°C 3°C to 5°C	VP or MAP (100% CO_2) MAP (70%O_2/30%CO_2) VP	≥3°C may occur for limited period during transport to cutting & packing plant
Lamb	0	77	-1°C to +4°C	VP	Deviation upwards from 3°C is not prolonged, e.g. hours. Lamb from New Zealand could be in the order of 60+ days, and at lower temperature

VP/MAP primals are distributed widely, including to manufacturers of further processed products, where specifications require receipt at no more than 3-5°C, to foodservice operations, to independent butchers, and to some retailers using vertically integrated supply chains and carrying out cutting operations in-store.

Packing for Retail – Time Considerations including Shelf Life

As stated earlier:

- BMPA's Quality Assured Pork Scheme (BMPA, 2018b) has stipulations which go beyond EU law.
- Annex III, Chapter II of 853/2004 (EC, 2004) requires FBOs producing minced meat, meat preparations or MSM to comply with requirements including that when prepared from chilled meat, minced meat must be prepared within no more than 15 days from the slaughter of the animals in the case of boned, vacuum-packed beef and veal.

In the UK the commercial storage of chilled foods must comply with local legislation by country, i.e.:

- The Food Hygiene (England) Regulations 2013 (Food Hygiene England, 2013)
- The Food Hygiene (Wales) Regulations 2006 [SI 2006/31 (W.5)] (Food Hygiene Wales, 2006)
- The Food Hygiene Regulations (Northern Ireland) 2006 [SR 2006 No 3] (Food Hygiene NI, 2006)

In England, Wales and Northern Ireland food that is likely to support the growth of pathogenic microorganisms or the formation of toxins must be kept at a temperature of 8°C or below. The requirement applies to the temperature of the food, not the surrounding air. The requirement applies to foods, including raw materials and ingredients, at all stages of preparation, processing, transport, storage and display for sale within the manufacture, retail and catering sectors. Schedule 4 provides exemptions from the chill holding requirement in defined circumstances for some foods even though they are inherently likely to support the growth of pathogenic microorganisms and/or the formation of toxins.

In Scotland the requirements are different. Any person in respect of any commercial operation or food premises who keeps food without a refrigerator, a refrigerated chamber or a cool ventilated place is guilty of an offence unless the food is held at over 63°C. As there is no specific temperature mentioned for the chilling of foods that are likely to support bacterial growth it is recommended that if the food storage place chosen exceeds 8°C then the shelf life of the foodstuff may need to be reduced. Food should be kept at ambient temperature for the shortest time possible. Schedule 4 of the Scottish Regulation does however contain a number of specific exemptions for chill holding.

FSA's Guidance on Temperature Control Legislation in the United Kingdom was published in May 2016 but is no longer available on its website.

The shelf life of products is generally determined by conducting storage trials under the temperature conditions that the product would be sold and expected to be stored to the end of the given shelf life.

Variables such as pH, water activity and storage temperatures are considered when decisions are made on the life of the product. However, the pH and water activity of fresh meat are as a rule non-controlling in relation to non-proteolytic *C. botulinum* (Table 2).

Table 2: Reported pH ranges of Fresh Beef, Pork and Lamb

Species	pH values	Reference
Beef	pH 5.5 to pH 5.7	Stringer *et al*. (2011)
	pH 5.0 to pH 5.5	Jones (2018)
Pork	pH 5.5 to pH 6.5	Homer & Matthews (1998)
	pH 6.0-6.5 (approx.)	Jones (2018)
Lamb	pH 5.5 to pH 5.6	Stringer *et al*. (2011)
	pH 5.0 to pH 5.7	Jones (2018)

Shelf life is assured in practice by applying high hygiene standards during production, including sourcing of the animals to result in low spore loadings (Barker *et al.*, 2016), of low distribution and storage temperatures to the point of purchase by the consumer, and limitation of shelf life.

For established products, shelf life is based on the history of the product and its composition.

The Australian State of Victoria has shelf life guidelines for meat products stored frozen or up to 5°C, with the reference temperature of 5°C being the minimum standard required by the Australian Standard of Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (AS 4696, 2007). Validation data from product specific trials and testing must be provided for any situation outside established guidelines. If process is adopted from scientific research, then verification records must be provided for the shelf life used (Prime Safe, 2018).

3.3.3 Discussion and Summary of Industry Practice

Overall, data on temperatures, holding times and shelf lives comprising industry practice in relation to UK retail was reviewed and found to be relatively consistent. The only divergences appear to be that a single company might have a different retail shelf life for different cuts of one type of meat, and that different companies might have a different retail shelf life for the same cut of one type of meat.

See Table 1 for maturation times and temperatures, and example production flow information is given in Appendices 3-5.

Currently, UK industry as represented by the project consortium typically applies a chilled retail shelf life of up to 11-13 days to packs of fresh beef, pork and lamb, with a maximum of 18-27 days (Table 3). Production temperature regimes are consistent as they are subject to EU regulation enhanced by industry and commercial codes. Given that retail life includes storage by the consumer, it was agreed by the consortium that this storage period should be assumed to be at 8°C.

Table 3: Minimum, Typical and Maximum Retail Shelf Lives for Fresh Beef, Pork and Lamb

BEEF	
Minimum	7 days (MAP)
Typical	8-13 days
Maximum	23 days
PORK	
Minimum	7 days
Typical	8-11 days
Maximum	18 days
LAMB	
Minimum	7 days
Typical	8-11 days
Maximum	27 days

<u>Foodservice</u>

Fresh meat is also purchased from abattoirs and processors by both wholesalers and large foodservice businesses. Foodservice is an often overlooked but still vital part of the meat industry.

Consumer Storage

Although consumer packs of VP non-cured meats are kept chilled in the commercial distribution chain, their useful shelf life is relatively short. This is due the lack of deep chill during storage by consumers. Without deep chill there is little protection against the principal microorganisms that cause meat spoilage.

Temperatures of domestic refrigerators can vary from front to back and top to bottom, and a mean overall temperature and maxima and minima are reported in studies.

Peck *et al*. (2006) reviewed international domestic refrigerator temperature performance and reported that the overall mean temperature of 39% of domestic fridges was <5°C, and ~80% were up to 7.9°C (Table 4). It was also reported that the mean temperature of 35% of UK domestic refrigerators at that time was <5°C, with the reported mean temperature being 6.6°C, which was comparable with the overall international position (Table 5).

Table 4: International Domestic Refrigerator Temperature Performance

Temperature reported (°C)	Number of fridges at specified temperature	Percentage of fridges at specified temperature	Cumulative percentage
<4°C	143	4.0	4.0
4.0-4.9°C	1,255	34.8	38.8
5.0-5.9°C	120	3.3	42.1
6.0-6.9°C	24	0.7	42.8
7.0-7.9°C	1,356	37.6	80.4
8.0-8.9°C	68	1.9	82.3
9.0-9.9°C	633	17.5	99.8
≥10°C	8	0.2	100

Table 5: UK Domestic Refrigerator Performance

Temperature reported (°C)	Number of fridges at specified temperature	Percentage of fridges at specified temperature
<5°C	397	34.5
≥5°C	755	65.5

Although there has been relatively little research into domestic refrigerator performance since the publication of Project B13006 report (Peck *et al*., 2006), a recent survey reporting on the temperature performance of 671 domestic refrigerators in England found the overall mean internal temperature to be 5.3°C, the maximum overall mean temperature in a single refrigerator was 14.3°C and the overall minimum mean temperature was -4.1°C (Biglia *et al*., 2018). 174 refrigerators (26% of the sample) were found to operate for all of the survey period at a temperature higher than 5°C. In contrast only 37 refrigerators (5% of the sample) were found to operate for all of the survey period within the recommended range of 0°C to 5°C. The appliance type had a significant effect on the refrigerator temperature; the refrigerator sections in fridge-freezers were significantly lower in mean temperature

than refrigerators with an ice-box and larder fridges. Mean temperatures in fridge-freezers were between 0°C and 5°C in 48% of cases whereas in other types of refrigerator this figure was 35%.

The latest data for English domestic refrigerators indicate improvement in their mean temperature performance over the past decade, and that despite improvements in compliance with a 5°C maximum target, a significant proportion were found to exceed this throughout the study period (Biglia *et al*., 2018).

3.4 Exposure Assessment (Market Sales Data - Consumption)

This risk assessment is based on the number of portions (i.e. person servings) of the meats in question consumed over the last decade or more. Since the strength of this work is where there is the greatest market data, it was agreed by the project consortium that the number of portions need to exceed 10^9 internationally for a product to be included.

The quantity (volumes and approximate number of portions) of VP/MAP fresh meat held at 3°C to 8°C sold in the UK and internationally that follows industry standard practice is summarised, building on market data in the 2006 report from Project B13006 (Peck *et al*., 2006), and incorporating additional recent information from various sources.

The pre-packed chilled fresh meat sector has existed for some 50 years in industrialised countries. The vast majority of chilled pre-packed fresh meat products are MAP or VP. Fresh meat primals tend to be VP throughout the world. VP coupled with deep chill, is the basis of the meat export industry (Peck *et al*., 2006).

Fresh beef, pork and lamb consumption data are used in this report to determine the level of protection (safety units) provided by industry practice (see section 3.8). This follows the approach used in Project B13006 (Peck *et al*., 2006), and is of the form that a total of "*x*" portions have been sold without incidence of foodborne botulism, and that the level of protection provided by current practice is that the risk is "1 in $>10^x$ packs are associated with botulism".

3.4.1 Data Sources

Data sources used include:

- Project report B13006 (Peck *et al*., 2006), which analysed markets during the period 1999-2005.
- BMPA compiled data for 2009-2018 for UK sales of beef, lamb and pork through the major multiples (BMPA, 2018a).
- AHDB supplied data (Kantar) for sales of fresh beef, pork and lamb in Great Britain August 2015-August 2018, split by the top 5 major retailers, other retailers and independent butchers
- MLA compiled data for 2005-2018 for Australian international exports of beef/veal and lamb
- OECD data for global per capita and regional consumption of beef, lamb and pork in 2017 (OECD, 2018)
- FAO data for global beef and pork consumption over the period 1964-2015, and projected to 2030 (FAO, 2018)

3.4.2 Portion Size

The number of portions of fresh meat from species within scope was agreed by the consortium to be based on each person serving being a portion of 250g.

The derivation of this was the need to be conservative yet reflect evidence:

- MLA data showed that 163g raw mass was the mean consumption rate in Australia (https://www.mlahealthymeals.com.au/library-new/meat-consumption-library/).
- UK NDNS data (2008-2016: https://www.gov.uk/government/statistics/ndns-results-from-years-7-and-8-combined) survey referred to 'Red and processed meat' which included ham, bacon and other meat-based items. For all the UK data going back to the 2008-9 NDNS survey, the 97.5%ile highest daily consumption of red and processed meat was 236g, which was by men 19-64 years old in 2008-9 (Table 6).

Table 6: UK Consumption of Red and Processed Meat - NDNS Data 2008-2016 for men 19-64 years

	Quantity of red & processed meat consumed in given period (g/day)			
	2008/09 - 2009/10	2010/11 - 2011/12	2012/13 - 2013/14	2014/15- 2015/16
Mean	91	82	84	77
Median	85	77	79	66
Standard Deviation	63	58	57	58
2.5th percentile	0	0	0	0
97.5th percentile	236	219	219	216

Rounding up to 250g meant that taking this value as being a standard portion size was a relatively conservative approach and has a rational basis.

3.4.3 Consumption Data

This review considered data from GB, the UK, Australia, EU28, OECD and global figures.

3.4.3.1 UK Consumption Data

Two datasets have been consolidated to establish the UK consumption of fresh beef, lamb and pork between 1999 and 2017 (except for 2006). Market data for 1999-2005 were summarised in 2006 (Peck *et al*., 2006). However, Project report B13006 (Peck *et al*., 2006) did not specifically refer to the number of portions, and these have been calculated based on the mass of meat sold (Appendix 6). It is estimated that 7.8 x 10^9 250g portions of fresh beef, pork and lamb were sold in the UK between 1999 and 2005 (Table 7).

BMPA data for 2007-2017 for UK sales of beef, lamb and pork through the major multiples confirmed that sufficient quantities (>10^9 portions of 250g) had been consumed in the UK alone to satisfy data quality requirements (Table 7). See Appendices 6-9 for data used to calculate these figures.

Data show that for fresh beef, pork and lamb, from 2007-2017 the total mass consumed in the UK sold through the major multiples was 1.0 x 10^7 tonnes, and the number of portions consumed in the UK sold through the major multiples was 4.1 x 10^{10} 250g portions. Correcting for retailers' 76% market share

(see Appendix 6), then from 2007-2017 the total mass consumed in the UK sold through the major multiples was 1.3 x 10^7 tonnes, and the number of portions consumed in the UK sold through the major multiples was 5.4 x 10^{10} 250g portions (Table 7).

Consolidating together the datasets from 1999-2005 and 2007-2017 gives combined total UK sales figures for fresh beef, pork and lamb of 1.6 x 10^7 tonnes, which is equivalent to 6.2 x 10^{10} 250g portions (Table 7).

Table 7: Consolidated 1999-2005 and 2007-2017 UK Consumption of Fresh Beef, Pork and Lamb

	1999-2005 From Peck *et al.* (2006)		2007-2017 Major retailer sales (76% UK market)		2007-2017 Sales corrected to total UK market		Total UK sales 1999-2005 and 2007-2017	
Meat	**Total tonnes**	**Total no. portions**	**Total tonnes**	**Total no. portions**	**Total tonnes**	**Total no. portions**	**Total tonnes**	**Total no. portions**
Beef	$5.6x10^5$	$2.2x10^9$	$5.5x10^6$	$2.2x10^{10}$	$7.2x10^6$	$2.9x10^{10}$	$7.8x10^6$	$3.1x10^{10}$
Lamb	$2.1x10^5$	$8.4x10^8$	$1.5x10^6$	$5.9 x10^9$	$2.0x10^6$	$7.8x10^9$	$2.2x10^6$	$8.6x10^9$
Pork	$1.2x10^6$	$4.8x10^9$	$3.3x10^6$	$1.3x10^{10}$	$4.3x10^6$	$1.7x10^{10}$	$5.5x10^6$	$2.2x10^{10}$
TOTAL	**$2.0x10^6$**	**$7.8x10^9$**	**$1.0x10^7$**	**$4.1x10^{10}$**	**$1.3x10^7$**	**$5.4x10^{10}$**	**$1.6x10^7$**	**$6.2x10^{10}$**

3.4.3.2 Global Consumption Data

OECD (Organisation for Economic Co-operation and Development) data for global per capita and regional consumption of beef, lamb and pork in 2017 (OECD, 2018) shows that EU28 has a high per capita consumption (Table 8).

Using the following population figures for 2017 enables calculation of the number of portions for each species of fresh meat consumed by each population group (Table 8).

- World population = 7.52 x 10^9 [https://www.populationpyramid.net/world/2017/ (accessed 12/9/18)]
- OECD population = 1.26 x 10^9 [https://data.oecd.org/pop/population.htm-latest data (accessed 28/11/18)]
- BRICS (five major emerging economies: Brazil, Russia, India, China and South Africa) population = 3.06 x10^9 [https://www.statista.com/statistics/254205/total-population-of-the-bric-countries/ (accessed 20/9/18)]
- EU28 population = 5.12 x 10^8 [https://www.statista.com/statistics/253372/total-population-of-the-european-union-eu/ (accessed 20/9/18)]

Table 8: 2017 Global Consumption data for Fresh Beef, Pork and Lamb

Location	Consumption in 2017		
	(kg/capita)	(Number 250g portions/capita)	Total number of 250g portions
BEEF			
World	6.5099	26.0	2.0×10^{11}
OECD	14.5375	58.1	7.3×10^{10}
BRICS	4.4559	17.8	5.5×10^{10}
EU28	10.9858	43.9	2.3×10^{10}
PORK			
World	12.2695	49.1	3.7×10^{11}
OECD	23.5686	94.3	1.2×10^{11}
BRICS	15.4992	62.0	1.9×10^{11}
EU28	32.4806	129.9	6.7×10^{10}
LAMB			
World	1.7268	6.9	5.2×10^{10}
OECD	1.4051	5.6	7.1×10^{9}
BRICS	1.6903	6.8	$2.1 X10^{10}$
EU28	1.9360	7.7	$4.0 X10^{9}$
TOTAL			
World	20.5063	82.0	6.2×10^{11}
OECD	39.5111	158.0	2.0×10^{11}
BRICS	21.6454	86.6	2.7×10^{11}
EU28	45.4024	181.5	9.3×10^{10}

These data (Table 8) show that global consumption of fresh beef, pork and lamb in 2017 totalled 6.2×10^{11} 250g portions, and that in EU28 was 9.3×10^{10} 250g portions. It should be noted that not all of the presentations of these will have been as fresh meat. However, where fresh meat is handled, the established technology for managing it prior to further handing is VP, particularly in well-developed countries

FAO data (www.fao.org/docrep/005/74252e/y4252e05b.htm (accessed 12/9/18)) show that global beef consumption and that of pork (excluding China) were at steady consumption levels over the period 1964-2015, and that projected to 2030 (Table 9). Ovine and caprine meat consumption has increased in recent years (Table 9). Based on the world population stated above, these data can be used to calculate the global number of 250g portions consumed in 2015 (Table 10). Note that not all will be sold fresh and/or VP or MAP, and the figures include caprine meat.

Table 9: FAO Data – Global Consumption of Meat by Type 1964-2015, with 2030 projection

Species	Global meat consumption by type (kg/capita, carcass weight equivalent)						
	1964/66	1974/76	1984/86	1994/96	1997/99	2015	2030
Bovine	10.0	11	10.5	9.8	9.8	10.1	10.6
Ovine & caprine	1.8	1.6	1.7	1.8	1.8	2.1	2.4
Pig	9.1	10.2	12.1	13.7	14.6	15.3	15.1
Pig excl. China	9.7	10.8	11.3	10.4	10.3	9.9	9.7

Table 10: Calculated 2015 Global Number of Portions of Meat Consumed by Type

Species	Global meat consumption in 2015		
	(kg/capita)	(kg total)	(no. 250g portions)
Bovine	10.1	7.60×10^{10}	3.04×10^{11}
Pig	15.3	1.15×10^{11}	4.60×10^{11}
Ovine & caprine	2.1	1.58×10^{10}	6.32×10^{10}
TOTAL	**27.5**	**2.07×10^{11}**	**8.27×10^{11}**

The estimate based on FAO data (Table 10) of 8.3×10^{11} 250g portions of fresh meat consumed globally in 2015 aligns closely with the estimate of 6.2×10^{11} 250g portions consumed globally in 2017 based on OECD data (Table 8).

Australia exported 1.6×10^{8} 250g portions of fresh lamb and 3.4×10^{8} portions of fresh beef to the UK between 2005 and October 2018 (Appendices 10 and 11).

3.4.4 Discussion of meat consumption data

It is estimated that in the UK over the period 1999 to 2017, excluding 2006, the total number of 250g portions of beef, pork and lamb consumed were 3.1×10^{10}, 2.2×10^{10} and 8.6×10^{9}, respectively. This is a total of 6.2×10^{10} 250g portions.

OECD consumption data indicates that in 2017, the global number of portions of beef, pork and lamb consumed were 2.0×10^{11}, 3.7×10^{11} and 5.2×10^{10}, respectively. This gives a total of 6.2×10^{11} 250g portions for 2017 global consumption. Using 2015 FAO data, the global number of portions of beef, pork and lamb consumed in 2015 were 3.0×10^{11}, 4.6×10^{11} and 6.3×10^{10}, respectively, totalling 8.2×10^{11} 250g portions. Both datasets demonstrate significant global consumption of fresh beef, pork and lamb each year.

3.5 Exposure assessment for *Clostridium botulinum* spore loadings in fresh chilled meat

The exposure assessment summarises *C. botulinum* spore loading information in fresh chilled meat. It builds on data summarised in CFA's SUSSLE1 (Sustainable Shelf Life Extension) project and our peer-reviewed publication (Barker *et al*., 2016) where spore loads for food materials were expected to centre on a concentration range of 1 to 10 spores kg^{-1}.

3.5.1 Method for data collection

An extensive literature search was carried out in August 2018. Records were retrieved from online databases, then combined with articles held in personal literature collections and references cited in/or citing eligible articles. Searches were not restricted by country or language. Literature surveys were conducted to determine the non-proteolytic *C. botulinum* spore concentration in meat. A database was constructed on incidence of spores of non-proteolytic *C. botulinum* types B, E or F in meat. The search was performed on the following electronic databases:

Web of Science (Advanced Search in All Databases, 2016 to August 2018)

Search	Field	Search terms	Results
1	Topic	Botulinum	4,331
2	Topic	enumerat* OR incidence OR prevalen* OR presence OR occur* OR level OR screen* OR survey OR isolation OR detection OR study OR quantifi*	5,060,574
3	Topic	meat OR beef OR cow OR cattle OR chicken OR turkey OR duck OR poultry OR pig OR pork OR bacon OR sausage OR ham OR lamb OR sheep OR mutton OR venison OR deer OR cured OR meal OR food OR fresh OR raw	634,756
1 AND 2 AND 3		Sets 1, 2 & 3 were combined	415

PubMed (Advanced Search, 2016 to August 2018)

Field	Search term
All fields	Botulinum
All fields	enumerat* OR incidence OR prevalen* OR presence OR occur* OR level OR screen* OR survey OR isolation OR detection OR study OR quantifi*
All fields	meat OR beef OR cow OR cattle OR chicken OR turkey OR duck OR poultry OR pig OR pork OR bacon OR sausage OR ham OR lamb OR sheep OR mutton OR venison OR deer OR cured OR meal OR food
Date	2016/01/01"[Date - Publication] - "3000"[Date - Publication]

201 articles were recovered by this search.

Google Scholar (2016 – August 2018)
An Advanced Search was performed with 'botulinum' anywhere in the article.

And with at least one of the following words anywhere in the article: enumerat* OR incidence OR prevalen* OR presence OR occur* OR level OR screen* OR survey OR isolation OR detection OR study OR quantifi*

And with at least one of the following words anywhere in the article: meat OR beef OR cow OR cattle OR chicken OR turkey OR duck OR poultry OR pig OR pork OR bacon OR sausage OR ham OR lamb OR sheep OR mutton OR venison OR deer OR cured OR meal OR food.

45 articles were generated by this search.

The initial list of references was filtered to recover 4 eligible sources that could contribute primary evidence on spore concentration: i.e. not reviews or summarised data. These sources, together with the sources initially identified by Barker *et al.* (2016) are presented in Table 11. Meat species included in the dataset were chicken, turkey, pork, lamb and beef; and included raw and mildly processed meat (samples excluded from the dataset were canned meat and seal and whale meat). In some cases, the reported presence of type B or F toxin did not allow differentiation between proteolytic and non-proteolytic *C. botulinum*. For those records, either was assumed to be present.

Primary data on the origin of materials, type of meat, experimental methods, the microbiology (including toxin type information), and the results were systematically extracted from these sources and entered into a database. The selected sources contributed 103 records to the database. Each record corresponds to a single test applied to a particular food material. Expert microbiologists scored each record, on a scale of 1 to 5, according to the quality of the microbiological method (as described by Barker *et al*., 2016)

3.5.2 Results of literature search

Four additional sources were identified during this literature survey on spore loadings in raw meat compared to those described in Barker *et al.* (2016). In these new references *C. botulinum* was not detected in in any of the nearly 250 meat samples tested (Table 11).

3.5.3 Discussion of spore loadings in fresh meat

Barker *et al*. (2016) provided a quantification of non-proteolytic *C. botulinum* spore loads in various food materials, including fresh meat. They reported that spore loads were expected to centre on a concentration range of 1 to 10 spores kg^{-1} for food materials, with a low contamination of meat. In fresh meat, the probability of the spore loading exceeding 10 spores kg^{-1} was estimated as 2×10^{-7} (Barker *et al*., 2016). A literature search of articles published in or after 2016, identified reports of additional tests for spores of non-proteolytic *C. botulinum* in meat, all of which were negative. Therefore, this increases the belief that small spore loads are to be expected in fresh meat at the expense of beliefs about large loads.

Table 11: Details from publications on *C. botulinum* spore loadings in meat

Reference	Location	Meat source	Sample weight & number	Heating Temp/time	Incubation Temp/time	Toxin test method	# positive	Group	Limit of detection
Abrahamsson & Riemann (1971)	USA	Semi-preserved meat:	30g	70°C or 75°C Time NG	30°C/4-5d	Mouse			≤10 spores/30g
		Bologna	36				5 type A	Prot	
		Cooked ham	100				1 type B	NG	
		Smoked turkey	41						
		Smoked chicken	50						
		Corned beef	20						
		Smoked beef	30						
		Liver sausage	14						
		Luncheon loaf	33						
		All beef salami	20						
		Smoked pastrami	10						
		Pork sausage	8						
		Beef franks	4						
		Smoked beef loaf	5						
		Ham salad spread	1						
Barker *et al.* (2016)	UK		200g	65°C/32 min	12°C/7d	PCR	0		1 spore/200g
		Chicken	21			A, B, E, F			
		Beef	16						
		Lamb	5						
		Pork	5						
		Turkey	50						
Böhnel *et al.* (2008)	Germany	**Slaughter cattle:**	5g	None	37°C/4d	Mouse		NG	NG
		Tonsils	10			(CDC)	2 ABE& C		

		Small intestines Large intestines **Slaughter pigs:** Tonsils Small intestines	10 10 10 10			A+B+E and C+D	0 1 ABE &CD 0 0		
Carlin *et al.* (2004)	France	Meat & poultry	25g or 50g 143		30°C/3d subculture: 30°C/18h	PCR-ELISA to A, B, E, F Mouse	6 type A 1 type B 1 types A+B	Prot NG Prot/NG	NG IAC used
Chukwu *et al.* (2016)	Nigeria	Raw Meat	20	None	37°C/24h	PCR A, B	0		NG
Greenberg *et al.* (1966)	USA & Canada	Raw beef Raw pork Raw chicken	3g 624 656 1078	60°C/15 min	37°C/3d	Mouse A, B, C	0 0 1 type C		NG
Grenda *et al.* (2017)	Poland	Smoked meat Pork meat	10g 56 21	'Pasteurisation process' Assume 70°C/ 15 min	30°C/3d subculture: 30°C/18h	PCR A, B, E, F Confirmed in mouse assay	0		NG
Hauschild & Hilsheimer (1980)	Canada	Pork, bacon	75±5g 416	± 75°C/ 20min	35°C/7d	Mouse	1 type A/B	NG	≈ 1 spore/75g
Hauschild & Hilsheimer (1983)	Canada	Liver, liver sausage	75g 552	± 75°C/ 20min	35°C/7d	Mouse?	5 type A	Prot	1-2 spores/75g
Müller (1967)	Denmark	Cattle liver Swine liver from slaughterhouse carcasses showing pathological signs	4-5g/100 4-5g/100	± 60°C/60 min	37°C/4-6d	Mouse A, B, C, D, E	3 type C 4 type C		NG

Peck *et al.* (2010)	NG	Ham & Bacon Fusilli con pollo Spagetti ragu	200g/45 200g/17 200g/10	65°C/32 min	12°C/7d	PCR A, B, E, F	0		5 spores/200g 79 spores/200g 1 spore/200g
Roberts & Smart (1976)	UK	Sliced bacon Unsliced bacon	25g/263 50g/110 175g/26	None	35°C/≈6d Subculture: 35°C/2d	Mouse	1 A, 10 B 3 A, 3 B 19 A	NG NG Prot	NG
Roberts & Smart (1977)	UK	Bacon	30g 684	None	35°C (6d?)	Mouse?	1 type B 9 untyped	NG	NG
Sathish & Swaminathan (2009)	South India	 Raw mutton meat Chicken carcass Raw chicken meat Mutton carcass Cattle raw meat Cattle carcass	10g 10 5 5 5 20 20	70% EtOH or 80°C/10min	35°C/10d	Mouse test (A, B, E) & PCR 31 on clostridia isolated from meats	0		NG

Note: NG = details not given in original publication

3.6 Review of foodborne botulism incidents related to commercial chilled foods

In 2006 an extensive literature search was performed to collect literature data on outbreaks of foodborne botulism as part of Project B13006 (Peck *et al*., 2006). The authors concluded that outbreaks of foodborne botulism were most likely to be associated with home-made foods where control measures to prevent growth of *C. botulinum* had not been properly implemented during food preparation. Shortly afterwards, Peck *et al*. (2008) reviewed foodborne botulism outbreaks in short shelf-life, commercial chilled foods; they identified 14 outbreaks that were attributable to foods intended to be served chilled. However, none of the outbreaks were due to correctly stored chilled foods; illness occurred when foods were time and/or temperature abused or when pre-formed botulinum toxin was inadvertently added, via another food component, to a correctly chilled product. This review updates the botulism outbreak data summarised in B13006 Project Report (Peck *et al*., 2006) that focussed on foods relevant to the UK/International market and conditions applicable to sourcing and storage of foods.

3.6.1 Method for data collection

An extensive literature search was carried out in August 2018 to collect data on outbreaks of foodborne botulism. Records were retrieved from online databases, then combined with articles held in personal literature collections and references cited in/or citing eligible articles. Searches were not restricted by country or language. The search was performed on the following electronic databases:

Web of Science (Advanced Search in All Databases, 1945 to August 2018)

Search	Field	Search terms	Results
1	Topic	Botulism	6,931
2	Topic	meat OR beef OR chicken OR turkey OR pork OR bacon OR sausage OR ham OR lamb OR mutton OR chilled OR 'ready meal' OR 'ready to eat' OR refrigerat* OR fridge OR 'temperature abuse'	1305141
1 AND 2		Sets 1 & 2 were combined	844

PubMed (Advanced Search, 2006 to August 2018)

Field	Search term
All fields	Botulism
All fields	meat OR beef OR chicken OR turkey OR pork OR bacon OR sausage OR ham OR lamb OR mutton OR chilled OR 'ready meal' OR 'ready to eat' OR refrigerat* OR fridge OR 'temperature abuse'
Date	2006/01/01"[Date - Publication] - "3000"[Date - Publication]

127 articles were recovered by this search.

Google Scholar (2006 – August 2018)
An Advanced Search was performed with 'botulism' in the title

Then with at least one of the following words also in the title: meat OR beef OR chicken OR turkey OR pork OR bacon OR sausage OR ham OR lamb OR mutton OR chilled OR "ready meal" OR "ready to eat" OR refrigeration OR refrigerated OR refrigerate OR fridge OR "temperature abuse"

31 articles were generated by this search.

References were checked for suitability based on a review of titles and abstracts, which identified a sub-set of 196 records. The entire article of each of these references was then individually assessed for relevance, and appropriate information on outbreaks of foodborne botulism in chilled foods extracted.

Additionally, foodborne botulism outbreaks in France between 1995 and 2016 were reviewed, as botulism in France is often associated with meat products. This involved a review of appropriate articles in Bulletin Épidémiologique Hebdomadaire (Carlier *et al*. 2001, 2007; Haeghebaert *et al*. 2003; Mazuet *et al*. 2011, 2012, 2018) and discussions with scientists at Institut Pasteur (Paris).

3.6.2 Results of literature search

Of the 196 references initially identified as potentially suitable to be included in the database, twelve were considered eligible as the outbreak vehicle fitted the criterion of a commercial food intended to be stored chilled. These records, combined with the foodborne botulism outbreaks identified by Peck *et al.* (2008), are presented in Table 12. Of the 26 outbreaks summarised in Table 12, 16 were associated with proteolytic *C. botulinum*, one with *C. baratii*, and four with non-proteolytic *C. botulinum* (all four due to type E toxin in vacuum-packed fish). In a further five outbreaks, the organism responsible for causing botulism was not reported, including three outbreaks caused by type B toxin (Table 12), and it is not clear whether proteolytic *C. botulinum* or non-proteolytic *C. botulinum* strains were involved. The dominance of proteolytic *C. botulinum* indicates the significance of temperature abuse. None of the 26 outbreaks of botulism implicated correctly stored commercial chilled foods (Table 12).

Meat products (family or artisan prepared) are associated with foodborne botulism in France, with commercial meat products also implicated in several outbreaks between 1998 and 2016 (Carlier *et al*. 2001, 2007; Haeghebaert *et al*. 2003; Mazuet *et al*. 2011, 2012, 2018). In outbreaks involving commercial sausages in 2003 (saucisson) and 2013 (chorizo), the sausages were intended to be stored at ambient temperature. A single case in 2012 was suspected to involve commercial pâté from Bulgaria, but product details are not available. A single botulism case in 2015 may have involved commercial jambon blanc intended to be stored chilled, although the origin of this botulism case is not clear, as a strain of non-proteolytic *C. botulinum* type B was found in a sample of jambon blanc and the patient stool, but botulinum neurotoxin was not detected in the product. In summary, no outbreaks of botulism have been confirmed in France with commercial industrial chilled products when the shelf-life and storage temperature have been respected.

3.6.3 Discussion of foodborne botulism related to commercial chilled foods

Non-proteolytic *C. botulinum* is the principal microbiological safety concern, in relation to spore-forming bacteria, in the manufacture of chilled foods as it has the ability to grow at chill temperatures (Peck *et*

al., 2008). Foodborne botulism outbreaks involving non-proteolytic *C. botulinum* have been most frequently associated with salted, vacuum-packed, smoked, or dried fish; home-cured sausages and hams and traditional “fermented” foods prepared by the peoples of Alaska and northern Canada (Peck *et al.*, 2006; Peck, 2010).

Importantly, however, none of the 26 outbreaks of botulism outbreaks identified in this study implicated correctly stored commercial chilled foods. Thus, commercially produced foods intended to be stored chilled do not appear to be implicated in foodborne botulism when the shelf-life and storage temperature are maintained as specified by manufacturers. There is no evidence of cases of botulism arising from the foods that are within the scope of this project, i.e. fresh beef, fresh pork, fresh lamb.

Table 12: Examples of foodborne botulism involving commercial foods intended to be stored chilled [based on, and updating, Peck *et al*. (2008)]

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)
UK (1989)	Hazelnut yoghurt	Prot (B)	27(1)	Toxin added with canned hazelnut conserve to correctly chilled yoghurt	O'Mahony *et al.* (1990)
USA (1989)	Chopped garlic-in-oil	Prot (A)	3	Temperature abuse (product not refrigerated)	Morse *et al.* (1990)
USA (1990)	Grilled fresh Palani (surgeon fish)	NR (B)	3	Temperature abuse	CDC (1991)
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)
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)
USA (1994)	Clam chowder	Prot (A)	2	Temperature abuse (product not refrigerated)	Sobel *et al.* (2004)
USA (1994)	Black bean dip	Prot (A)	1	Temperature abuse (product not refrigerated)	Sobel *et al.* (2004)
Canada (1995)	Country-style pâté	NR (B)	2	Temperature abuse (product not refrigerated)	Leclair *et al.* (2013)
Italy (1996)	Mascarpone cheese	Prot (A)	8(1)	Temperature abuse; pH > 6	Aureli *et al.* (2000)
Germany (1997)	Hot-smoked, vacuum-packed fish	NP (E)	2	Suspected temperature abuse	Korkeala *et al.* (1998)
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)
France (1999)	Fish soup	Prot (A)	1	Temperature abuse at home	Carlier *et al.* (2001)
Canada (2001)	Cooked boneless pork product	Prot (A)	1	Temperature abuse (product not refrigerated)	Leclair *et al.* (2013)
Germany (2004)	Vacuum-packed smoked salmon	NP (E)	1	Consumed 3 days after ‘‘use by date’’	Dressler (2005)
UK (2004)	Organic hummus	NR	1	Time/temperature abuse	McLauchlin *et al.* (2006)

Canada/USA (2006)	Refrigerated carrot juice	Prot (A)	6	Temperature abuse; product pH between 6 and 7	Sheth *et al.* (2008)
Finland (2006)	Vacuum-packed smoked whitefish	NP (E)	1	Suspected temperature abuse	Lindström *et al.* (2006)
China (2007)	Sausages	Prot (A)	66	Temperature abuse (product not refrigerated)	Zhang *et al.* (2010)
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)
France (2009)	Vacuum packed hot-smoked whitefish	NP (E)	3	Suspected temperature abuse during travel and home storage	King *et al.* (2009)
Italy (2010)	Cream of vegetable soup	NR (B)	1	Temperature abuse (product not refrigerated); long shelf-life	Daminelli *et al.* (2011)
USA (2011)	Potato soup	Prot (A)	2	Temperature abuse (product not refrigerated)	CDC (2011)
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)
Slovakia (2015)	Hummus spread	Prot (A)	1	Suspected temperature abuse	Mad'arova *et al.* (2017)
France (2015)	Frozen minced beef used in restaurant Bolognese sauce	*C. baratii* (F)	3	Time/temperature abuse (sauce prepared ≥24h in advance, left at room temperature for several hours)	Mazuet *et al.* (2017)

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

3.7 Summary of data on growth of, and neurotoxin formation by, Clostridium botulinum in chilled fresh meat

An extensive literature search carried out in 2011 provided a strong summary of the knowledge available at that time on growth and neurotoxin formation on fresh chilled meats by non-proteolytic *C. botulinum* (Stringer *et al.*, 2011). The report concluded that the published data available were limited and sometimes contradictory. We have now performed a new literature search, and also include unpublished data kindly provided by industry. The literature data have been supplemented with data from a new challenge test on the ability of non-proteolytic *C. botulinum* to form neurotoxin in chilled fresh meat.

3.7.1. Literature Review

3.7.1.1 Method for data collection for literature review

A literature search was conducted in October 2018 to collect data on neurotoxin formation and growth on fresh meat by non-proteolytic *C. botulinum*. Records were retrieved from online databases, then combined with articles held in personal literature collections and references cited in/or citing eligible articles. Searches were not restricted by country or language. The search was performed on the following electronic databases:

Web of Science (Advanced Search in All Databases, between 1945 to 1970 and 2011 to 2018)

Search	Field	Search terms	Results
1	Topic	Botulinum	8035
2	Topic	meat OR pork OR lamb OR beef OR chicken OR turkey	1,230,233
1 AND 2		Sets 1 & 2 were combined	445

PubMed (Advanced Search, 2011 to 2018)

Field	Search term
All fields	Botulinum
All fields	meat OR pork OR lamb OR beef OR chicken OR turkey
Date	2011/01/01"[Date - Publication] - "3000"[Date - Publication]

259 articles were recovered by this search.

Google Scholar (2011 –2018)

An Advanced Search was performed with ‘botulinum’ in the title. Then with at least one of the following words also in the title: meat OR pork OR lamb OR beef OR chicken OR turkey. 15 articles were generated by this search.

References were checked for suitability based on a review of titles and abstracts, which identified a sub-set of eight records. The entire article of each of these references was then individually assessed for relevance, and appropriate information was extracted.

3.7.1.2 Results of the literature search

In a previous summary, Stringer *et al.* (2011) reported that only limited literature data were available on toxin formation by and growth of non-proteolytic *C. botulinum* at chill temperature on fresh meat. In the current literature survey, only one additional article out of the 719 records generated by the literature searches was considered eligible for inclusion, as it fitted the criterion of determination of toxin formation and/or growth of non-proteolytic *C. botulinum* on raw meat at a temperature of ≤8°C. The findings from published articles and additional unpublished data kindly donated by members of the project consortium are summarised below.

The ability of a non-proteolytic *C. botulinum* type E strain to form botulinum toxin in 250g samples of ground beef was investigated by Warnecke *et al.* (1967). After inoculation with 3 x 10^6 cfu per pack, samples were vacuum packed in oxygen impermeable bags and stored for up to 18 days at 41°F, 55°F and 72°F (stated as equivalent to 3°C, 13°C and 23°C in the article, but confusingly actually equivalent to 5°C, 13°C and 22°C). Samples were inspected at three-day intervals, and when excessive gas formation was observed, samples were frozen for subsequent toxin testing. Toxin was tested for using the mouse bioassay, and positive samples were confirmed by mixing meat extract with type E antiserum prior to injecting into mice. Samples held at 41°F (ca. 5°C) for 11 days had an acceptable appearance with slight gas formation and sour odour. While samples held at 55°F and 72°F (ca. 13°C and 22°C) for 3 days were wholly unacceptable with gas formation, sour odour and dark appearance. All tested ground beef samples caused mortality in mice, including negative controls. However, interpretation of the data is difficult as the authors did not indicate the sampling time for samples that were positive for botulinum toxin. Furthermore, the mouse deaths from samples held at 41°F and 55°F were deemed non-specific and not attributable to botulinum toxin since; (i) addition of type E antiserum did not prevent mouse death, and (ii) when the samples were refrozen and later re-injected into mice, mortality was not observed.

Ajmal (1968) reported on toxin formation by a non-proteolytic *C. botulinum* type E strain on irradiated horsemeat. In this challenge test, 5g samples of horsemeat were placed into 1oz bottles, surface inoculated with spores of type E strain FDA 70 at a concentration of either 10^3 or 10^5 spores g^{-1} meat and incubated at 4°C or 30°C either aerobically or in anaerobic jars. At 30°C, all inoculated samples were toxic by mouse bioassay after 1, 2, 4 and 7 days incubation. However, neither growth nor toxin formation by type E strain FDA 70, as measured by viable count and mouse assay, were observed on samples stored at 4°C for 12 weeks.

A thesis abstract (in English) by Schocken-Iturrino (1980) reported growth of *C. botulinum* in nearly all inoculated samples of vacuum packed meat products tested at >3°C. A translation (via Google Translate) of the original thesis (written in Portuguese) suggests that the meat samples tested in this work were sausages, ham and bacon, rather than fresh chilled meat, and are outside of the scope of this review.

Moorhead and Bell (1999) surface inoculated boneless lamb chumps at a concentration of 10^4 spores cm^{-2} (total = 5 x 10^6 spores per pack) with a cocktail of five botulinum toxin-producing strains (one proteolytic *C. botulinum* type A, two non-proteolytic *C. botulinum* type B, one non-proteolytic *C. botulinum* type E, and one *C. butyricum* type E). Vacuum packed samples were heat shrunk for 2 - 3s at 80 - 85°C and duplicate samples were stored at nine temperatures from -1.5°C to 15°C for up to 84 days. Packs were tested for botulinum toxin when they appeared blown (Table 13). All blown packs were positive for botulinum toxin, using the mouse bioassay (Table 13). The accepted minimum growth

temperature for non-proteolytic *C. botulinum* is 3°C (Peck, 2009), thus the report of neurotoxin formation at 2°C is unexpected. Moorhead and Bell (1999) stated that temperature in the packs did go above 2.0°C during weekly defrost cycles, but only for short time periods, and these would not be expected to lead to toxin formation based on studies in broth systems. Packs tested after incubation for 11 days at 8°C were positive for botulinum toxin (Table 13), but there was no report of the last day when packs were non-toxic. Moorhead and Bell (1999) also reported that only samples inoculated with *C. botulinum* became toxic (i.e. uninoculated control samples did not become toxic), but there is no mention of the use of toxin neutralising antibodies in the mouse bioassay to confirm that the toxic effect was due to botulinum toxin rather than a non-specific toxic effect. In the absence of toxin neutralisation tests, it is not known which strain(s) was responsible for toxin formation.

Table 13: Time to blown pack and presence of botulinum neurotoxin in duplicate samples of vacuum-packed lamb chumps (Moorhead & Bell, 1999)

	Time to blown pack and botulinum neurotoxin (days)								
Incubation temperature	**-1.5°C**	**0°C**	**2°C**	**4°C**	**6°C**	**8°C**	**10°C**	**12°C**	**15°C**
Duplicate sample A	>84	>84	55	27	17	11	6	3	3
Duplicate sample B	>84	>84	55	27	20	11	7	3	3

Later, Moorhead and Bell (2000) compared the effects of vacuum packing and modified atmosphere packing under carbon dioxide (1.5L CO_2 per kg meat) on toxin formation by *C. botulinum* in lamb chumps stored at 2°C or 4°C for 84 days. Duplicate packs were tested for toxin formation (using mouse bioassay) at various time points between 21 and 84 days storage. All tested samples were toxic in mice (Table 14). It was not confirmed that mouse death was specifically caused by botulinum toxin, rather than being a non-specific toxic effect. Given results obtained in broth systems (Peck, 2009), it is unexpected that botulinum toxin was formed at 2°C. The authors did not report the last day when packs were not toxic.

Table 14: Time to botulinum toxin detection in duplicate samples of vacuum- or modified atmosphere packed lamb chumps (Moorhead & Bell, 2000)

	Storage conditions			
	2°C		4°C	
Storage time (days)	**Vacuum**	**CO_2**	**Vacuum**	**CO_2**
21			TT	TT
28			TT	TT
35			TT	TT
48	TT	TT		
55	TT	TT		
63	TT	TT		
84	TT	TT	TT	TT

TT = both duplicate packs positive for botulinum neurotoxin. No symbol = not tested

In challenge tests conducted by Hyytiä-Trees *et al.* (2000), unheated ground beef was surface-inoculated with 10^2 or $10^{5.3}$ spores kg^{-1} of a mixture of non-proteolytic *C. botulinum* strains (one type B, three type

E, and one type F). Vacuum-packed samples (1.5 kg) were stored at 4°C or 8°C, and tested for toxin formation (using mouse bioassay) and growth (measured by quantitative PCR). With the lower spore inoculum (10^2 spores kg^{-1}), toxin was not detected, and growth was only detected after 28 days at 8°C (Table 15). With the higher spore inoculum ($10^{5.3}$ spores kg^{-1}), toxin was only detected after 28 days storage at 8°C, and growth was detected in all conditions (Table 15).

Table 15: Toxin formation and growth of non-proteolytic *C. botulinum* in unheated chilled ground beef (Hyytiä-Trees *et al.,* 2000)

Inoculum (spores kg^{-1})	Toxin formation and growth in storage conditions			
Storage temperature	**4°C**		**8°C**	
Storage time	**21 days**	**28 days**	**21 days**	**28 days**
10^2	xx	xx	xx	xG
$10^{5.3}$	xG	xG	xG	TG

xx = no toxin or growth detected
xG = no toxin detected, but growth of type E strain detected
TG = type E toxin detected, growth of type E strain detected
Neither toxin formation nor growth were detected of type B or type F strain

Stringer *et al.* (2011) conducted a challenge test using three samples of beef, and two samples each of pork and lamb (Table 16). Triplicate samples (ca. 100g) of each meat type were surface inoculated with 10^6 spores of an eight-strain cocktail of non-proteolytic *C. botulinum*, comprising three type B and five type E strains, and vacuum packed in gas impermeable plastic bags (ca. 15 × 18 cm). Also, one sample each of beef, pork and lamb was irradiated prior to inoculation (Table 16). Cooked meat medium (CMM) served as a positive control. Packs were subject to one of four storage regimes; (i) 2°C for 21 days then 5°C for 42 days, (ii) 5°C, (iii) 8°C, or (iv) 10°C. Triplicate samples were removed at defined time periods. The packs were visually inspected for signs of spoilage, and frozen at -20°C for subsequent toxin testing. The presence of type B and type E botulinum neurotoxins in the sample was tested for using an ELISA. Samples were scored as positive if the concentration of botulinum neurotoxin exceeded 240 pg g^{-1} of beef or CMM, and 720 pg g^{-1} of lamb or pork (Table 17).

Stringer *et al.* (2011) reported that all packs lost their vacuum-packed appearance by day 10. Beef samples were judged visually acceptable until day 56, and the lamb samples were visually acceptable throughout the experiment. Pork samples were visually unacceptable (thick creamy exudate) at day 24 (5°C), and day 17 (8°C & 10°C). Toxin production in the test samples stored at 5°C (both with/without prior storage at 2°C) was sporadic, with toxin formation not detected to day 24, and two toxic samples detected at day 28. In test samples stored at 8°C, botulinum neurotoxin was not detected to day 21, but three samples were positive at day 28 days (one pack each of beef 1, pork 5 and lamb 7 (Table 17)). At 10°C, toxin formation was observed from day 10 in packs of pork 4 and lamb 6. Subsequent samples of these two meats were also positive for botulinum toxin, as was beef 1 at day 28 (Table 17). All irradiated samples were visually acceptable throughout the test, with no exudate. However, gas formation was detected, especially in the inoculated samples. Botulinum neurotoxin was not detected in any irradiated meat samples. Given the formation of gas in inoculated and uninoculated packs, it is possible that a gas-forming psychrotrophic spoilage organism had survived irradiation and grown on the meat, alternatively it is conceivable that non-proteolytic *C. botulinum* may have grown without forming toxin, or that

formed toxin had been degraded. This latter possibility raises the question of false-negative results. Toxin formation was detected after 10 days storage at 8°C or 10°C in CMM (positive control), although when stored at 5°C toxin was only detected after 63 days incubation (Table 17). The slow formation of toxin at 5°C is probably because CMM is under a head space of air, and it was not sufficiently reduced to permit growth and toxin formation at this temperature. Surveys have indicated that most strains grow and form toxin at 5°C in suitably reduced media (Stringer *et al*. 2013).

Table 16: Fresh meat samples tested in C. *botulinum* challenge test conducted by Stringer *et al.* (2011)

Meat sample	Description
Beef 1	longissimus - sirloin from a multi species plant, delivered and cut at old MLC demonstration shop to replicate "dirty" plant cutting room. Temp >7°C
Beef 2	Vastus - thick flank from multi species plant, cut and prepared on site to replicate "clean" plant cutting room. Temp <7°C
Beef 3	Gluteus medius - main rump muscle from multi species plant, cut and prepared on site to replicate "clean" plant (as beef 2)
Pork 4	longissimus - loin from multi species plant, cut and prepared on site to replicate "clean" plant (as beef 2)
Pork 5	longissimus - loin from multi species plant, cut and prepared on site to replicate "clean" plant (as beef 2)
Lamb 6	A mix of chump (gluteus medius) thick flank (vastus) topside (semimembranosus) silverside (gluteobiceps) loin (longissimus) selected from a single species lamb plant. Delivered and prepared as beef 1
Lamb 7	As lamb 6, but cut under strict hygiene conditions in chiller area. room temperature <7°C
Beef 8	as beef 1, but frozen and irradiated (25 to 40 kGy) prior to inoculation
Pork 9	as pork 4, but frozen and irradiated (25 to 40 kGy) prior to inoculation
Lamb 10	as lamb 6, but frozen and irradiated (25 to 40 kGy) prior to inoculation
CMM 11	Microbiological (Robertson's) cooked meat medium (positive control)

Table 17: Effect of storage temperature and time on formation of botulinum neurotoxin by *C. botulinum* in a fresh chilled meat challenge test conducted by Stringer *et al.* (2011)

	Number of samples (out of 3) positive for toxin at specified storage temp/time																
Stor. Temp (°C)	--	**2°C/5°C**		**5°C**						**8°C**				**10°C**			
Stor. Time (d)	**0**	**56**	**63**	**17**	**21**	**24**	**28**	**35**	**42**	**10**	**14**	**21**	**28**	**10**	**14**	**21**	**28**
Beef 1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Beef 2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Beef 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pork 4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	2
Pork 5	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Lamb 6	0	1	0	0	0	0	1	0	0	0	0	0	0	2	1	1	1
Lamb 7	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Beef 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pork 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lamb 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CMM 11	0	0	1	0	0	0	0	0	0	3	3	3	3	3	3	3	3

Note that triplicate packs were tested, and the number of packs positive for botulinum neurotoxin (concentration exceeding 240 pg g^{-1} of beef or CMM, and 720 pg g^{-1} of lamb or pork) is shown for each storage temperature and time.

Jones (2018) carried out a challenge test study on four samples of fresh chilled meat for the British Meat Processors Association (Table 18). Tested samples of approximately $2cm^3$ (ca. 1g) were vacuum-packed and held at 1°C. Half of the samples were irradiated (25 kGy). For inoculation, each sample was removed from its vacuum pack and placed into a fresh vacuum bag, and ca. 10^4 spores of non-proteolytic *C. botulinum* (one type B strain, one type E strain) were added per bag. The samples were then re-packed under vacuum and stored at 3°C for two days, then 5°C for one day, and finally at 7°C for the remainder of the test. Triplicate samples were removed at days 0, 10, 14, 21 and 28, and enumerated for sulphite-reducing clostridia (SRC). Data for individual packs are not reported, only the geometric mean value. Uninoculated packs served as an indicator of naturally occurring sulphite-reducing clostridia (SRC). The SRC count is not specific for non-proteolytic *C. botulinum*, and this test relies on the presence of *C. botulinum* at a higher concentration than that of naturally occurring SRC. Provided that there is low background SRC contamination in uninoculated control packs, then the SRC count in the inoculated packs is taken to be that of non-proteolytic *C. botulinum*. However, the result is considered invalid if there is a high concentration of naturally occurring background SRC. Jones (2018) determined whether there had been an increase of >0.5 log cfu/g in SRC count (geometric mean value from the triplicate packs of product) at any of the time points when compared to the mean SRC count at day 0, and stated that “an increase of >0.5 log is greater than the natural variation in levels that may be expected for biological systems and thus is indicative of growth”. Test samples were scored as showing “growth”, “no growth” or “invalid” (Table 18). Following 28 days of storage, growth of non-proteolytic *C. botulinum* was not detected in any of the beef samples, nor in any of the other non-irradiated meat samples (but note data for pork not complete after day 14). Growth was reported in the irradiated lamb sample at day 28 (but not day 21) and in the irradiated pork samples at day 14 (but not day 10) (Table 18). Selected samples were also tested for botulinum neurotoxin, but it was not detected. However, the limit of detection for type B toxin was reported as 50ng g^{-1} meat. This limit of detection is very high and is of limited utility (as ca. 30ng toxin is a human lethal dose). Given the reported ability of non-proteolytic *C. botulinum* to form toxin in the absence of an increase in viable count in other challenge test experiments (e.g. Bell & Kyriakides 2000, Brown & Gaze 1990, Brown *et al.* 1991, Hyytiä *et al.* 1999; Keto-Timonen *et al*. 2012), and the exceptionally high detection limit for botulinum toxin reported by Jones (2018), the possibility that significant quantities of undetected toxin may have been formed cannot be ruled out.

Table 18: Effect of storage time on growth of non-proteolytic *C. botulinum* in a fresh chilled meat challenge test conducted by Jones (2018)

Meat		Growth of non-proteolytic C*. botulinum* in fresh chilled meat at day indicated			
		10	**14**	**21**	**28**
Beef	**irradiated**	NG	NG	NG	NG
	Non-irradiated	NG	NG	NG	NG
Lamb	**irradiated**	NG	NG	NG	G
	Non-irradiated	NG	NG	NG	NG
Pork (rind-off)	**irradiated**	NG	G	NG	G
	Non-irradiated	NG	NG	i	i
Pork (rind-on)	**irradiated**	NG	G	NG	G
	Non-irradiated	NG	NG	NG	i

G = Growth reported (more than 0.5 log increase in mean SRC count compared to day 0)
NG = No growth (less than 0.5 log increase in mean SRC count compared to day 0)
i = invalid result (increase in background SRC count too large for valid result)

The results of four confidential challenge test studies on chilled fresh meat were kindly provided by members of the project consortium. In each challenge test, the experimental approach was similar to that described by Jones (2018) with viable counts of SRC taken to be indicative of viable counts of non-proteolytic *C. botulinum*. However, the arithmetic mean was now used rather than the geometric mean used by Jones (2018). The formation of botulinum neurotoxin was not measured. Thus, given the reported ability of non-proteolytic *C. botulinum* to form toxin in the absence of an increase in viable count in other challenge test experiments (see above), then there is the possibility that undetected toxin may have been present.

- Confidential Study 1 involved the challenge test of three products (vacuum skin packed beef rump, topside of beef and lamb). Packs (size not stated) were inoculated with ca. 10^4 spores of non-proteolytic *C. botulinum* (two type B strains, one type E strain) per gram, vacuum packed, stored at 22°C for 2 hours, and then at 8°C for the remainder of the test. Triplicate samples of inoculated and uninoculated control samples were enumerated for SRC at day 0, and daily from day 11 to 20. The SRC counts for individual packs were reported, and in some cases were very different for the triplicate packs (e.g. for one sample the mean was 6 x 10^3 cfu g^{-1} meat, and the SRC counts for each pack were <10, 3x10^1, and 1x10^4 cfu g^{-1} meat). Overall the mean SRC count did not increase by >0.5 log cfu/g for any of the products, and thus non-proteolytic *C. botulinum* was deemed not to have grown in the products in the test conditions.
- Confidential Study 2 was concerned with the challenge test of two pork products (rind-on shoulder and rind-on leg). Packs (200g) were surface inoculated with ca. 10^3 spores of non-proteolytic *C. botulinum* (one type B strain, one type E strain) per gram, vacuum packed, stored at 3°C for two days, then 5°C for one day, and finally at 7°C for the remainder of the test. Triplicate samples of inoculated and uninoculated control samples were enumerated for SRC at days 0, 10, 12, 14, and 16. The SRC counts for individual packs were reported, and again in some cases were very different for the triplicate samples. For rind-on shoulder, the mean SRC count had increased by >0.5 log cfu/g at day 12 (but not day 10), and for rind-on leg, the mean SRC count had increased by >0.5 log cfu/g

at day 14 (but not day 12). These increases were taken to be indicative of growth of non-proteolytic *C. botulinum*.

- Confidential Study 3 consisted of the challenge test of a beef joint. Packs (300g) were inoculated with ca. 10^3 spores of non-proteolytic *C. botulinum* (one type B strain, one type E strain, one type F strain) per gram, vacuum packed, and stored at 5°C for three days, and then at 7°C for the remainder of the test. Triplicate samples of inoculated and uninoculated control samples were enumerated for SRC at days 0, 11, 12, 13, and 14. All three replicates and the mean SRC count had increased by >0.5 log cfu/g at day 11 (mean = $7x10^4$ cfu/g; replicate values = $9x10^3$, $6x10^4$, $2x10^5$), and this increase was judged indicative of growth of non-proteolytic *C. botulinum*. However, at days 12, 13 and 14, the mean SRC count was less than 10^3 cfu/g. In this study, many of the SRC counts were estimated, and outside a standard range for plate counts.
- Confidential Study 4 involved the challenge test of VP sirloin steak. Packs (size not given) were inoculated with ca. 10^3 spores of non-proteolytic *C. botulinum* (one type B strain, one type E strain) per gram, and stored at 8°C. Triplicate samples of inoculated and uninoculated control samples were enumerated for SRC at days 0, 12, 13, 14, and 15. The mean SRC count did not increase by >0.5 log cfu/g to day 14, and thus non-proteolytic *C. botulinum* was deemed not to have grown in the product to this day. High growth of background SRC gave an invalid result at day 15.

3.7.1.3 Discussion of Literature review

A review of toxin formation by non-proteolytic *C. botulinum* in a range of chilled food and food materials reported that 100 out of 514 independent challenge tests were positive for botulinum toxin in 10 days at 8°C (Peck *et al.*, 2006, Peck *et al.*, 2008). ComBase and other predictive models also indicate the potential for growth and toxin formation by non-proteolytic *C. botulinum* in chilled foods (Peck *et al.*, 2006, Peck *et al.*, 2008), including fresh chilled meat (Stringer *et al*., 2011). However, prior to the challenge test carried out by Stringer *et al*. (2011), there were few data on toxin formation by non-proteolytic *C. botulinum* in chilled fresh meat. A review has been carried out to summarise data from the published literature and other sources.

Table 19 provides a summary of results from challenge test experiments carried out primarily at 7°C or 8°C. It should be noted that there are significant differences in the experimental protocols followed and also some limitations to the reported results (further details are summarised above). For example:

- the spore inoculum concentration (typically 10^5-10^7 spores kg^{-1}) used in the challenge test experiments was often several orders of magnitude higher than that reported for fresh meat in the literature (Barker *et al*., 2016) and in section 3.5 of this report
- the mass of meat used in some challenge tests does not reflect that sold commercially, and the mass used may impact on the results. For example a small mass (e.g. 1g portion) would have a larger surface area to volume/mass ratio compared to cuts of meat sold commercially. It is possible that smaller portions of meat with a greater number of cut surfaces could mean that more nutrients were available to *C. botulinum* and other microbes
- many of the challenge tests also included a period at 5°C (and in some cases 3°C too)
- some meat was irradiated prior to inoculation
- many studies measured growth, and an absence of growth may not equate to an absence of botulinum toxin
- data within some individual studies were inconsistent

- most challenge test were not carried out in accordance with a practice developed in conjunction with industry (Appendix 12) to reflect the approach used internationally (Doyle 1991, NACMCF 1992, Health Canada Food Directorate 2010, NACMCF 2010)

Care must therefore be taken not to over interpret these data. Based on this analysis of these published and unpublished results, many, but not all challenge tests, found beef and lamb to be acceptable to day 20 or 21 at 7°C or 8°C (Table 19). Pork was found to support toxin formation and growth by non-proteolytic *C. botulinum* more readily than beef or lamb (Table 19). There is also evidence that the potential for toxin formation by *C. botulinum* in red meats may depend on properties of each meat, with some samples (e.g. irradiated meat) supporting more rapid toxin formation/growth than other samples of the same meat type. Thus, in view of the unknown provence of the samples used in previous challenge test experiments, and the potential for batch to batch variation, then the application of the findings to all batches of fresh chilled meat is unclear.

Table 19: Summary of time to toxin formation/growth by non-proteolytic *C. botulinum* in fresh chilled meat principally stored at 7°C/8°C[a]

Meat type	Last day acceptable[b]	First day unacceptable[c]	reference
Beef	21	28	Hyytia-Trees *et al.* (2000)
	--[d]	21	
	21	28	Stringer *et al*. (2011)
	28	--	
	28	--	
	28	--	
	28	--	Jones (2018)
	28	--	
	20	--	Confidential study 1
	20	--	
	--	11	Confidential study 3
	14	--	Confidential study 4
Lamb	--	11	Moorhead & Bell (1999)
	21	28	Stringer *et al*. (2011)
	28	--	
	28	--	
	21	28	Jones (2018)
	28	--	
	20	--	Confidential study 1
Pork	21	28	Stringer *et al*. (2011)
	28	--	
	28	--	
	10	14	Jones (2018)
	10	14	
	14	--	
	21	--	
	10	12	Confidential study 2
	12	14	

[a] Care should be taken in the interpretation of these data, due to differences and limitations in the experimental protocol (e.g. quantity meat tested, spore inoculum concentration, temperature storage regime, method to detect toxin formation/growth). Further details are given in the text.

[b] Last day acceptable = last day when toxin formation or growth not detected, according to the criterion adopted by the authors (see text for further details)

[c] First day unacceptable = first day when toxin formation or growth was detected, according to the criterion adopted by the authors (see text for further details)

[d] Information not available on last day acceptable or first day unacceptable

3.7.2. Challenge test on neurotoxin formation by C. botulinum in chilled fresh meat

3.7.2.1 Methodology of challenge test experiment

The challenge test was carried out in accordance with a practice developed in conjunction with industry (Appendix 12), and reflected peer reviewed methods we have used previously, and was consistent with the approach recommended and used by others internationally (Doyle 1991, NACMCF 1992, Health Canada Food Directorate 2010, NACMCF 2010). Guidance documents produced in the USA/Canada for challenge test studies emphasise the importance of verifying that neurotoxin formation can be prevented (Doyle 1991, NACMCF 1992, Health Canada Food Directorate 2010, NACMCF 2010). The experimental protocol was discussed and optimised with industry members of the project consortium.

Spores were produced of twelve strains (seven type B and five type E) of non-proteolytic *C. botulinum* that formed a good quantity of botulinum neurotoxin (Table 20). The selected strains were commonly used in challenge tests, and where possible were relevant to the meat matrix and/or reference strains. The spores were produced and washed free of toxin using standard methods, and enumerated by viable counts. The spore cocktail was prepared in 0.85% saline and contained an equal number of spores of each strain, and the final concentration of spores added to the meat was confirmed by viable count

Table 20: Non-proteolytic *Clostridium botulinum* strains used in the challenge test experiments

Our name	Original name	Source
Type B		
83/01	Eklund 2B	USA - Pacific sediments
81/30	Eklund 17B	USA - Pacific sediments
87/02	CDC 3875	Iceland - Human stool from botulinum case (dried meat)
90/04	Prevot 59	France – (unknown)
93/10	Kapchunka B2	USA - Fish outbreak
05/20	IFR 05/020	Canada - Scallops
05/25	IFR 05/025	UK – Dried egg pasta (fettucine)
Type E		
81/26	Beluga	USA - Fermented Beluga whale
81/31	Hazen 36208	USA - Labrador smoked salmon
86/21	Prevot P34	France - Freshwater perch
87/01	Dolman VH	Canada - Pickled herring
93/08	CDC 8073	USA - Human stool from botulinum case

Six examples of fresh meat (two each of beef, lamb and pork) were tested (Table 21). Each of the six meat samples was from a different supplier (source selected by BMPA to reflect UK market). For each meat species, meat from a short maturation period and a long maturation period were tested (selected by BMPA to reflect UK market). Following slaughter, the meat was held at <3°C prior to shipping, during shipping, and prior to inoculation. It was intended that the supplied meat samples would all be of 125g (selected by project consortium members to reflect the lowest mass sold commercially), but due to variation in the size and shape of meat supplied, all packs of meat were weighed. Details of the mass of meat tested are given in Table 21. The initial pH of each meat was measured after the meat sample had been homogenised with an equal mass of distilled water (Table 21).

Table 21: Summary of fresh chilled meat samples used in the challenge test experiment

BMPA code	Our code	Animal slaughter date	Challenge test inoculation date	Maturation time[a]	Mass (g) of meat per pack [mean +/-StdDev]	Mean initial pH of meat
6	Beef 1	20/07/2018	12/09/2018	54 days	127 ± 3	5.57
5	Beef 2	05/09/2018	12/09/2018	7 days	118 ± 6	5.53
3	Lamb 1	04/09/2018	12/09/2018	8 days	131 ± 5	5.71
4	Lamb 2	10/07/2018	12/09/2018	64 days	123 ± 3	5.76
1	Pork 1	07/09/2018	12/09/2018	5 days	117 ± 5	5.68
2	Pork 2	20/08/2018	12/09/2018	23 days	121 ± 4	5.46

[a] Maturation time = number of days between animal slaughter and challenge test inoculation. During this time the meat was held at <3°C in chilled primal and/or retail packs. Note that all inoculated/uninoculated meat samples were incubated at <3°C for 1 further day, then at 5°C for 1 day, 22°C for 2 hours, and then at 8°C for the remaining incubation period.

For inoculation, the bags of meat were opened and 2 x 100µl of spores (heat activated (70°C/10 mins) spore cocktail) were added to each bag of meat to a final concentration of 500 spores per bag of meat (4 spores/g meat). The spore inoculum was plated to confirm that the correct inoculum had been added. The spore inoculum concentration was selected to be both conservative and also to be more representative of natural contamination than that used in previous challenge tests with fresh meat (note that the concentration was more than 1000-times higher than 30 spores per kg which Barker *et al*. (2016) determined had a probability of natural occurrence of 3.8×10^{-20}, see section 3.5). The bags were then vacuum packed, and gently massaged to distribute the spores. Uninoculated packs of each meat served as the negative control. Anaerobic microbiological broth (Robertson's cooked meat medium) inoculated with the spore cocktail served as the positive control (to demonstrate toxin formation at chill temperature). A conservative approach for temperature storage was agreed by consortium members. All inoculated/uninoculated meat samples and microbiological broth were incubated at <3°C for 1 day, then at 5°C for 1 day, 22°C for 2 hours (to simulate potential abuse during consumer purchase and transportation), and then at 8°C for the remaining incubation period (to reflect domestic storage). Altogether there were 6 meats x 8 sampling times x 3 (triplicate) packs x 2 (inoculated & uninoculated) = 288 packs of meat, plus one extra sampling time (50 days) for beef (6 packs) to give 294 packs of meat. There were also 1 positive microbiological broth x 8 sampling times x 2 (duplicate bottles) x 2 (inoculated & uninoculated) = 32 bottles of positive control microbiological broth.

Triplicate packs of inoculated and uninoculated meat (and duplicate bottles of positive control microbiological broths) were removed at each of eight sampling times (nine for beef), and observed for signs of visual spoilage. The sampling times were days 0, 10, 12, 15, 18, 21, 25, 35 for all meat types (day 0 is the last day at <3°C, day 1 is after 24h at 5°C), with an additional sample time of day 50 for beef. Samples were frozen on the day of sampling, thawed later, and botulinum toxin extracted from the entire sample using gelatin phosphate buffer. Tests for botulinum toxin were carried out in a type B toxin ELISA and type E toxin ELISA, and compared with standard curves in the meat extract to take account of food matrix effects. Each sample was scored as containing more toxin or less toxin than the ELISA detection limit.

3.7.2.2 Results of challenge test experiment

Storage temperature

Storage temperature was monitored throughout the experiment (Table 22).

Table 22: Summary of storage temperatures in the fresh chilled meat challenge test experiment

	Intended storage temperature					
	<3°C		5°C		8°C	
	Test	Control	Test	Control	Test	Control
Mean temperature (°C)	1.7	1.6	4.9	4.9	8.0	8.0
Standard Deviation (°C)	0.1	0.5	0.1	0.1	0.1	0.1
Minimum Temperature (°C)	1.6	1.2	4.7	4.7	7.6	7.7
Maximum Temperature (°C)	2.3	3.1	5.6	5.2	8.3	8.9

Test = packs inoculated with spores (also included all Day 50 samples and microbiological broth controls)
Control = uninoculated packs (except Day 50)

Observations for visual signs of spoilage

Growth of *C. botulinum* can be associated with gas production that may be observed in vacuum-packed meat products. All packs of meat were examined for gas production, exudate formation and discolouration of meat at each sampling point. Sample packs started to lose their tight vacuum-packed appearance within 12 days. Pork 1 samples appeared unacceptable after 12 days at 8°C due to the production of a thick, creamy exudate. Gas production in packs of Lamb 2 was observed from Day 12. By Day 21, gas production was evident in packs of Pork 2. Both types of beef tested and Lamb 1 were judged to look acceptable until Day 35, when all samples appeared spoiled and smelled off (Table 23).

Table 23: Effect of storage time on visual spoilage in the fresh chilled meat challenge test

Sample	BMPA code		Detection of visual spoilage at specified storage time (days)								
			0	10	12	15	18	21	25	35	50
Beef 1	6	Inoculated	NS	NS	NS	NS	NS	NS	NS	S	S
		Uninoculated	NS	NS	NS	NS	NS	NS	NS	S	S
Beef 2	5	Inoculated	NS	NS	NS	NS	NS	NS	NS	S	S
		Uninoculated	NS	NS	NS	NS	NS	NS	NS	S	S
Lamb 1	3	Inoculated	NS	NS	NS	NS	NS	NS	NS	S	--
		Uninoculated	NS	NS	NS	NS	NS	NS	NS	S	--
Lamb 2	4	Inoculated	NS	NS	NS	NS	NS	S	S	S	--
		Uninoculated	NS	NS	S	S	S	S	S	S	--
Pork 1	1	Inoculated	NS	NS	S	S	S	S	S	S	--
		Uninoculated	NS	NS	S	S	S	S	S	S	--
Pork 2	2	Inoculated	NS	NS	NS	NS	NS	S	S	S	--
		Uninoculated	NS	NS	NS	NS	NS	S	S	S	--

NS = No spoilage detected in any of the three replicate packs
S = Spoilage detected in at least one of the three replicate packs
-- = not tested (lamb and pork were not tested at day 50)

Note that day 0 is the last day at <3°C, day 1 is after 24h at 5°C, day 2 is after 24h at 8°C etc.

Detection of botulinum neurotoxin

All samples (inoculated packs, uninoculated packs, and positive control microbiological broths) were tested for the presence of botulinum neurotoxin type B and type E. Botulinum neurotoxins were extracted from each pack, diluted in buffer (total dilution 1:4), tested in duplicate wells in the type B ELISA and type E ELISA, and the mean absorbance of each test sample was calculated. To quantify the amount of toxin formed, each test plate contained a standard curve constructed using a neurotoxin standard in the same meat extract. Samples were considered positive for botulinum neurotoxin if the mean absorbance of the test sample was greater than the absorbance given by a defined concentration of neurotoxin, prepared in an extract of the same meat, and included in the same ELISA plate. The detection limit for each ELISA was based on: (i) standard curves generated for each assay, (ii) the mean plus three standard deviations of the background absorbance, and (iii) previous experience with these ELISA tests. A concentration of botulinum toxin of about 20-40pg toxin g^{-1} food has been previously accepted as a reasonable detection limit with tests carried out using the mouse test according to the FDA BAM manual. Similar detection limits have been achieved in the present project for the type B ELISA and for the type E ELISA (10 pg toxin ml^{-1} meat extract, 40 pg toxin g^{-1} meat). The detection limit is several orders of magnitude lower than the human lethal oral dose of botulinum toxin (approximately 30 ng).

Type B botulinum neurotoxin was not detected in any of uninoculated packs of fresh chilled meat, but was detected in positive controls of microbiological broth from day 12 onwards (Table 24). At day 10, the estimated concentration of botulinum neurotoxin (5 and 14 pg toxin g^{-1} broth) in the microbiological broth was below the detection threshold. All packs of inoculated chilled fresh meat contained <40 pg type B toxin g^{-1} of meat, except for Pork 1 at day 35 (Table 24). In one pack of Pork 1 at day 35, the concentration of type B toxin was ca. 450 pg toxin g^{-1} of meat. In one pack of Beef 2 at day 12, the estimated concentration of type B toxin was ca. 31 pg toxin g^{-1} of meat (below the detection threshold). Type E botulinum neurotoxin was not detected in any of inoculated or uninoculated packs of fresh chilled meat, but was detected in positive controls of microbiological broth from day 15 onwards (Table 24).

Table 24: Effect of storage time on formation of botulinum neurotoxin by *C. botulinum* in the fresh chilled meat challenge test

Sample	BMPA code		Detection of botulinum toxin at specified storage time (days)[a]								
			0	10	12	15	18	21	25	35	50
Beef 1	6	Inoculated	NT[b]	NT	NT	NT	NT	NT	NT	NT	NT
		Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT
Beef 2	5	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT
		Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT
Lamb 1	3	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	--[d]
		Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	--
Lamb 2	4	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	--
		Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	--
Pork 1	1	Inoculated	NT	NT	NT	NT	NT	NT	NT	T[c]	--
		Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	--
Pork 2	2	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	--
		Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	--
Positive control	--	Inoculated	NT	NT	T	T	T	T	T	T	T
	--	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT

[a] day 0 is the last day at <3°C, day 1 is after 24h at 5°C, day 2 is after 24h at 8°C etc.

[b]NT = No botulinum toxin detected (i.e. all three replicate packs contained <40 pg type B toxin g^{-1} of meat and <40 pg type E toxin g^{-1} of meat)

[c]T = Botulinum toxin detected in at least one of the three replicate packs (Pork 1 sample at day 35 contained >40 pg type B toxin g^{-1} of meat; positive control microbiological broth contained >40 pg type B toxin g^{-1} from day 12 onwards, and also >40 pg type E toxin g^{-1} from day 15 onwards)

[d]-- = not tested (lamb and pork were not tested at day 50)

3.7.2.3 Discussion of challenge test experiment

The concentration of type B botulinum neurotoxin was below the detection limit of 40 pg toxin g^{-1} of meat in all inoculated and uninoculated packs of meat, except for one inoculated pack of Pork 1 at day 35. Here the concentration of type B toxin was ca. 450 pg toxin g^{-1} of meat. Pork 1 was deemed spoiled at day 12. The concentration of type E botulinum neurotoxin was below the detection limit of 40 pg toxin g^{-1} of meat in all inoculated and uninoculated packs of meat. Since the tested meats were selected by the BMPA to give a broad representation of the UK market (e.g. current UK maturation times), then it is likely that the findings of the challenge test experiment can be taken to be representative of that of fresh chilled beef, lamb and pork presently sold in the UK.

3.7.3. Discussion of the position with regard to growth of, and neurotoxin formation by, non-proteolytic C. botulinum in chilled fresh meat (based on literature review and challenge test experiment)

The literature review and challenge test reported here build on the work of Stringer *et al.* (2011), who acknowledged that very limited literature data were available at that time on toxin formation and growth of non-proteolytic *C. botulinum* in fresh chilled red meat.

More recent challenge test studies provide information on toxin formation and growth of non-proteolytic *C. botulinum* in fresh chilled red meat, but some are subject to various limitations. These include, most notably, the use of lack of growth to be a proxy for lack of toxin formation (foodborne botulism being an intoxication caused by consumption of botulinum neurotoxin and not an infection caused by cells), and also the use of very high spore concentrations during inoculation, that are not representative of natural contamination.

A new challenge test study has now been carried out as part of this project. Six examples of fresh meat (two each of beef, lamb and pork) were tested, and for each species, meat of a short maturation period and a long maturation period were tested. Samples were inoculated with spores of strains of non-proteolytic *C. botulinum* type B and E. All tests for the presence of type B neurotoxin were negative at day 25 (i.e. <40 pg type B toxin g^{-1} of meat). Pork was positive for type B neurotoxin at day 35 (i.e. >40 pg type B toxin g^{-1} of meat). Samples of lamb and beef were negative for type B neurotoxin on the last sampling day (days 35 and 50, respectively). All tests for the presence of type E neurotoxin were negative (i.e. <40 pg type E toxin g^{-1} of meat).

The variation in previous challenge test data inevitably mean that the findings from the new challenge tests do not align well with all previous results. Toxin/growth were often reported more rapidly in previous challenge test than in the new challenge test. Possible explanations include the high spore inoculum used previously and the small mass leading to a larger surface area to mass ratio.

3.8 Estimated level of protection (safety units) provided by current practice for chilled, VP/MAP fresh meat held at 3°C to 8°C

The FSA document on the safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to non-proteolytic *Clostridium botulinum* (FSA, 2017) stated 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*

For those foods that do not meet with this guidance, it is necessary to demonstrate safety with respect to *C. botulinum*, such as through risk assessment, predictive modelling or challenge test. Risk assessment is a term often used when attempting to define the potential of any object or system to cause harm. In food safety this term is often applied to an assessment of the potential for any food to cause an adverse effect to the consumer (CODEX, 1999; ICMSF, 2002; Membre *et al*., 2018). A limited

number of risk assessments have been previously carried out for *C. botulinum* (e.g. Carlin *et al.,* 2000; Barker *et al*., 2005; Membre *et al*., 2015). Risk assessments for *C. botulinum* carried out by Hauschild and Simonsen (1985, 1986) and later by Peck *et al.* (2006, 2008) estimated the level of protection (safety units) provided by current practice for specific foods with respect to C*. botulinum*, and this approach is used in the present work.

3.8.1 Previous risk assessments that estimated the level of protection (safety units) provided by existing practice for specific foods with respect to *C. botulinum*

Hauschild and Simonsen (1985, 1986) conducted a risk assessment with respect to *C. botulinum* of shelf stable canned cured meats. While these authors considered proteolytic *C. botulinum*, rather than non-proteolytic *C. botulinum*, the basic principles are the same. It was noted that there were few published datasets (e.g. challenge test data) on *C. botulinum* in shelf stable canned cured meats and that this made a safety assessment difficult to achieve. However, they recognised that if the wealth of data generated by the food industry were used, then a safety assessment could be made (Hauschild and Simonsen, 1985, 1986). A novel method was described that used industry data on the numbers of individual packs of product produced over many years, together with recorded cases of botulism attributed to those products, to calculate the level of protection (safety units). This provided a meaningful assessment of actual risks from those products in the past, and as long as existing commercial practices are described, then this could show that the controls used are valid and lead to a safe product. Estimated safety units (decimal number of products marketed per number causing botulism) for defined luncheon meats, canned cured ham and sausages ranged from >7 to >9 (i.e. $>10^7$ to $>10^9$ products) (Hauschild and Simonsen, 1985, 1986).

Peck *et al*. (2006, 2008) used the same approach based on commercial practices and experience for their risk assessment that estimated the level of protection (safety units) provided by existing practice for defined cooked chilled foods with respect to non-proteolytic *C. botulinum*. This included:

- Characterisation of the hazard
- Calculation of the number of packs of chilled foods sold in the UK and internationally (VP, MAP and low oxygen),
- Summary of current commercial practice (e.g. temperature control at various parts of the food chain),
- Ascertaining the number of cases of botulism related to those products,
- Estimating the level of protection (safety units) provided by current practice

This work considered the hazard presented by non-proteolytic *C. botulinum* in cooked chilled foods with a shelf-life of ≤10 days and no specific single controls (pH, Aw, salt etc.), other than storage temperature and shelf life. It should be recognised, however, that although control over a maximum 10 day life at ≤8°C is specified, some of this product will be consumed before the end of its 10 day life, and for some of its life the storage temperature may be less than 8°C (although once the product is moved from commercial distribution to consumer storage, it is possible that some product is stored at temperatures in excess of 8°C). Furthermore, some products will be cooked or reheated before consumption, and if toxin had been formed in such product and sufficient heat applied, then it is possible that the toxin will have been denatured before consumption (Peck *et al*., 2006, 2008).

It was established that $8.3x10^9$ ($10^{9.8}$) packs of cooked chilled food had been sold with a maximum shelf-life of 10 days at ≤8°C between 1986 and 2005, with no associated cases of foodborne botulism (Peck *et al*., 2006, 2008). Thus:

- 1 in $>10^{9.8}$ packs are associated with botulism.
- The number of safety units (decimal number of products marketed per number causing botulism) was calculated as $10^{9.8}$/<1, or >9.8

These findings were reported to the ACMSF, who endorsed the findings and supported a 10-day shelf life (i.e. 10-day rule), with the VP/MAP guidance revised from ≤5 days at ≤8°C to ≤10 days at ≤8°C (ACMSF, 2006).

There are some qualifications to this approach that must be noted. For example, it is assumed that the existing practice remains unchanged (both commercial and consumer practice). Also, although the probability of botulism incidents remaining undetected is low, reporting of the illness cannot be guaranteed to be complete.

3.8.2 Risk assessment to estimate the level of protection (safety units) provided by existing practice for chilled, VP/MAP fresh meat held at 3°C to 8°C with respect to C. botulinum

The level of protection (safety units) provided by current practice for fresh chilled meat has now been estimated using the approach described in section 3.8.1 (above):

- It is estimated that the total UK sales of fresh chilled beef, pork and lamb between 1999-2005 and 2007-2017 was $6.2x10^{10}$ portions each of 250g (Table 7)
- It is estimated that the total global sales of beef, pork and lamb in 2017 was $6.2x10^{11}$ portions of 250g (Table 8)
- There were no cases of foodborne botulism associated with this product (Section 3.6).

Since $6.2x10^{10}$ ($10^{10.8}$) portions of fresh chilled meat (beef, pork and lamb) were sold in the UK between 1999-2005 and 2007-2017 according to current commercial practice, with no associated cases of foodborne botulism, then:

- 1 in $>10^{10.8}$ packs are associated with botulism
- The number of safety units (decimal number of products marketed per number causing botulism) is $10^{10.8}$/<1, or >10.8
- The level of protection (safety units) with respect to *C. botulinum* provided by existing practice in the UK for chilled, VP/MAP fresh meat held at 3°C to 8°C is >10.8

Since $6.2x10^{11}$ ($10^{11.8}$) portions of fresh chilled meat (beef, pork and lamb) were sold globally in 2017, with no associated cases of foodborne botulism, then:

- 1 in $>10^{11.8}$ packs are associated with botulism
- The number of safety units (decimal number of products marketed per number causing botulism) is $10^{11.8}$/<1, or >11.8.
- The level of protection (safety units) with respect to *C. botulinum* provided by existing practice for chilled, VP/MAP fresh meat sold globally in 2017 is >11.8

3.8.3 Discussion of level of protection (safety units) provided by existing practice for chilled, VP/MAP fresh meat held at 3°C to 8°C with respect to C. botulinum

The level of protection (safety units - decimal number of products marketed per number causing botulism) with respect to *C. botulinum* provided by existing practice in the UK for chilled, VP/MAP fresh meat held at 3°C to 8°C is >10.8 (based on sales between 1999-2005 and 2007-2017). While, the level of protection (safety units) with respect to *C. botulinum* provided by existing practice for chilled, VP/MAP fresh meat sold globally in 2017 is >11.8. These levels of protection are greater than that reported in other assessments of this type for defined luncheon meats, canned cured ham and sausages (Hauschild and Simonsen, 1985, 1986), and for cooked chilled food with a maximum shelf-life of 10 days at ≤8°C (Peck *et al*., 2006, 2008).

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4 Overall Project Discussion

4.1 Discussion of Project Outcomes

Currently, UK industry as represented by the project consortium typically applies a chilled retail shelf life of up to 11-13 days to packs of fresh beef, pork and lamb, with a maximum of 23 days for beef, 27 days for lamb, and 18 days for pork. Production temperature regimes are consistent as they are subject to EU regulation enhanced by industry and commercial codes. Given that retail life includes storage by the consumer, it was agreed by the consortium that this storage period should be assumed to be at 8°C.

The level of protection (safety units - decimal number of products marketed per number causing botulism) with respect to *C. botulinum* provided by existing practice in the UK for chilled, VP/MAP fresh meat held at 3°C to 8°C is >10.8; that is more than $10^{10.8}$ person servings have been marketed without incidence of botulism. This level of protection is greater than that reported in other assessments of this type for defined meat products, and for cooked chilled food produced according to the 10-day rule (Hauschild and Simonsen, 1985, 1986; Peck *et al*., 2006, 2008).

Challenge test studies reported in the literature provide information on toxin formation and growth of non-proteolytic *C. botulinum* in fresh chilled red meat, but some are subject to various limitations. A new challenge test study has been carried out with six examples of fresh meat (two each of beef, lamb and pork). Samples were inoculated with spores of strains of non-proteolytic *C. botulinum* type B and E, and incubated at 8°C to day 50 (beef) or day 35 (lamb and pork). All samples of lamb and beef were negative for type B and type E neurotoxin (i.e. <40 pg type B toxin g^{-1} of meat and <40 pg type E toxin g^{-1} of meat). All samples of pork were negative for type B and type E neurotoxin (i.e. <40 pg type B toxin g^{-1} of meat and <40 pg type E toxin g^{-1} of meat) at day 25, with one sample positive for type B neurotoxin at day 35 (i.e. >40 pg type B toxin g^{-1} of meat).

The project conclusions could be strengthened by further challenge tests of fresh beef, lamb and pork. This would support the conclusions from the current challenge test, contributing to counter the literature data that is subject to various limitations.

The estimation of the level of protection and the results from the challenge test experiment both support a shelf-life of greater than 10 days for fresh chilled beef, lamb and pork held at 3°C to 8°C.

4.2 Progress Made Against Project Milestones

Milestones	**Progress made**
1. On execution of contract, subject to MLA receiving the matching contribution from the funding partner)	Completed in full
2. Complete part one of risk assessment (including (1) hazard characterisation, (2) summary project scope, (3) summary industry best practice, (4) exposure assessment (market sales data), (5) exposure assessment (spore loading), and (6) review foodborne botulism related to chilled fresh meat)	Completed in full. Described in sections 3.1-3.6 of this report
3. Complete part two of risk assessment (summary of data on growth of and toxin formation by *C. botulinum* in chilled fresh meat)	Completed in full. Described in section 3.7 of this report
4. Complete part three of risk assessment (estimate the level of protection (safety units) provided by current practice for fresh meat), and finalise project report	Completed in full. Described in section 3.8 of this report
5. Submit final project report to MLA/BMPA	Completed in full. This document is the final project report

5 Conclusions/recommendations

UK industry typically applies a chilled retail shelf life at 3°C to 8°C of up to 11-13 days to packs of fresh beef, pork and lamb, with a maximum of 23 days for beef, 27 days for lamb, and 18 days for pork. This current practice provides a high level of protection with respect to *C. botulinum*, estimated as >10.8 safety units (decimal number of products marketed per number causing botulism), i.e. more than $10^{10.8}$ products have been marketed per number causing botulism.

Some previous challenge test studies are subject to various limitations. A new challenge test study carried out in the current project demonstrated that samples of beef and lamb inoculated with spores of non-proteolytic *C. botulinum* and incubated at 8°C did not become toxic to day 50 (beef) or day 35 (lamb) (i.e. <40 pg type B toxin g^{-1} of meat and <40 pg type E toxin g^{-1} of meat). All samples of pork were negative for type B and type E neurotoxin (i.e. <40 pg type B toxin g^{-1} of meat and <40 pg type E toxin g^{-1} of meat) at day 25, with one sample positive for type B neurotoxin at day 35 (i.e. >40 pg type B toxin g^{-1} of meat). The positive pork sample had spoiled by day 12.

The shelf-life of fresh red meat held at 3°C to 8°C is of great significance to industry. The estimation of the level of protection and the results from the challenge test experiment both support a shelf-life of greater than 10 days for fresh chilled beef, lamb and pork held at 3°C to 8°C.

The project conclusions could be strengthened by further challenge tests of fresh beef, lamb and pork. This would support the conclusions from the current challenge test, contributing to counter the literature data that is subject to various limitations.

The project findings should be widely disseminated in order to maximise benefit and value from this project to the red meat industry.

6 Key messages

The shelf-life of fresh red meat held at 3°C to 8°C is of great significance to industry. The estimated level of protection and the results from the challenge test experiment both support a shelf-life of greater than 10 days for fresh chilled beef, lamb and pork held at 3°C to 8°C.

Using a risk assessment approach, it was established that the current practice provides a high level of protection with respect to non-proteolytic *Clostridium botulinum*; thus there is no evidence that currently-applied shelf lives of fresh chilled beef, pork and lamb combined with current production standards are unsafe.

The ability not to be constrained by a 10-day shelf-life, as indicated in present FSA (2017) guidelines, and the freedom to adopt a shelf-life greater than 10 days at 3°C to 8°C for fresh chilled beef, lamb and pork is of significant economic/social/sustainability benefits to producers/processors/retailers. There may also be environmental/consumer benefits through lower wastage.

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Normal text

8 Appendix

8.1 Appendix 1: Definitions

The following definitions apply throughout:

Term	Definition	Source
Abattoir	A building which is licensed for the slaughter of animals and initial preparation of carcases for human consumption, also commonly called a slaughterhouse. The premises would normally contain the following facilities: accommodation for animals awaiting slaughter (called a lairage), a slaughter area (termed a slaughter hall), an area for emergency slaughter (see casualty slaughter), a refrigerated area, detained meat area with adequate space for holding suspect meat (see condemnation), offal, gut and tripe area, hide and skin area, cutting room, despatch area, amenities for personnel, and a veterinary officers room. Abattoirs are subject to licensing and compliance with EU and UK legislation.	AHDB (2012)
Ageing	Ageing (conditioning, maturation, or hanging) the holding of carcases or primal joints at refrigerated temperatures (0 to 4°C) to improve eating quality (particularly tenderness and flavour). The rate and degree of post mortem tenderisation (ageing) during refrigerated storage (see refrigeration) varies markedly between species and muscles.	AHDB (2012)
Cured meat	Meat that has been treated with NaCl and a source of nitrite.	Based on FAO: http://www.fao.org/docrep/010/ai407e/AI407E14.htm
Dressing	The process of removing various parts of the body of an animal following slaughter. Following this process, the body of the animal is generally referred to as a carcase.	AHDB (2012)
Dry ageing	A method of ageing whereby the carcase, side or cut is left exposed to the air in a chill room for a required period of time. This can lead to high evaporative losses and a reduced lean meat percentage; however it can also promote a stronger meat flavour, which some consumers find desirable.	AHDB (2012)
Fresh meat	Meat that has not undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is vacuum-wrapped or wrapped in a controlled atmosphere.	853/2004 (EC, 2014)

Hot boning	Also referred to as hot de-boning is the separation of the bones from the meat in a carcase as carried out immediately following slaughter, before the carcase has been chilled and the meat time to set (rigor mortis). Hot de-boning of individual muscles removes them from the restraint of attachment to the skeleton and will allow them to shorten if chilled too rapidly (see cold shortening) which causes the meat following cooking to be of a reduced tenderness compared to conventionally (cold) deboned meat (see boning).	AHDB (2012)
Hot processing	Also referred to as pre-rigor processing is the term given to the processes of boning of a carcase and the manufacture of meat products immediately following slaughter, before the carcase has had time to cool and the meat to set (rigor mortis).	AHDB (2012)
Jointing	Also referred to as cutting is the process of sectioning the carcase. Following splitting the carcase into sides, it is then cut into primal joints which is then followed by the process of cutting into retail cuts.	AHDB (2012)
Lairage	The area of an abattoir where animals are held (rested) before slaughter.	AHDB (2012)
Maturation (see also ageing)	Holding primals prior under controlled temperature conditions and under VP or MAP up to retail sale	Industry
Meat	Skeletal muscles of mammalian and bird species recognised as fit for human consumption with naturally included or adherent tissue, where the total fat and connective tissue content does not exceed the values indicated below and where the meat constitutes an ingredient of another food.	1169/2011 (EC, 2011)
Meat Preparations	Fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat.	853/2004 (EC, 2014)
Meat Products	Processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat.	853/2004 (EC, 2014)
Minced Meat	Boned meat that has been minced into fragments and contains <1% salt.	853/2004 (EC, 2014)
Primals	See jointing	AHDB (2012)
Retail cuts	Portions of meat prepared for sale to the consumer in the raw state.	AHDB (2012)
Safety Units	Measure of the level of protection. Safety units are defined as the decimal number of products marketed per number causing illness. For example, a safety unit of >10.8 indicates that more than $10^{10.8}$ person servings have been marketed without incidence of illness.	Hauschild & Simonsen (1985, 1986)

8.2 Appendix 2: Legislative Context of EU Regulation 853/2004 (EC, 2004)

Regulation (EC) No 853/2004 (1) relates to the following Community legislation, in particular regarding the principles and definitions:

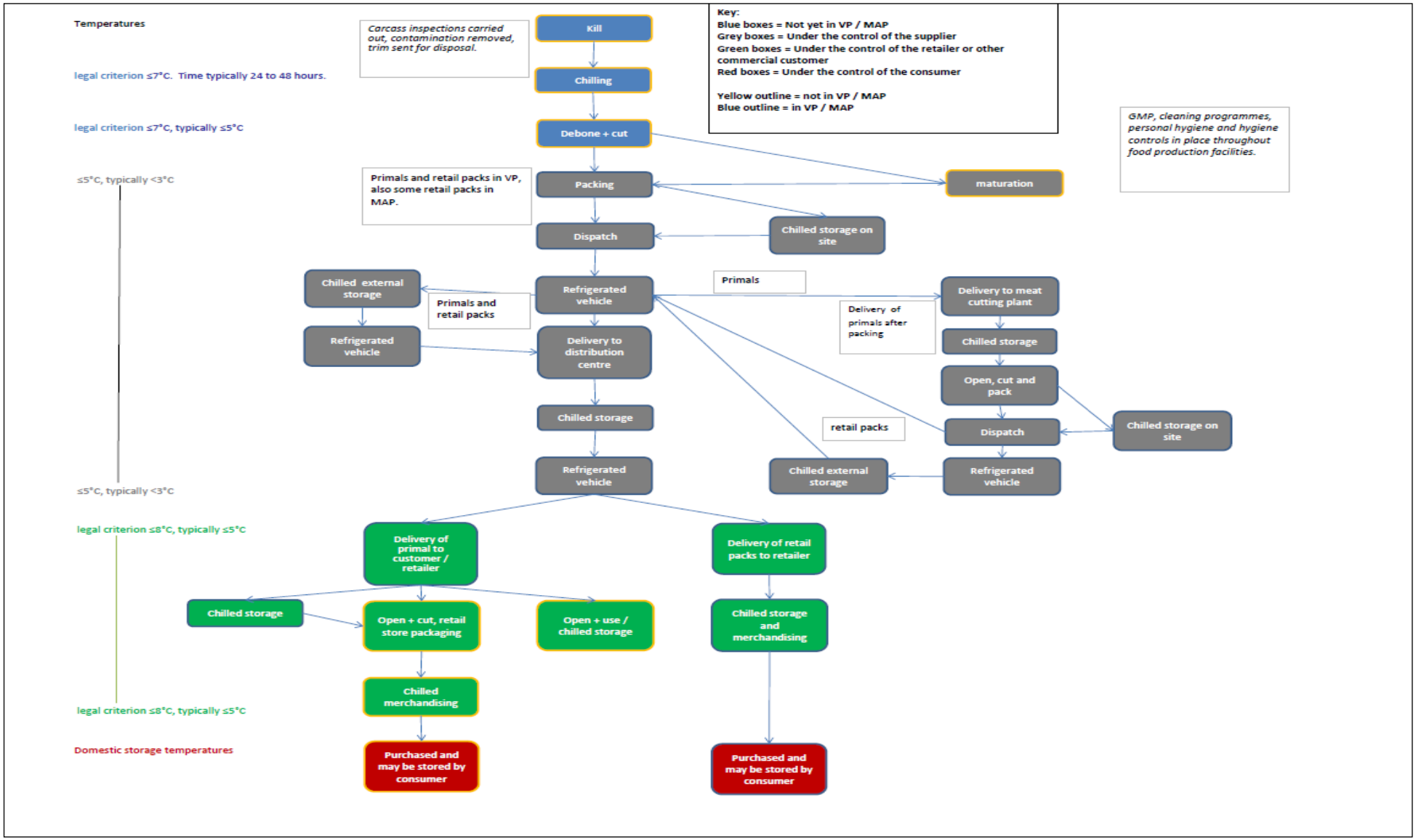
- Regulation (EC) No 178/2002 (2) of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety (also referred to as the General Food Law),
- Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April on the hygiene of foodstuffs (3), and Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare (4),
- Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (5),
- Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004, for the organisation of official controls under Regulations (EC) No 854/2004 and (EC) No 882/2004, derogating from Regulation (EC) No 852/2004 and amending Regulations (EC) No 853/2004 and (EC) No 854/20046 (6),
- Commission Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat (7),
- Commission Regulation (EC) No 2076/2005 of 5 December 2005 laying down transitional arrangements for the implementation of Regulations (EC) No 853/2004, (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and No 854/2004 (8).

Separate guidance documents on Regulations (EC) No 178/2002 and (EC) No 852/2004 have been established.

References:

(1) OJ No L 226, 25.6.2004, p.22
(2) OJ No L 31, 1.2.2002, p. 1
(3) OJ No L 226 of 25.6.2004, p. 3
(4) OJ No L 165, 30.4.2004, p.1
(5) OJ No L 338, 22.12.2005, p. 1
(6) OJ No L 338, 22.12.2005, p. 27
(7) OJ No L 338, 22.12.2005, p. 60
(8) OJ No L 338, 22.12.2005, p. 83

8.3 Appendix 3: Example flowchart for fresh chilled beef in VP/MAP



8.4 Appendix 4: Example flowchart for fresh chilled pork in VP/MAP

Kill to butchery:
- Pigs delivered and killed on day 0 (max 25% of daily kill kept overnight in lairage) seasonal temp depends on time the of year
- Pigs go through pre-chill (-25°C for approx 90 mins)
- Pigs kept overnight (approx 16-18hrs) in the chiller 0-3°C
- Pigs released from chill <5°C
- Pigs butchered to product specification. End product temp average <5°C
- Primal packed to VP (max life P+10) or MAP (max life P+10) in bulk format (dolav 500kg, bags 25kg)

Raw Material (In house):
- Stored at 0°C to +4°C
- Min pack+1 Max pack+9
- Gas flush or VP (format depends on the customer)

Raw Material Intake (Approved Suppliers):
- **Pork temperature range 0°C to +4°C**
- **Pork UK Min pack+1 Max pack +8 (VP and Gas flush)**
- **Pork EU Min pack+6 (VP and Gas flush)**

Raw Material Storage (post intake):
- Chilled Storage set at 0°C to +3°C
- Max storage Pork UK pack +9 (VP and Gas flush)
- Max storage Pork EU pack +8 (VP and Gas flush

Raw Material prior to slice:
- Temperature set range -0°C to +4°C (typically achieving below 3°C)
- Opened used within 24 hours, unused back to the chiller storage 0°C to +2°C

Retail pack:
- Fresh Pork temp max 4°C (crust frozen below 2°C)
- Product packed from VP or MAP to any of the below finished pack formats
- Shelf life:
  - Fresh Pork MAP Max Pack+ 9
  - Fresh Pork VP (Joints) Pack+12
  - Fresh Pork Skin Pack Pack + 10
  - Frozen Pack+12 months

Retail Pack Storage:
- Chilled Storage set at 0°C to +3°C
- Max storage time:
  - Fresh Pork Pack+7
  - VP joint Pack+9

Despatch to retailer depot:
- Trailers set below 3°C
- Max transport time 24 hours

8.5 Appendix 5: Example flowchart for fresh chilled lamb in VP/MAP

[1] Delivery and lairage of Livestock
[2] Abattoir Dirty Process – stun and stick, head and tail removal, strips and legging, anal vacuum, hind feet removal, shoulder pull, release weasands, punch arm, hide removal.
[3] Abattoir Clean Process – Rodding and weasand clipping, skin flaps, back feet removal, brisket saw, inversion, remove forelegs.
[4] Evisceration
[5] Pluck removal, Kidney release →red offal → (not in scope)
[6] Trim, FSA inspection, weighing and classification
[7] Carcase Electrical stimulation (if used)
[8] Carcase Chilling (no less than 10°C within the first 10 hours – ref AHDB Optimising Lamb Meat Quality 2016. Note – if electrical stimulation is used temperature fall can be quicker than this). Final temperature <3°C
[9] Carcase Storage <3°C (0 days – 7 days depending on maturation / processor. Note – this step is minimised as far as possible where long life is required.)
[10] Cutting into primals
[11] Vacuum pack (VP) or CAPTEC MAP* or Retail MAP***.
[12] Storage in deep chill <3°C 0 days – 84 days depending on origin and process. (Deep chill temperature typical practice is -1°C to 0°C. Typical maximum life is 70 days under VP and 84 days under CAPTEC MAP)
[13] Primals cut or minced into retail or business-to-business (B2B) products VP or Retail MAP
[14] Storage in chill <3°C (<2°C for mince) 0 days – balance of life from stage 12**.
[15] Dispatch (B2B) <3°C maximum life as for stage 14 or placed into the retail supply chain/unknown transit/commercial or domestic kitchens 3 to 8°C (ref FSA guidance June 2017) 10 day life applied not exceeding total life as at stage 14.

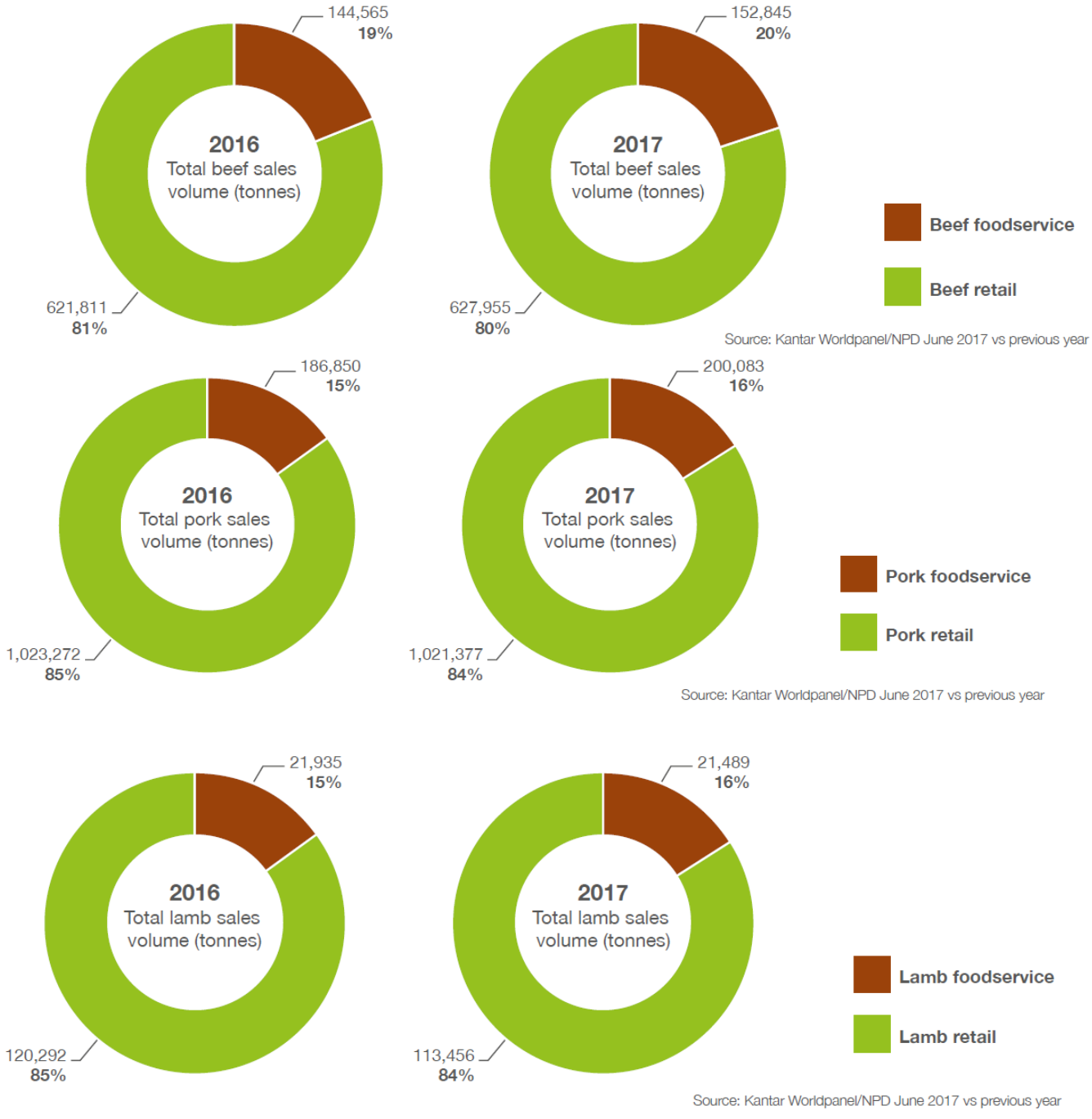
Notes:
*CAPTEC MAP – gas flushed 70% CO_2/30% N_2
**Product (typically from the Southern Hemisphere) will be given a typical deep chill life of up to 84 days. This is further processed in the UK and given another shelf life but this cannot exceed the total 84 day shelf-life allocated at point of pack in the Southern Hemisphere.
***Retail MAP typically 25%CO_2/75%O_2

Typical Shortest Life	**Typical Longest Life**
Day 0 – kill – temperature to <3°C	Day 0 – kill – temperature to <3°C
Day 1 – cut and pack into Retail MAP	Day 1 – cut and pack into CAPTEC MAP deep chill <3°C
Day 8 – end of life 3 to 8°C	Day 74 – cut and pack into retail VP pack
	Day 84 – end of life 3 to 8°C

8.6 Appendix 6: Data Used to Calculate UK Consumption of Fresh Beef, Pork and Lamb 1999-2005 and 2007-2017

AHDB data (Fig. A1) show the market split between sales through retailers and foodservice for each species of interest. Approximately 80-85% of sales of each species is through retailers. Importantly, the data for pork is "top level pork sales" and also includes processed pork such as bacon and sausage. It is assumed that inclusion of processed meat does not impact on the proportion of market segment at multiple retail in the calculations that follow.

Fig. A1: Fresh Beef, Pork and Lamb 2016 and 2017 UK Sales via Retail and Foodservice (AHDB, 2017).



AHDB (Kantar) data for sales of fresh beef, pork and lamb in Great Britain August 2016-August 2018, gives the split by the top five major (multiple) retailers, other retailers and independent butchers (Table A1).

Table A1: AHDB (Kantar) data - sales of fresh meat by outlet type from August 2016-August 2018 in Great Britain (AHDB, 2017)

	Percentage of volume (52 weeks ending period)					
	Beef		Lamb		Pork	
	13-Aug-17	12-Aug-18	13-Aug-17	12-Aug-18	13-Aug-17	12-Aug-18
Total Butchers	6.1	6.3	12.5	13.7	8.8	7.9
Top 5 Retailers*	63.8	63.1	63.5	61.3	63.5	63.8
Other retailers	30.2	30.6	23.9	25.1	27.7	28.3

* Top 5 in terms of volume sold (Tesco, Sainsbury's, ASDA, Morrison's, Co-op)

Given that these data exclude Northern Ireland, which had a population of 1.87 million in 2017 (https://www.nisra.gov.uk/sites/nisra.gov.uk/files/publications/MYE17_POP_TOTALS.xlsx (accessed 20/9/18)) or 2.7% of the UK's total population of 65.6 million (https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/articles/overviewoftheukpopulation/july2017 (accessed 20/9/18)), then it is assumed that applying the same market splits to the entire UK market does not introduce significant errors.

By combining AHDB datasets (Fig. A1 and Table A1), UK sales of beef, lamb and pork sold through major multiples, independent butchers and foodservice can be calculated for each of these markets between August 2016 and August 2018 (Table A2).

Table A2: Percentage Total UK Market Sales by Volume for Fresh Beef, Pork and Lamb, by Outlet Type (August 2016 – August 2018). Note that the data for pork is "top level pork sales" and also includes processed pork such as bacon and sausage

Meat	Aug 2016-Aug 2017		Aug 2017-Aug 2018	
	Tonnes	Proportion of segment	Tonnes	Proportion of segment
Beef				
Total Retail	621,811	81%	627,955	80%
Independent butchers	37,930	5%	39,561	5%
Multiple retail	583,881	76%	588,394	75%
Foodservice	144,565	19%	152,845	20%
Beef Total	**766,376**		**780,800**	
Pork				
Total Retail	1,023,272	85%	1,021,377	84%
Independent butchers	90,048	7%	80,689	7%
Multiple retail	933,224	77%	940,688	77%
Foodservice	186,850	15%	200,083	16%
Pork Total	**1,210,122**		**1,221,460**	
Lamb				
Total Retail	120,292	85%	113,456	84%
Independent butchers	15,037	11%	15,543	12%
Multiple retail	105,256	74%	97,913	73%
Foodservice	21,935	15%	21,489	16%
Lamb Total	**142,228**		**134,945**	
Beef&Pork&Lamb Total	**2,118,726**		**2,137,205**	
Beef&Pork&Lamb Portions @250g	$8.47X10^9$		$8.55X10^9$	

From this dataset, the proportion of sales in the UK from August 2016-August 2018 through multiple retailers was calculated to be 76% (Table A3).

Table A3: Proportion of Sales of UK Fresh Beef, Lamb and Pork Sales by Multiple Retailers August 2016-August 2018 (Calculated from AHDB Data). Note that the data for pork is "top level pork sales" and also includes processed pork such as bacon and sausage

	Multiple retail sales	
	Tonnes	**% Total market**
Beef		
2016-2017	583,881	76%
2017-2018	588,394	75%
Pork		
2016-2017	933,224	77%
2017-2018	940,688	77%
Lamb		
2016-2017	105,256	74%
2017-2018	97,913	73%
Total	**3,249,356**	**76%**

BMPA data for sales by the major retailers of fresh beef, pork and lamb, from 2008-2017 totalled 10,241,496 tonnes (Appendices 7 to 9). Applying the calculated retailers' market share (76%) to the BMPA UK data brings total UK estimated sales through retailers, foodservice and independent butchers to 13,475,565 tonnes, which is equivalent to 5.4×10^{10} portions from 2008-2017.

An earlier report (Peck *et al*., 2016) provides details of 1999-2005 UK fresh beef, pork and lamb consumption data (Table A4). Combining the two datasets gives total UK sales figures for fresh beef, pork and lamb figures for the period 1999-2005 and December 2007-December 2017 of 1.6×10^{7} tonnes, which is equivalent to 6.2×10^{10} 250g portions.

Table A4: UK Raw Red Meat Sales (1999-2005) (Updated from Peck *et al*., 2006)

Raw red meat	Total tonnes	Total number of packs	Total number of 250g portions
Beef	5.6×10^{5}	4.2×10^{9}	2.2×10^{9}
Lamb	2.1×10^{5}	1.2×10^{9}	8.4×10^{8}
Pork	12.0×10^{5}	2.5×10^{9}	4.8×10^{9}
TOTAL	1.97×10^{6}	7.9×10^{9}	7.8×10^{9}

8.7 Appendix 7: UK Beef Market and Consumption Data 2007-2018

BMPA data for fresh beef sales in the UK through major multiples for the ten year period from 52 weeks ending 28 December 2008 to the year ending 31 December 2017, by retailer show that the total number of 250g portions of fresh beef sold by UK major multiples from 2008-2017 was ~2.19 x10^{10} (Table A5).

Table A5: UK Major Retailer Sales of Fresh Beef 2007-2017

Retailer	FRESH BEEF (Tonnes) Dec 2007-Dec 2017	FRESH BEEF (250g portions) Dec 2007-Dec 2017
1	2,892,607	11,570,428,000
2	748,298	2,993,192,000
3	420,272	1,681,088,000
4	441,221	1,764,884,000
5	376,814	1,507,256,000
6	124,197	496,788,000
7	67,866	271,464,000
8	97,675	390,700,000
9	129,601	518,404,000
10	120,389	481,556,000
11	52,750	211,000,000
Grand totals	**5,471,690**	**21,886,760,000**

Yearly figures from BMPA (Table A6) used to generate these totals show that comparing the years from 52 weeks ending 28 December 2008 with the year ending 31 December 2017 fresh beef consumption was lower by ca. 4%.

Table A6: BMPA Data – Fresh Beef Sales through Major Retailers December 2008-December 2017

Retailer	52 w/e 28/12/08	52 w/e 27/12/09	52 w/e 12/12/10	52 w/e 11/12/11	52 w/e 9/12/12	52 w/e 8/12/13	52 w/e 7/12/14	52 w/e 6/12/15	52 w/e 4/12/16	52 w/e 31/12/17	Total Tonnes	Total 250g Portions
1	300,864	294,622	300,658	301,540	288,936	280,479	278,334	277,697	284,372	285,105	2,892,607	11,570,428,000
2	82,562	81,403	86,353	85,326	81,200	73,303	63,233	64,902	64,976	65,040	748,298	2,993,192,000
3	50,557	49,868	46,052	44,842	38,644	37,704	39,339	37,299	37,428	38,539	420,272	1,681,088,000
4	45,815	48,455	50,889	48,412	48,283	45,821	45,345	38,936	36,390	32,875	441,221	1,764,884,000
5	35,178	38,856	42,529	41,758	38,976	39,575	37,150	35,305	34,275	33,212	376,814	1,507,256,000
6	22,900	18,154	13,534	13,590	10,102	9,078	8,756	8,864	9,825	9,394	124,197	496,788,000
7	6,037	5,509	5,854	6,032	6,483	6,972	7,468	7,413	8,108	7,990	67,866	271,464,000
8	8,934	8,639	9,068	9,056	9,599	9,483	10,573	11,194	10,431	10,698	97,675	390,700,000
9	2,144	2,809	3,430	6,089	8,954	10,503	15,583	22,451	27,266	30,372	129,601	518,404,000
10	7,013	4,822	6,613	9,633	9,256	10,492	12,503	15,854	21,293	22,910	120,389	481,556,000
11	3,796	5,338	5,199	5,348	4,977	5,083	6,329	5,637	5,172	5,871	52,750	211,000,000
Total	**565,800**	**558,475**	**570,179**	**571,626**	**545,410**	**528,493**	**524,613**	**525,552**	**539,536**	**542,006**	**5,471,690**	**21,886,760,000**

8.8 Appendix 8: UK Pork Market and Consumption Data 2007-2018

BMPA data for fresh pork sales in the UK through major multiples from 2008-2017, by retailer show that the total number of 250g portions of fresh pork sold by UK major multiples from the 52 weeks ending 28 December 2008 with the year ending 31 December 2017 was ~ 1.31 x10^{10} (Table A7).

Table A7: UK Major Retailer Sales of Fresh Pork Dec 2007-Dec 2017

Retailer	FRESH PORK (Tonnes) Dec 2007-Dec 2017	FRESH PORK (250g portions Dec 2007-Dec 2017
1	1,755,589	7,022,356,000
2	422,523	1,690,092,000
3	270,795	1,083,180,000
4	240,402	961,608,000
5	262,945	1,051,780,000
6	77,731	310,924,000
7	44,304	177,216,000
8	46,233	184,932,000
9	61,234	244,936,000
10	84,762	339,048,000
11	20,008	80,032,000
Grand totals	**3,286,526**	**13,146,104,000**

Yearly figures from BMPA used to generate these totals show that comparing the years from 52 weeks ending 28 December 2008 with the year ending 31 December 2017 fresh pork consumption was lower by ca. 6% in 2017 (Table A8).

Table A8: UK Major Retailer Sales of Fresh Pork 2007-2017

Retailer	52 w/e 28/12/08	52 w/e 27/12/09	52 w/e 12/12/10	52 w/e 11/12/11	52 w/e 9/12/12	52 w/e 8/12/13	52 w/e 7/12/14	52 w/e 6/12/15	52 w/e 4/12/16	52 w/e 31/12/17	Total Tonnes	Total 250g portions
1	177,206	182,559	183,189	187,005	180,235	174,097	178,136	169,068	163,495	160,599	1,755,589	7,022,356,000
2	44,627	48,232	49,506	47,416	42,986	39,490	38,582	36,790	37,446	37,448	422,523	1,690,092,000
3	29,061	30,997	31,196	29,296	28,736	26,682	26,891	25,233	21,686	21,017	270,795	1,083,180,000
4	25,752	26,035	25,450	26,554	23,674	24,131	23,195	23,165	21,997	20,449	240,402	961,608,000
5	22,754	28,879	29,865	29,845	29,042	28,361	29,073	25,853	21,227	18,046	262,945	1,051,780,000
6	13,402	10,957	8,877	8,445	9,251	7,822	6,447	4,748	4,476	3,306	77,731	310,924,000
7	4,543	4,435	4,232	4,311	4,171	4,513	4,383	4,272	4,706	4,738	44,304	177,216,000
8	4,346	4,742	5,094	5,373	5,234	4,826	4,206	4,084	4,368	3,960	46,233	184,932,000
9	1,636	1,742	1,468	2,225	3,858	5,562	8,868	9,719	11,653	14,503	61,234	244,936,000
10	3,398	3,471	4,409	7,689	7,778	8,229	10,944	12,635	12,370	13,839	84,762	339,048,000
11	1,215	1,226	1,498	1,993	1,702	1,370	2,824	2,604	2,944	2,632	20,008	80,032,000
Total	**327,940**	**343,275**	**344,784**	**350,152**	**336,667**	**325,083**	**333,549**	**318,171**	**306,368**	**300,537**	**3,286,526**	**13,146,104,000**

8.9 Appendix 9: UK Lamb Consumption Data 2007-2017

BMPA data for fresh lamb sales in the UK through major multiples from 2008-2017, by retailer show that the total number of 250g portions of fresh lamb sold by UK major multiples for the ten years for the 52 weeks ending 28 December 2008 to the year ending 31 December 2017 was ~ 5.9 x10^9 (Table A9).

Table A9: UK Major Retailer Sales of Fresh Lamb Dec 2007-Dec 2017

Retailer	FRESH LAMB (Tonnes) Dec 2007-Dec 2017	FRESH LAMB (250g portions) Dec 2007-Dec 2017
1	811,271	3,245,084,000
2	208,361	833,444,000
3	95,580	382,320,000
4	139,067	556,268,000
5	84,201	336,804,000
6	32,201	128,804,000
7	25,796	103,184,000
8	33,526	134,104,000
9	22,037	88,148,000
10	19,593	78,372,000
11	11,647	46,588,000
Grand totals	**1,483,280**	**5,933,120,000**

Yearly figures from BMPA used to generate these totals show that comparing the years from 52 weeks ending 28 December 2008 with the year ending 31 December 2017 fresh lamb consumption was lower by ca. 35% in 2017 (Table A10).

Table A10: BMPA Data – Annual UK Sales of Fresh Lamb Sold by Major Multiples

Retailer	52 w/e 28/12/08	52 w/e 27/12/09	52 w/e 12/12/10	52 w/e 11/12/11	52 w/e 9/12/12	52 w/e 8/12/13	52 w/e 7/12/14	52 w/e 6/12/15	52 w/e 4/12/16	52 w/e 31/12/17	Total Tonnes	Total 250g portions
1	102,110	93,601	86,914	71,305	73,866	84,380	79,070	78,643	74,559	66,823	811,271	3,245,084,000
2	24,711	25,371	24,757	20,667	20,942	23,245	19,533	17,099	16,495	15,541	208,361	833,444,000
3	11,181	11,275	9,870	7,885	8,216	8,825	9,603	11,012	9,527	8,186	95,580	382,320,000
4	18,459	17,348	16,542	12,934	13,312	15,952	12,598	11,252	11,132	9,538	139,067	556,268,000
5	10,557	9,737	9,,290	8,179	8,107	8,148	7,434	8,125	7,316	7,308	84,201	336,804,000
6	7,674	5,633	4,067	2,627	2,195	2,751	2,120	1,986	2,220	928	32,201	128,804,000
7	3,565	3,120	2,425	1,539	2,279	3,084	2,409	2,495	2,487	2,393	25,796	103,184,000
8	4,428	4,061	3,756	3,214	3,053	3,473	3,165	3,163	2,898	2,315	33,526	134,104,000
9	1,340	1,279	1,089	947	1,004	1,639	3,314	3,907	3,780	3,738	22,037	88,148,000
10	1,678	1,123	1,203	651	1,092	1,562	2,065	3,692	3,838	2,689	19,593	78,372,000
11	833	1,005	1,161	985	732	694	1,641	1,626	1,479	1,491	11,647	46,588,000
Total	**186,536**	**173,553**	**161,074**	**130,933**	**134,798**	**153,753**	**142,952**	**143,000**	**135,731**	**120,950**	**1,483,280**	**5,933,120,000**

8.10 Appendix 10: Australian Fresh Lamb exports to UK 2005-2018

Australia exported 3.98 x 10^4 tonnes or 1.59 x 10^8 portions of fresh lamb to the UK between 2005 and October 2018 (Table A11).

Table A11: Australian Chilled Fresh Lamb Exports to the UK, January 2005 to October 2018

Year	Quantity exported (Tonnes)				Total number 250g portions
	Multipacked/VP	Individually VP	VP Primals	Total	
2005		2,076		2,076	8.30×10^6
2006	10	2,315		2,325	9.30×10^6
2007	113	2,996		3,110	1.24×10^7
2008	52	2,413		2,465	9.86×10^6
2009	38	3,171		3,208	1.28×10^7
2010	82	2,990	21	3,093	1.24×10^7
2011	28	3,329	26	3,383	1.35×10^7
2012	192	3,846		4,037	1.61×10^7
2013	391	3,298		3,689	1.48×10^7
2014	523	3,365		3,888	1.56×10^7
2015	486	3,043		3,529	1.41×10^7
2016	362	2,144		2,506	1.00×10^7
2017	336	1,709		2,045	8.18×10^6
2018 (to October)	106	375		481	1.93×10^6
Grand Total	**2,720**	**37,070**	**47**	**39,836**	**1.59×10^8**

Source: DAWR, via MLA

8.11 Appendix 11: Australian Fresh Beef exports to UK 2005-2018

Australia exported 8.55 x 10^4 tonnes or 3.42 x 10^8 portions of fresh beef and veal to the UK between 2005 and October 2018 (Table A12).

Table A12: Australian Chilled Beef and Veal Exports to the UK January 2005 - October 2018

Year	Quantity exported (Tonnes)				Total number
	Multipacked/VP	Individually VP	VP Primals	Total	250g portions
2005	0	4,568		4,568	$1.83x10^7$
2006	8	6,208	23	6,240	$2.50x10^7$
2007	0	3,302	169	3,472	$1.39x10^7$
2008	0	4,680	422	5,102	$2.04x10^7$
2009	1	3,577	501	4,078	$1.63x10^7$
2010	28	4,296	313	4,637	$1.85x10^7$
2011	116	5,378	252	5,745	$2.30x10^7$
2012	261	6,724	102	7,087	$2.83x10^7$
2013	509	9,336	72	9,916	$3.97x10^7$
2014	710	9,417	290	10,417	$4.17x10^7$
2015	598	8,755	46	9,399	$3.76x10^7$
2016	539	7,100	8	7,648	$3.06x10^7$
2017	475	5,566		6,041	$2.42x10^7$
2018 (to end Oct)	82	1,094		1,176	$4.71x10^6$
Grand Total	**3,327**	**80,002**	**2,197**	**85,525**	**$3.42x10^8$**

Source: DAWR, via MLA

8.12 Appendix 12: Guidelines for Setting Shelf Life of Chilled Foods in Relation to Non-proteolytic *Clostridium botulinum*

(https://www.chilledfood.org/new-publication-guidelines-for-setting-shelf-life-of-chilled-foods-in-relation-to-non-proteolytic-clostridium-botulinum/).

Guidelines for Setting Shelf Life of Chilled Foods in Relation to Non-proteolytic *Clostridium botulinum*

First edition

9 July 2018

Guidelines for Setting Shelf Life of Chilled Foods in Relation to Non-proteolytic *Clostridium botulinum*

Introduction

The primary responsibility for food safety rests with the food business operator (FBO). General implementation of procedures based on HACCP principles, together with the application of good hygiene practice, should reinforce FBOs' responsibility.

This document has been developed by a number of FBOs, trade bodies, associations and laboratories in the UK and Australia to provide guidance in relation to non-proteolytic *Clostridium botulinum* and:

- Shelf life establishment for chilled foods by FBOs, and
- On challenge testing foods by laboratories.

The guidance provides information to enable FBOs to consider:

- How to establish shelf life in relation to non-proteolytic *Clostridium botulinum*
- What needs to be considered and what actions need to be taken to determine whether challenge testing is appropriate before contacting a laboratory,
- What issues the laboratory should take into consideration for challenge testing to be carried out appropriately and give valid scientific data, and
- How to use these data to establish safe shelf life with respect to non-proteolytic *Clostridium botulinum*

The guidance is designed to also ensure that sufficient information is provided by FBOs and laboratories to arrive at valid decisions and to support FBOs when challenged by Competent Authorities.

Contents

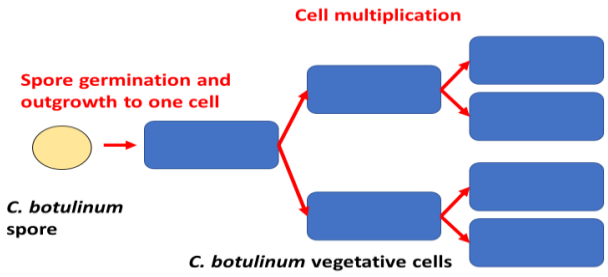
1. *Clostridium botulinum*: Key Facts

Clostridium botulinum (*C. botulinum*) is a bacterium that produces spores that survive for an extended period under harsh conditions, and may survive some heat processes applied by the food industry. The spores are ubiquitous in the natural environment, being present in soil, for example. Spores can germinate and outgrow to form new vegetative cells that produce botulinum neurotoxin and/or multiply (Figure 1). Foodborne botulism is an intoxication caused by consumption of botulinum neurotoxin that has been pre-formed by *C. botulinum* in the food. Thus, botulinum neurotoxin is the hazard. Botulinum neurotoxin is the most powerful toxin known, 30 ng of which can be fatal to a human. Although tests in laboratory medium indicate that neurotoxin is formed in late exponential/early stationary phase, there is evidence from challenge tests that botulinum neurotoxin may be formed in food without a measured increase in bacterial viable count (Bell & Kyriakides 2000[1], Brown & Gaze 1990[2], Brown *et al* 1991[3], Hyytiä *et al* 1999[4]). Possible explanations include that the measured viable count fails to recognise prior cell growth and subsequent death, and/or the cells have been metabolically active and formed neurotoxin but the viable count has not increased. Guidance documents produced in the USA/Canada for challenge test studies emphasise the importance of verifying that neurotoxin formation can be prevented (Doyle 1991[5], NACMCF 2010[6], Health Canada Food Directorate 2010[7], NACMCF 1992[8]), and one recently stated that "detection of toxins is measured rather than growth, as toxin can be produced without an increase in numbers" (NACMCF 2010[6]). Most published challenge tests measure neurotoxin formation rather than viable count. Although ACMSF/FSA documents refer to the prevention of growth and neurotoxin production (FSA, 2017[9]), it is generally accepted that it is sufficient to demonstrate the prevention of neurotoxin production, as stated above.

There are two main types of *C. botulinum*: psychrotrophic (also known as non-proteolytic), which is capable of neurotoxin formation during cold storage, and mesophilic (also known as proteolytic) which require higher temperatures to form neurotoxin. Strains of psychrotrophic (non-proteolytic) *C. botulinum* form botulinum neurotoxin of types B, E or F, while strains of mesophilic (proteolytic) *C. botulinum* form botulinum neurotoxin of types A, B, and/or F. Foodborne botulism is most commonly associated with botulinum neurotoxin of types A, B, or E. Reviews have been published on *C. botulinum* (Hauschild 1989[10], Bell & Kyriakides 2000[1], Peck 2009[11], Johnson 2013[12], Peck 2014[13], Peck *et al* 2008[14]).

The minimum temperature at which non-proteolytic *C. botulinum* forms neurotoxin is 3°C, while for proteolytic *C. botulinum* this is 10-12°C. Thus, in correctly stored chilled foods (maximum of 8°C), there is the potential for non-proteolytic *C. botulinum* but not proteolytic *C. botulinum* to form neurotoxin (Peck 2006[15], Peck *et al* 2008[14], Lindström *et al* 2006[16]). Note this guidance focuses on non-proteolytic *C. botulinum*, but if the storage temperature may reach or exceed 10°C, then the risk of production of botulinum neurotoxin from proteolytic *C. botulinum* must be considered and included in the risk assessment. Note: fermented products (e.g. cheese maturation, yogurt production, fermented meat, etc.) rely on holding at higher temperatures to result in sufficiently rapid pH and water activity (A_w) decline, to assure safety.

Figure 1: *Clostridium botulinum* cell cycle



2. Definitions (see also Figure 1)

Definitions are adapted from various sources including ISO[17], EURL[18] and legislation[19].

Bacterial spore: resistant form of bacteria which is dormant until it germinates

Challenge test: study of the fate of microorganisms (or their spores) artificially inoculated in a food, including the formation of neurotoxins

Exponential growth phase: growth phase in which the bacterial cells are multiplying exponentially. A period within the growth cycle where the viable count is said to double, i.e. 1, 2, 4, 8, 16 etc.

Germination: mechanism by which a bacterial spore starts becoming a vegetative cell

MLD_{50}: median lethal dose of botulinum neurotoxin required to kill half the members of a tested mice population after a specified test duration. Typically, 10 pg botulinum neurotoxin is equivalent to approximately 1 MLD_{50}. The human lethal dose is approximately 30 ng or 3,000 MLD_{50}

Organising laboratory: laboratory with responsibility for managing the challenge tests

Outgrowth: steps in the transition of a germinated spore to the first cellular division of a vegetative cell (includes cell wall synthesis, cellular elongation)

pH: a measure of the acidity or alkalinity of a food. The pH 7 is defined as neutral. Values of pH less than 7 are considered acidic, and those with greater than 7 are considered basic (alkaline)

Sampling: selection of one or more unit or portions of food such that the units or portions selected are representative of that food

Sampling point (date): selected date to collect data in the study design

Shelf life: either the period corresponding to the period preceding the 'use by' or the minimum durability date[19]. In practice this means the period during which the product maintains its microbiological safety and sensory qualities at a specific storage temperature. It is based on identified hazards for the product, heat or other preservation treatments, packaging method and other inhibitory or inhibiting factors that may be used

Stationary phase: phase in which the bacterial population is at its maximum level; the number of bacterial cells dying and being produced is at equilibrium

Test portion: measured (volume or mass) amount of the representative sample taken from the test unit for use in the analysis

Test unit: measured (volume or mass) amount of the food used for inoculation

Vegetative cell (of *C. botulinum*): bacterial form which is capable of forming botulinum neurotoxin and/or multiplying under favourable environmental conditions

Water activity (A_w): a measure of the water in a food that is available to microbes. It is not the same as the water content of the food, as some of the water in food can be bound to other molecules. Only unbound water can support the growth of microbes. The water activity scale

extends from 0 to 1.0 (pure water) but most foods have a water activity level in the range of 0.2 for very dry foods to 0.99 for moist fresh foods

3. Factors for Food Business Operators to consider regarding setting shelf life in relation to non-proteolytic *Clostridium botulinum*

Setting shelf life requires FBOs to carefully consider a wide variety of factors and hurdles − raw material quality, hygienic processing, temperature, water activity (A_w), acidity, packaging atmosphere − to determine how to control microbiological growth and/or the development of conditions that can lead to foodborne illness or spoilage. The appropriate choice and combination of these factors enables the optimum shelf life to be set for a food in combination with a food's usage conditions.

The following section provides information on the establishment of shelf life.

In some cases, where necessary, non-proteolytic *C. botulinum* challenge testing may be part of the approach to data generation. However, before challenge testing is commissioned by the customer they must discuss the proposed work in detail with the organising laboratory and understand the following:

- Why a challenge test is needed, and if other options (e.g. modelling, risk assessment, literature review) are more appropriate
- Whether the proposed approach is sufficiently scientifically rigorous to provide valid information
- How generated data will answer the question asked and that these data can be used in risk management decisions (e.g. converted to shelf life)
- How the project maximises value for money in terms of the number of samples to be tested and the wider applicability of results to products

Q1 How could the shelf life of my product be extended, e.g. beyond 10 days?

The Advisory Committee for the Microbiological Safety of Foods (ACMSF[20]) recommends in relation to vacuum and modified atmosphere packed chilled foods 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 neurotoxin 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 coolest point in the food, or
- A pH of 5.0 or less throughout the food and throughout all components of complex foods, or
- A minimum salt level of 3.5% in the aqueous phase throughout the food and throughout all components of complex foods, or
- An A_w of 0.97 or less throughout the food and throughout all components of complex foods, or
- A combination of heat and preservative factors which can be shown consistently to prevent growth and neurotoxin production by non-proteolytic *C. botulinum*

Although ACMSF/FSA documents refer to the prevention of growth and neurotoxin production (FSA 2017[9]), it is generally accepted that it is sufficient to demonstrate only the prevention of neurotoxin production, as foodborne botulism is an intoxication with

botulinum neurotoxin (the hazard). Guidance documents for challenge test studies produced in the USA/Canada emphasise the importance of demonstrating that neurotoxin formation can be prevented (Doyle 1991[5], NACMCF 2010[6], Health Canada Food Directorate 2010[7], NACMCF 1992[8]). If viable counts are carried out, then a significant increase in viable count should be taken to indicate a hazardous scenario even when neurotoxin formation is not detected. Importantly, however, a failure to measure an increase in viable count does not prove that neurotoxin has not been formed.

Guidance on considerations in relation to non-proteolytic and proteolytic *C. botulinum* and cheese has been published by the Specialist Cheesemakers Association: http://www.specialistcheesemakers.co.uk/media/download.aspx?MediaId=151

See Appendix 1 for a worked example.

Q2 How can I defend an existing product's shelf life if it is beyond 10 days, and what options do I have to extend shelf life beyond 10 days?

HACCP implementation must be in place from New Product Development onwards to ensure that relevant hazards and their necessary controls have been identified from the outset.

It is important that each food type is made consistently so that the inherent characteristic (e.g. thermal process received, pH, A_w) do not vary significantly. If consistency is not achievable, the worst possible case should be assessed as below.

When defending or extending a product's shelf life, information should be available covering the following elements:

a) **<u>Risk Assessment</u>** to consider parameters including:

Recipe control	pH, A_w, salt, preservatives, moisture content
Shelf life	desired duration and storage conditions (temperatures) and how these affect the types of pathogens that could grow or produce neurotoxin
Ingredients	sourcing location, dried or fresh, published risk data associated with ingredients, e.g. Barker *et al* 2016[21]
Hygiene measures	including area segregation, cleaning and disinfection regime, i.e. appropriate use of biocides
Process	type (e.g. thermal, high pressure) process duration, thermal profile, aseptic or clean fill, pre- or post-process packing

b) **<u>Modelling</u>** likelihood of microbial activity using Risk Assessment data

Free online software such as ComBase (www.combase.cc) can provide data on the likelihood of microbial growth, but does not give an indication of neurotoxin production. A prediction of growth can be taken as indicative of neurotoxin formation, however a prediction of no increase in viable count may not correlate to an absence of neurotoxin formation. Therefore, predictive models should be used with caution, and expert advice taken on interpreting the results. It is noted that there are several reports in the scientific literature that neurotoxin formation by *C. botulinum* can occur in the absence of an increase in viable count (e.g. Brown & Gaze 1990[2], Brown *et al* 1991[3], Hyytiä *et al* 1999[4], Bell & Kyriakides 2000[1]). Possible explanations include (i) the measured viable count fails to recognise prior cell growth and subsequent death, and/or (ii) the cells have been metabolically active and

formed neurotoxin although the viable count has not increased. A failure to measure (or predict) an increase in viable count does not therefore prove that neurotoxin has not been (or will not be) formed.

c) Shelf Life Studies

Shelf life studies involve storing non-inoculated foods under expected conditions and assessing their microbiological, organoleptic, chemical and physical quality. This should be done prior to production and by way of ongoing monitoring during production runs. This provides valuable information on product quality, but is not primarily concerned with microbiological safety, and certainly not with safety with respect to *C. botulinum*.

d) Risk Review

Review of data from Risk Assessment, modelling, and literature data from refereed publications to determine whether the pathogen could produce neurotoxin over the intended shelf life and storage conditions, including taking account of variation in pH and/or A_w over product life. If the risk review shows that controlling factors for non-proteolytic *C. botulinum* are not in place then a challenge test should be considered.

Controlling factors in addition to chilled storage (at ≥3°C to 8°C max):

- ≤10 days shelf life
- pH 5.0 max throughout the food, or
- A_w 0.97 max throughout the food, or
- Salt (NaCl) 3.5% minimum throughout the aqueous phase of the food, or
- A combination of heat and preservative factors which can be shown consistently to prevent growth and neurotoxin production by non-proteolytic *C. botulinum*, e.g. combination of pH/A_w/NaCl/preservatives, the SUSSLE Process giving the SUSSLE Shelf Life
- Thermal process 90°C for 10 mins equivalent (e.g. 42 days max is indicative in foods where it is suspected to contain lysozyme (Fernandez & Peck 1999[22])

Additional but sometimes less easily quantifiable factors that can play a role in control of non-proteolytic *C. botulinum*, e.g.:

- Competition from other microorganisms
- Nitrate and/or nitrite
- Other permitted preservatives

e) Challenge Testing

The Risk Review can inform where challenge testing can provide further data required to establish the food's safety under expected storage conditions.

Q3 What is a *Clostridium botulinum* challenge test?

A *C. botulinum* challenge test involves the addition of relevant organisms (or their spores – in this case non-proteolytic *C. botulinum*) to a food, to determine whether neurotoxin production occurs under expected storage conditions for that food's formulation, production and packaging method, assessing the safety and stability of the food. Guidance

produced in the USA/Canada emphasises the importance of demonstrating that neurotoxin formation is prevented, and this recommendation is based on neurotoxin being the hazard, and the observation that neurotoxin can be formed without an increase in viable count (Doyle 1991[5], NACMCF 2010[6], Health Canada Food Directorate 2010[7], NACMCF 1992[8], Brown & Gaze 1990[2], Brown *et al* 1991[3], Hyytiä *et al* 1999[4], Bell & Kyriakides 2000[1]). If viable counts are carried out, then a significant increase in viable count should be taken to indicate a hazardous scenario even when neurotoxin formation is not detected. Importantly, however, a failure to measure an increase in viable count does not prove that neurotoxin has not been formed.

Q4 In what circumstances would a challenge test be appropriate?

It should be noted that there is no legal requirement in the EU or UK to carry out challenge testing for any microorganism. The primary responsibility for food safety rests with the FBO. General implementation of procedures based on the HACCP principles, together with the application of good hygiene practice, should reinforce FBOs' responsibility.

A challenge test may be advisable:

- If risk assessment (see below) identifies areas where further data are needed, or
- If changes are made to a food's formulation, thermal process, packaging system used, distribution or storage regime, or shelf life, and risk assessment identifies reduction in the level of control, or
- If a primary controlling factor is reduced so that it may cease to be effective (e.g. salt reduced), or
- If food has been produced in a standard way over a period of time but is found not to comply with guidance

Q5 How might the results of a challenge test help set shelf life for a new/existing product?

In the case of *C. botulinum*, absence of neurotoxin production provides an indication of the absolute limit for safety under the inoculum level, storage conditions, product formulation, production process and packaging technology assessed. However and very importantly, given the toxicity of botulinum neurotoxin, allowable shelf life will always be significantly shorter than the time when neurotoxin production occurs.

Note that if viable counts are carried out, then a significant increase in viable count should be taken to indicate a hazardous scenario even when neurotoxin formation is not detected. Importantly, however, a failure to measure an increase in viable count does not prove that neurotoxin has not been formed.

Examples of this include setting shelf life:

- By taking time (days) off the last negative challenge test result
- As a proportion of the time before the last negative challenge test result
- A combination of the above

Section 4 provides organising laboratories with guidance on how to present information to FBOs so they can decide the maximum allowable shelf life.

4. Factors for an organising laboratory to consider when designing a challenge test of chilled foods with non-proteolytic *Clostridium botulinum*

The laboratory protocol needs to be agreed with the FBO in advance of any testing being carried out. This should include the provision of valid samples by the FBO, conditions for the transportation of foods to the organising laboratory, at what point during shelf life sampling will take place, in addition to satisfactorily addressing the points below. This document reflects guidance produced in the USA/Canada to consider when designing a challenge test with *C. botulinum* and the current ISO standard for challenge testing (Doyle 1991[5], NACMCF 2010[6], Health Canada Food Directorate 2010[7], NACMCF 1992[8,] ISO/DIS 20976-1:2016[17]).

4.1 Background

4.1.1 Purpose of challenge test and scope

- Ensure that the customer understands why a challenge test is needed, and that other options (e.g. modelling, risk assessment, review of literature data) are not more appropriate
- Ensure that the customer understands how generated data will answer the question asked and that these data can be used in risk management decisions (e.g. converted to shelf life)
- Ensure that customer understands how the project maximises value for money in terms of the number of samples to be tested and the wider applicability of results to products

4.1.2 General information

- A *C. botulinum* challenge test should only be carried out by an organising laboratory with specialised containment facilities, and with suitable safety precautions
- A production flow diagram setting out CCPs including any thermal processes should be provided by the customer to enable the challenge test protocol to be designed appropriately

4.2. *C. botulinum* strains to be used

4.2.1 Selection of strains of non-proteolytic *C. botulinum* to be used in spore cocktail

- Number of strains (e.g. 5-10)
- Origin of strains/traceability – where possible, source should be appropriate to tested product matrix. Use of reference strains (e.g. Eklund 17B and Beluga)
- Toxin types - strains forming type B or E neurotoxin should always be included, with strains forming type F neurotoxin sometimes also included. It should be checked at regular intervals (e.g. annually) that strains form good quantities of neurotoxin (e.g. >1000 MLD_{50}/ml)
- Suitability of strains – proven neurotoxin formation at specific temperature, pH, salt concentration, etc.

4.2.2 Production, enumeration and storage of spores

- Suitable spore production method (e.g. broth/plate, duration, incubation temperature)
- Washing of spores so that they are free of neurotoxin, vegetative cells and sporulation medium
- Any prior adaptation of strains to growth/germination at specific temperature/pH/salt/other
- Spore enumeration by viable count determination
- Storage of spore crops for valid periods of time, temperature, at optimal levels of spores
- Preparation of spore cocktail containing equal number of spores of each strain, and confirmation of viable count

4.3. Food product and its inoculation

4.3.1 Optimising experimental design

- Number of replicates – for example 3 or 5
- Negative control samples – un-inoculated, or inoculated with inoculum carrier
- Positive control broths (or other suitable test samples) – to confirm neurotoxin formation at test temperature(s) or other control conditions
- Storage temperature of samples to reflect real situation, e.g. one temperature or change in temperature to reflect manufacture, retail, consumer storage (could include abuse step to simulate purchase)
- Duration of test
- Frequency of test points (minimum of 4-6 time points, including T=0)

4.3.2 Selection and preparation of realistic worst-case food products

- Test portion, pack size of product to be tested (e.g. 10g – 100g)
- Consider variability of pH/A_w/other e.g. preservative levels across batches/samples, particularly for multicomponent products, over their shelf lives
- If consistency between batches can be proved then test a single batch. If inconsistent batches, or multi-phasic or composite product then identify worst case scenario or test multiple batches (consider effect of age of individual batches / stage in shelf life)

4.3.3 Selection of suitable spore concentration for inoculation of food product

- Representative spore concentration, but recovery must be repeatable/reproducible
- Suggest 10-100 spores/g product or 100-1000 spores/pack on the basis of known food spore loadings[21] and laboratory practicalities

4.3.4 Selection of method of inoculation

- Spore heat activation or spore heat damage to mimic food production process prior to inoculation, or heat in product. It is recommended that spores are used immediately after heating
- Volume of inoculum (recommended volume-to-sample ratio ≤1:100, e.g. 100µl in 10g test portion)
- It is common practice to inoculate the product at a number of sites
- Distribution of inoculum in the product (e.g. surface inoculation, specific components within product)
- Inoculation likely to be product/packaging dependent. Such as: (i) initial packaging with septum and needle, or (ii) open pack inoculate (homogenise if appropriate) and repack under same VP/MAP using same packaging materials (or alternative with same technical properties), or (iii) open, aliquot, inoculate, pack under same conditions (e.g. for large volumes/masses)
- Consideration of effect of inoculation on pH /A_w/VP/MAP conditions – confirm effect through analysis of pH/A_w/gas

4.3.5 Processing of food post inoculation

- Does a process(es) need to be applied post inoculation to represent manufacturing process?
- If a heat treatment is applied, then it must reflect the manufacturing process
- Should the product be VP/MAP to reflect the manufacturing process? (need to consider worst-case situation – low or no oxygen)

4.3.6 Incubation of the food product (time and temperature)

- Note optimisation of experimental design (section 4.4.1)
- Storage temperatures to reflect practice. Monitoring and reporting of temperature profiling. Temperature tolerances

- Packing of product within storage equipment

4.4 Sampling the inoculated food product

4.4.1 Optimising sampling design

- See sections 4.1.1 and 4.3.1 above
- Also consider frequency of sampling, relationship of sampling to desired shelf life (e.g. demonstrate safety by subtracting time (days) off last negative challenge test result, or as a proportion of the time before the last negative challenge test result, or as combination of the two). It is common practice to test more frequently towards the end of shelf life

4.4.2 Testing for botulinum neurotoxin

- Extract whole test portion for botulinum neurotoxin
- Test for botulinum neurotoxin using an ELISA, and compare with standard calibration curves to take account of food matrix effects. Using the mouse bioassay (according to the BAM manual (Solomon & Lilly[23])) the detection limit for botulinum neurotoxin is *ca.* 20-40pg neurotoxin/g food. This has previously been accepted as the detection limit in challenge tests. The detection limit of the ELISA for botulinum neurotoxin should be within an order of magnitude of the mouse bioassay, i.e. 0.2-0.4 ng neurotoxin/g food (Solomon & Lilly[23], Ferreira *et al* 2001[24]). This detection limit is *ca.* one hundredth of a human lethal dose (approximately 30ng) of botulinum neurotoxin
- Alternative methods to the ELISA that have a similar sensitivity (e.g. endo Mass Spectrometry) may also be suitable to detect botulinum neurotoxin

4.4.3 Additional tests to support or in addition to measurement of botulinum neurotoxin

- Anaerobic plate counts to be correlated with neurotoxin results – consider media, incubation time/temp., replicates, etc.
- Detection of genomic botulinum neurotoxin [§]genes using PCR (Solomon & Lilly 2001[23], ISO 2017[25])
- Intrinsic analysis – pH/A_w etc., particularly where these are controlling factors
- Analysis of other key controlling parameters e.g. salt, preservatives

[§] The spore inoculum used in the challenge test is unlikely to give a positive result, as (i) most methods will not extract genomic DNA from spores, (ii) the low inoculum level will mean that the number of copies of genomic DNA will be below the detection limit.

4.5 Presenting the experimental findings

4.5.1 Need for customer to understand findings

- The customer needs to understand how results can be converted to risk management decisions, and the limitations of findings (must be clear before project starts, see section 4.1). Two examples of how this might be applied are: set shelf life by either taking time (days) off the last negative challenge test result, and/or as a proportion of the time before the last negative challenge test result. In addition to considering the result of the challenge test, the FBO may wish to take account of other information (e.g. safety record of existing similar products) in setting shelf life.
- Expert advice must be sought from the organising laboratory, taking into account the agreed *a priori* criteria for the challenge test

4.5.2 Final report

- Should clearly detail the results and their limitations, and provide a basis for risk management decisions
- Suitable sections for the report include: aim and type of test, experimental protocol, sample analysis, results and conclusions

Appendix 1

Worked Example: Smoked Salmon Pâté Jars

The Smokin' Seafood Co. are developing a new smoked salmon pâté. The proposal is a "cold mixed" product, made up from the following ingredients:

Smoked salmon (Aq. salt 3.5% frozen), **poached salmon** (cooked to 70°C/2 minutes, stored at <5°C, 10 days shelf life), **soft cheese** (cooked and hot filled >70°C, pH 4.5, stored at <5°C, 60 days shelf life), **cream** (pasteurised 80°C/15s stored at <5°C 10 days shelf life), **mayonnaise** pH 4.2 ambient stable (containing oil, egg yolk, cornflour, spirit vinegar, sugar, salt).

The process is summarised as:

Goods in
Storage of ingredients (chilled, frozen, ambient)
Preparation of components (shredding of salmon)
Weighing into batches
Mixing and blending
Prepared batches stored chilled
Transfer to hopper
Inline metal detected
Fill jars
Vacuum Lidded
Batch and date inkjet coded
Boxed and palletised
Despatch chiller (3-5°C)

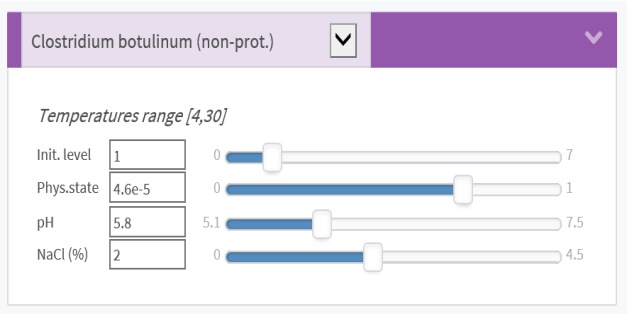
The shelf life starts when the product is ≥3.0°C. The desired shelf life is Production+12 days at 5-8°C (storage will be 2 days at up to 5°C on site and in retail depots, after which it could be purchased by a consumer and stored in a fridge at up to 8°C).

The product's parameters are given below against the ACMSF's requirements to prevent *C. botulinum* growth and neurotoxin formation in a chilled food:

ACMSF requirement	**Process/product parameters**	**Requirement met?**
A heat treatment of 90°C for 10 minutes or equivalent	Cold mixed	×
A pH of 5.0 or less throughout the food	5.6 - 5.8	×
A water activity (A_w) of 0.97 or less throughout the food	0.98	×
An aqueous salt concentration of 3.5% throughout the food	2.0 - 2.2	×
A combination of heat and preservative factors which can be shown consistently to prevent growth and toxin production by non-proteolytic *C. botulinum*	?	?

The effect of the combination of preservative factors is unknown, therefore the company seeks expert advice, and obtains a prediction of growth of *C. botulinum* in the product, using the product's

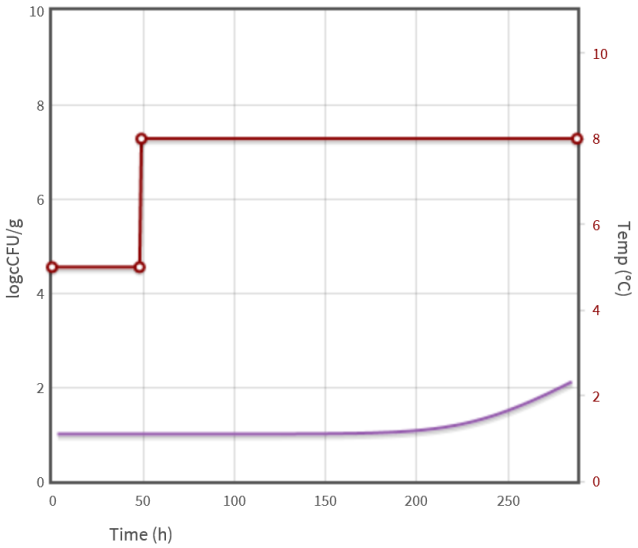
"worst case" parameters (lowest salt, highest pH, assume that a customer buys the product straight away and stores it at 8°C):



The prediction shows that growth starts to occur around 200 hours (8 days). The product does not prevent growth of *C. botulinum.* Therefore, unless a challenge test were performed to demonstrate that neurotoxin is not produced, could only be given a shelf life of up to 10 days in accordance with the FSA guidance.

As the product is cold mixed however, the shelf life of the ingredients should also be considered. All of the ingredients inhibit growth/toxin formation, apart from the poached salmon and the cream.

Therefore the shelf life of these ingredients before and after addition to the product must not exceed 10 days (e.g. poached salmon must be used at 2 days old if the final product has 8 days shelf life).



New Product Development options

The following options are trialled:

- Heating the product in its final packaging to a temperature of 90°C/10 minutes
- Increasing the salt to 3.5%
- Reducing the pH to 5.0 using lemon juice

The first two options result in poor organoleptic quality, but reducing the pH still gives a good quality product. To ensure that the pH never exceeds 5.0, a target of 4.8 is set.

Providing due diligence

pH becomes a CCP for control of *C. botulinum.* The company routinely monitors and records this on every batch on a number of pots to confirm correct ingredient control and thorough mixing. pH is also checked before launch on several batches, throughout shelf life and periodically thereafter at the end of shelf life, to ensure it does not change with time. pH is monitored using a calibrated probe. Because neurotoxin formation by *C. botulinum* is arrested in the product, theoretically the poached salmon and cream ingredients could be used up to 10 days after preparation, this being extended in the final product owing to appropriate acidification and reduction of pH to become a controlling factor for non-proteolytic *C. botulinum**.

*In this example *Listeria monocytogenes* (and potentially other pathogens, e.g. *Bacillus cereus)* is another potential hazard, and growth in the finished product would be predicted, therefore would need to be considered for ingredient and finished product shelf life.

Appendix 2

Drafting Group Membership

Mr Kim Matthews	Agriculture & Horticulture Development Board
Dr Andrew Millman	ASDA Stores Ltd
Mr Mark Young	Centre of Innovation Excellence in Livestock
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Dr Sam Kirk	Tesco Stores Ltd
Mrs Karen Sims	Waitrose Ltd
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Appendix 3

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