Advisory Committee on the Microbiological Safety of Food

Subgroup on non-proteolytic *Clostridium botulinum* and vacuum and modified atmosphere packaged foods

Final report

Advises the Food Standards Agency on the Microbiological Safety of Food

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Contents

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Executive summary

1. Foodborne botulism is caused by botulinum toxin produced by *C. botulinum*. Spores of *C. botulinum* are widely distributed in the environment and germinate, leading to growth and toxin formation, at low oxygen concentrations. Outbreaks of foodborne botulism have been associated with foods sealed in airtight containers including vacuum-packaged (VP) and modified-atmosphere-packaged (MAP) foods.

2. The Food Standards Agency has published guidance for food business operators and enforcement officers regarding 'The safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to non-proteolytic *Clostridium botulinum*'. This advises that, in the absence of other controlling factors, the shelf life be set to a maximum of ten days. These guidelines were first published in 2008 following consultation with the ACMSF and were subsequently updated in 2017, during which fresh meat was specifically mentioned for the first time.

3. The explicit inclusion of fresh meat, which always fell within the scope of the guidelines, has led to challenge by industry, as other countries do not provide similar guidelines in relation to fresh meat. The British Meat Processors Association and Meat Livestock Australia have recently published a study concerning VP and MAP fresh meat. It was agreed in June 2019 that it was appropriate for the ACMSF to establish a short-life subgroup to review the evidence on key aspects relating to the risk of non-proteolytic *C. botulinum* and VP/MAP foods.

4. The subgroup has reviewed three areas underpinning the current FSA guidance more broadly; thermal inactivation parameters, challenge testing and spore loading, as well as the industry funded report concerning fresh meat. The review of challenge testing and spore loading did not generate any major recommendations for the guidance outside of current best practice.

5. Drawing on a review of thermal inactivation parameters, the subgroup found evidence to recommend a change in the *z*-value within the range of 6.7-7.7C° for calculation of equivalent thermal processes below 90°C. If adopted, this would increase processing time at temperatures below 90°C.

6. The subgroup review of the industry funded report found evidence that could support an increase of the shelf life of fresh beef, lamb and pork from ten to thirteen days. This is based on the safety record of current industrial practice and supported by new challenge test data.

7. The subgroup has provided a series of recommendations concerning the guidance document, as well as observations on other areas which fell outside the scope of this review. A key observation is that the 1992 ACMSF 'Report on Vacuum Packaging and Associated Processes' would benefit from a full review.

Introduction

Non-proteolytic *C. botulinum*

8. Non-proteolytic *Clostridium botulinum* (*C. botulinum*, also known as psychrotrophic *C. botulinum*) is a spore-forming anaerobic bacterium capable of producing a neurotoxin, botulinum toxin, that is the most potent biological toxin known, with an estimated median lethal dose of 1 ng per kg bodyweight. Foodborne botulism is an intoxication caused by consumption of botulinum toxin formed by *C. botulinum* in food. Foodborne botulism is a potentially fatal form of food poisoning that leads to paralysis and is fatal in approximately 10% of cases.

9. Spores of *C. botulinum* are widely distributed in the environment and may be present in a variety of foods. Spores germinate, leading to growth and toxin formation, at low oxygen concentrations and in foods with a low redox potential. Outbreaks of foodborne botulism have been associated with foods sealed in airtight containers including vacuum-packaged (VP) and modified-atmosphere-packaged (MAP) foods. It is important to note that the presence of air, or a similar oxygen-containing atmosphere, cannot be relied upon to prevent growth and toxin formation by non-proteolytic *C. botulinum*. Such foods can contain areas of low oxygen and low redox potential that will allow *C. botulinum* to grow and form toxin.

Current guidance

ACMSF involvement

10. In 1992 the Advisory Committee on the Microbiological Safety of Food (ACMSF) issued a comprehensive 'Report on Vacuum Packaging and Associated Processes' (ACMSF, 1992). Evidence and recommendations from this report were used by the Food Standards Agency (FSA), with advice from other parties, in drafting of guidelines for food business operators and enforcement officers regarding 'The safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to non-proteolytic *Clostridium botulinum*'. These guidelines were first published in 2008 following consultation with the ACMSF and were subsequently updated in 2017 to improve clarity (Food Standards Agency, 2017). The ACMSF did not specifically review the changes made in the 2017 update, although the document was put out for public consultation.

Current guidelines

11. The current guidelines in this area advise that, unless suitable grounds for extension are proven, the shelf-life of VP and MAP chilled foods, including fresh meat, held at temperatures from 3 to 8°C is a maximum of ten days. The suitable grounds for a longer shelf life as detailed in the current guidelines are as follows:

    - a heat treatment of 90°C for ten 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*

12. Informed by the 1992 ACMSF report (ACMSF, 1992), these guidelines were established in 2008 following consultation involving the ACMSF. In 2017 the guidelines were revised to improve clarity (Food Standards Agency, 2017), during which fresh meat was specifically mentioned for the first time. The explicit inclusion of fresh meat, which always fell within the scope of the guidelines, has led to challenge by industry. Other countries do not provide similar guidelines in relation to fresh meat. The Food Safety Authority of Ireland specifically states that the ten-day rule does not apply to "*chilled VP/MAP raw meats sold as whole joints or cuts*" (Food Safety Authority of Ireland, 2019). There have also been scientific advances in several areas, including *z*-values, challenge testing and spore loading, which the subgroup identified as worthy of revisiting.

Terms of reference

13. It was agreed in June 2019 that it was appropriate for the ACMSF to establish a short-life subgroup to review the evidence on key aspects relating to the risk of non-proteolytic *C. botulinum* and VP/MAP foods.

14. The following terms of reference were agreed by the subgroup in September 2019:

- Review the FSA guidelines for the shelf-life of vacuum and modified atmosphere packaged foods and the risk posed by non-proteolytic *C. botulinum*, and other pathogens where appropriate, from these foods. This group will consider the 1992 ACMSF *Report on Vacuum Packaging and Associated Processes*, but it is outside the scope of this group to review that document.
- Specifically review the industry funded risk assessment of botulism from chilled, VP/MAP (Vacuum Packed/Modified Atmosphere Packed) fresh meat held at 3°C to 8°C (Peck, 2019).
- Where appropriate consider other risk-related evidence relevant to this topic made available to the FSA and the ACMSF during the lifetime of the group.

Review of the FSA guidelines

Thermal inactivation parameters – z and D-values

15. Microbial thermal inactivation calculations involve two values, the D-value and the z-value. The D-value is a measure of the heat resistance of a microorganism. It is the time in minutes at a given temperature required to destroy one-log (90%) of the target microorganism. The *z*-value is defined as the temperature change required to change the D-value by a factor of ten. The choice of a scientifically valid *z*-value and a reference D-value is essential for safe processing of chilled food stored at reduced oxygen levels, such as VP and MAP. The FSA recommends a thermal

process to provide at least a six-log reduction in the spores of non-proteolytic *C. botulinum* in the absence of other controlling factors.

16. In the current FSA Guidelines (Food Standards Agency, 2017) the following statement is made:

17. "*If heat treatment is to be used as the single controlling factor, the minimum heat treatment that should be used to manufacture a chilled VP/MAP product is 90°C for 10 minutes or equivalent achieved at the slowest heating point in the product.*"

18. The equivalence table in the FSA guidance is calculated using a *z*-value of 10.0 Celsius degrees (C°) to calculate equivalent thermal processes to 90°C for ten minutes for cooking temperatures between 90°C and 100°C based on Chilled Food Association (CFA) data (Chilled Food Association, 2006). A *z*-value of 9.0C° is used to calculate equivalent thermal processes to 90°C for ten minutes for cooking temperatures between 80°C and 90°C based on ACMSF data (ACMSF, 1992).

19. A literature search by the FSA made available to the ACMSF (ACMSF, 2018) and updated for the subgroup (VPMAP/12) highlighted a single key publication which was an extensive review of the first order inactivation kinetics of non-proteolytic *C. botulinum* based on data from 46 papers which met the study criteria (Wachnicka et al., 2016). These studies were conducted in foods, laboratory buffer solution or laboratory growth media over a heating range of 50°C to 95°C, using various recovery media. They found or derived 753 D-values (253 non-proteolytic Type B *C.* botulinum and 375 non-proteolytic Type E, 65 non-proteolytic Type F and 60 non-proteolytic mixed Types). A total of 436 *z*-values were also collected from these studies. The presence of lysozyme in the recovery media and the resulting biphasic inactivation curve was accounted for by taking or deriving *z*-values for the heat sensitive and heat resistant fractions separately.

***z*-values**

20. The published *z*-values were modelled by Wachnicka *et al.* using a beta probability density function to represent the uncertainty and were reported as a range around mean values. They reported a mean *z*-value of 6.7C° (range: 4.4C° to 10C°) for spores recovered in the absence of lysozyme.

21. The authors concluded that "*A suitable z value of 6.7°C is indicated from the analysis of the literature data (for recovery in the absence of lysozyme) and is consistent with the z value of 7°C advocated by the Chilled Food Association, compared to the z value of 9.2°C advocated by the United Kingdom Food Standards Agency* (The *z*-value used in both the ACMSF report (ACMSF, 1992) and FSA guidance (Food Standards Agency, 2017) is in fact 9.0C°.) A *z*-value of 7.0C° is also advocated by the European Chilled Food Federation (ECFF).

22. In further work, Wachnicka *et al.* also derived an alternative *z*-value based on a statistical analysis of the published D-value data for temperatures of 83°C and below and concluded that *"analysis of the reported D values … indicates a z value of 7.7°C*".

23. The subgroup considered this work and found that there was insufficient evidence to change the current recommended *z*-value of 10.0C° used for calculation of equivalent thermal processes above 90°C. However, there was sufficient information for the subgroup to consider *z*-values within the range of 6.7-7.7C° for calculation of equivalent thermal processes below 90°C. The equivalent time temperature combinations arising from a change in these values is included in Table 1.

Table 1: Equivalent heating processes resulting from z-values used by the Food Standards Agency (2017) and Chilled Food Association (2006), and proposed by Wachnicka et al (2016).

	Equivalent Thermal Inactivation Processes (minutes, 1 d.p.)			
Temp. (ºC)	**FSA 2017** z=9.0C^{o}	**CFA 2006** z=7.0C^{o}	**Wachnicka A** z=7.7C^{o}	**Wachnicka B** z=6.7C^{o}
90	10.0	10.0	10.0	10.0
89	13.0	13.9	13.5	14.1
88	17.0	19.3	18.2	19.9
87	22.0	26.8	24.5	28.0
86	28.0	37.3	33.1	39.5
85	36.0	51.8	44.6	55.8
84	46.0	72.0	60.2	78.6
83	60.0	100.0	81.1	110.9
82	77.0	139.0	109.4	156.3
81	100.0	193.1	147.5	220.4
80	129.0	268.3	198.9	310.8
Wachnicka A: FSA reference process time with Wachnicka *z*-value derived from analysis of published D-values (at 83°C and below) Wachnicka B: FSA reference process time with Wachnicka *z*-value derived from the average of published *z*-values				

D-values

24. Published log D-values were also modelled by Wachnicka *et al.* using a normal distribution at each heating temperature. At 90°C there were only eight published D-values and the mean log measured in minutes was -0.24 with a standard deviation (σ_{logD}) of 0.42 for spores recovered in the absence of lysozyme.

25. Based on further modelling, the authors concluded that "*On the basis of the 99% upper confidence limit (UCL) of predicted D values (in the absence of lysozyme and for heating temperatures below 83°C), the time required to reduce the spore concentration by a factor of 10^6 at 90°C is ~5 min*" (the actual value is 4.9 minutes) so that the reference value included in the FSA guidelines is conservative in relation to safety. Further, they suggested that independent analysis may be needed for heating temperatures above 83°C.

26. However, in considering this proposed D-value, the subgroup was mindful of differences in the heat resistance of spores in the different matrices used in the different studies reviewed by Wachnicka *et al*. (2016). D-values derived from inactivation studies in food tend to be higher than those derived in laboratory medium and buffer. In certain types of food, the D-values may be higher than the value derived in the review and are being diluted out by the larger body of data that

is derived from laboratory media and buffer. The subgroup agreed that further work is needed before deviating from the long-established reference inactivation process for non-proteolytic *C. botulinum* of 90°C for 10 minutes.

The effect of lysozyme on thermal inactivation of non-proteolytic *C. botulinum*

27. Lysozyme is an enzyme that is found naturally in some foods and, under certain conditions, has been shown to aid the germination of a susceptible fraction of heated spores. Wachnicka et al (2016) noted that the presence of lysozyme in the recovery media resulted in biphasic inactivation curves and this was accounted for by taking or deriving *z*-values for the heat-sensitive and heat-resistant spore fractions separately. The published *z*-values were modelled by Wachnicka *et al*, using a beta probability density function to represent the uncertainty and they reported a mean *z*-value of 9.3C° (range: 5.4C° to 14.3C°) for the heat-resistant fraction of spores recovered in the presence of lysozyme. For the heat-sensitive fraction of spores recovered in the presence of lysozyme, the mean log D-value was -0.03 (σ_{logD} = 0.21) and for the heat-resistant fraction of spores recovered in the presence of lysozyme, the log D-value was 1.29 (σ_{logD} = 0.20). Wachnicka *et al.* concluded that separate advice on heat inactivation of non-proteolytic *C. botulinum* spores should be given for foods containing lysozyme.

28. Lysozyme naturally present in or added to foods may survive mild heat processes, such that a heat treatment of 90°C for ten minutes (or equivalent) fails to deliver the intended six-log reduction. For example, Peterson *et al.* (1997) reported that heat treatments of 88.9°C for 65 minutes, 92.2°C for 45 minutes, or 94.4°C for 25 minutes were required to deliver a six-log reduction for pasteurised crabmeat, presumably because of naturally-present lysozyme. When hen egg white lysozyme was added to meat slurry prior to heating, heat treatments of 80°C for 230 minutes, 85°C for 184 minutes, or 90°C for 34 minutes were required to deliver a six-log reduction (Fernández and Peck, 1999). Furthermore, when a heat treatment of 90°C for ten minutes (or equivalent down to 80°C; data not available above 90°C) were applied to tubes containing 10^6 spores, growth was observed at 8°C in 48-54 days (Fernández and Peck, 1999).

29. In view of this evidence, the subgroup proposed that the maximum shelf-life of foods given a heat process of 90°C for ten minutes (or equivalent) should be limited to 42 days, unless it can be shown that lysozyme is absent from the food. The subgroup also agreed that expert advice should be sought if a shelf-life in excess of 42 days is desired.

Challenge testing

30. The present document (Food Standards Agency, 2017) provides information on the use of predictive growth models and challenge testing in shelf-life determination but does not make reference to alternative approaches that can be used to assess *C. botulinum* risk. The subgroup proposed that the document should be revised to recognise the importance of other established approaches such as risk assessment and exposure assessment, and that these approaches require expert advice.

31. Predictive microbiology models are important tools for food safety management as they provide a scientific basis to underpin key aspects of HACCP-based food safety management procedures. Predictive models available include those that describe growth and growth limits of non-proteolytic *C. botulinum*. It is important to recognise that models can only provide accurate information when interpreted by microbiologists with appropriate skills and experience, particularly as the models relate to growth and not toxin formation. Where a business does not have such skill and expertise it should consult an expert in food microbiology. The models are of particular benefit in providing a guide for the need for challenge testing or to enable the effective targeting of a challenge test study. Where results from predictive models and challenge testing are in conflict, the results of challenge testing should always take precedence.

32. The ACMSF 1992 VP/MAP report (ACMSF, 1992) was produced at a time when botulinum toxin testing was predominantly based on the mouse bioassay, a test that requires specialist animal handling facilities and is complex to perform. The mouse bioassay is viewed as the "gold standard" method, but in the UK is now only used for clinical investigations. The complexity of the bioassay, along with a reduction in the use of animal testing, led to the development of alternative methods to detect toxin and challenge tests based on observations of growth. Over recent years methods for botulinum toxin testing based on immunoassays (and other suitable methods) have become available. Such tests do not require the use of animal testing and makes testing for toxin more widely available. However, the specificity and sensitivity of alternative methods should be similar to that of the mouse bioassay.

33. The ACMSF 1992 VP/MAP report frequently refers to "*the prevention of growth and toxin formation*" but provides no explicit guidance with respect to challenge testing, whilst newer references advocate detection of toxin in challenge test experiments (Chilled Food Association, 2018; Health Canada, 2010; National Advisory Committee on Microbiological Criteria for Foods, 2010). Furthermore, the National Advisory Committee on Microbiological Criteria for Foods (2010) stated that "*detection of toxins is measured rather than growth, as neurotoxin can be produced without an increase in numbers*".

34. Foodborne botulism is an intoxication caused by consumption of pre-formed botulinum neurotoxin, and as noted previously recent guidance documents for challenge test studies emphasise the importance of verifying that neurotoxin formation can be prevented (Chilled Food Association, 2018; Doyle, 1991; Health Canada, 2010; National Advisory Committee on Microbiological Criteria for Foods, 2010, 1992). The clearer guidance on toxin detection has been stimulated by a number of publications that have reported that botulinum neurotoxin may be formed in some circumstances, in an absence of a measured increase in growth (Bell and Kyriakides, 2000; Brown et al., 1991; Brown and Gaze, 1990; Carlin and Peck, 1996; Hyytiä et al., 1999; Keto-Timonen et al., 2012; National Advisory Committee on Microbiological Criteria for Foods, 2010). A majority of published challenge tests have measured formation of botulinum neurotoxin rather than increase in viable count (Peck et al., 2008).

35. Various guidance documents have been produced on the conducting of challenge tests and setting of shelf-life. In June 2018, the UK food industry, in collaboration with partners, produced a guidance document on setting the shelf-life of chilled foods (Chilled Food Association, 2018). This document has been endorsed by the British Retail Consortium (BRC), the European Chilled Food Federation (ECFF), Meat and Livestock Australia (MLA) and others.

36. In the FSA current guidance (Food Standards Agency, 2017) there is a lack of clarity about whether there is the need in a challenge test to demonstrate that there is no production of botulinum neurotoxin as well as no increase in *C. botulinum* viable count (and in several places it is stated that only growth must be prevented). Given the above information, it is recommended that establishing the safety of a product through a challenge test must rely on demonstrating the absence of formation of botulinum neurotoxin.

37. It is also clear that in some circumstances detection of growth of *C. botulinum* may precede that of toxin formation (Carlin and Peck, 1996; Hyytiä et al., 1999; Keto-Timonen et al., 2012) and growth of the organism does indicate a potentially hazardous situation. An increase in viable counts over the course of the challenge test would indicate that *C. botulinum* can grow within the product and that there is a potential for toxin to be formed. An increase in viable count should be taken to indicate a potentially hazardous scenario even when toxin formation is not detected or measured. Concerns were raised by the sub-group with respect to the statistical power of the ISO 20976-1 standard on challenge testing to detect population growth of *C. botulinum* as an increase of 0.5 log in a test with three replicates. The level of change required to indicate growth may need to be reviewed, and this should be considered in future experimental work. Importantly, a failure to measure an increase in viable count does not prove that toxin has not been formed.

38. It is therefore recommended that detection of toxin is a minimum requirement for challenge testing, and that measuring viable counts is of merit in ensuring safety.

Spore loading

39. The spores of *C. botulinum* are considered to be ubiquitous within the environment and their presence in food materials cannot be discounted. However, isolation and detection of spores is technically challenging so estimates of spore numbers are always uncertain. In 2016 spore loads of non-proteolytic *C. botulinum* in food materials were reviewed and quantified in order to support improved understanding of risks associated with foodborne botulism (Barker et al., 2016).

40. The quantification included a literature review that captured 100 primary sources and 1090 estimates of spore loads in foods, an extensive programme of more than 450 laboratory tests to detect spores in food samples and an integrated scheme to establish the limit of detection for spores of non-proteolytic *C. botulinum* in food materials. The quantification gave a probabilistic estimate for the concentrations of spores, for non-proteolytic *C. botulinum*, in batches of food materials. The materials examined were explicitly associated with the manufacturing of minimally processed food and were categorized as Meat, Fish, Shellfish, Plant based foods, Cereals, Mushrooms and Fungi, Dairy liquids, Dairy non liquids and Herbs and Spices (with

an assumption of homogeneous classes). The review did not include any information for some food materials such as eggs and honey.

41. Subsequent (posterior) beliefs concerning concentrations of *C. botulinum* spores in food materials indicated that typical loads are smaller than many previously reported in the scientific literature. This shift reflects new evidence including significant numbers of negative results following microbiological tests on food samples and detailed evidence concerning the limit of detection. Current beliefs cannot rule out undetected spore loads with concentrations ~10 spores kg^{-1} but they provide increased confidence concerning the small probability for very heavily contaminated batches of materials.

42. Quantification did not identify significantly different spore loads in different food materials although some evidence pointed to smaller loads in meat and larger loads in herbs and spices (Barker et al., 2016). The limit of detection for spores of non-proteolytic *C. botulinum* in meat was particularly small (estimated as a single spore in a 200g sample). Herbs and Spices (dried) presented experimental issues due to product density, so probabilities for larger spore loads cannot be ignored.

43. Although the size of spore loads has direct relevance to the hazards associated with foodborne botulism; the actual hazard involves preformed toxin in food, their quantification is not always apparent in corresponding consideration of risks. Load sizes are not explicitly identified in the current FSA guidance on the safety of vacuum-packaged foods. The severity of botulism drives extreme vigilance so that safety considerations are usually based on complete inactivation of all possible spore populations in foods. The recently improved understanding of spore loads in food materials adds some confidence concerning the small probability of high spore concentrations and, in principle, this information could contribute to assessment of risk. However, incorporating improved quantification of spore loads into decisions about food safety is a complex step, which requires a structured approach, not currently included in the development of guidelines. In this respect an important component of complexity is the uncertain relationship between delay time (the period prior to germination and growth) and the size of a small population of, potentially damaged (adaptive), spores of non-proteolytic *C. botulinum*.

Review of the BMPA/MLA funded risk assessment

Scope

44. The subgroup agreed to "*specifically review the industry funded risk assessment of botulism from chilled, VP/MAP (Vacuum Packed/Modified Atmosphere Packed) fresh meat held at 3°C to 8°C*" (Peck, 2019). This risk assessment was presented to the subgroup by the British Meat Processors Association (BMPA) and by the author of the report Prof. Mike Peck, a co-opted member of the subgroup. The report was funded by BMPA, Meat & Livestock Australia (MLA) and donor companies in the food manufacturing and retail industries and is currently undergoing independent peer review. The scope of the report focussed on fresh red meat (beef, lamb and pork) using the following definition; "*meat that has not*

undergone any preserving process other than chilling, freezing or quick-freezing, including meat that is VP or MAP wrapped".

Background to the risk from non-proteolytic *C. botulinum* in raw meat

45. In the 1992 ACMSF report on vacuum packaging and associated processes, the ACMSF categorised chilled foods according to their "*composition, processing, and the form of packaging and the usual controlling factors"* and then prioritised them into those "*considered to present a high, medium, or low risk from growth and toxin production by psychrotrophic strains of C. botulinum"* as a result of the presence of these factors. Product categories regarded as low priority for attention were those where the organism was either unlikely to occur or where factors controlling growth or preventing toxin formation may be present singly or in combination. Foods were also considered to be low priority if they are susceptible to overt spoilage prior to growth and toxin formation by *C. botulinum*. The report defined "*raw animal products e.g. fish, poultry, shellfish and meat"* to be a low priority for attention although the report did highlight that the prioritisation regarding specific chilled food categories was "*intended as a working reference and it is not intended to be used independently of the report"*.

46. Since the publication of 1992 ACMSF report (ACMSF, 1992) the industry has generally focussed controls on foods in the high and medium risk categories and therefore less focus has been placed on those in low risk groups including raw animal products such as meat. The recent publication of the FSA guidance on the safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to non-proteolytic *Clostridium botulinum* (Food Standards Agency, 2017) did not differentiate between the risk presented by different chilled food groups and clearly identified raw meat as being at risk from growth of the organism and within scope of the ten-day guidance in the absence of controlling factors other than temperature. Accordingly, the shelf-life of VP/MAP foods (including fresh meat) held at 3°C to 8°C should be a maximum of ten days, unless suitable grounds for a longer shelf-life can be identified.

The 2019 BMPA / MLA study

47. Fresh chilled meat lacks any single factor controlling the growth of *C. botulinum* as defined by the FSA (Food Standards Agency, 2017). The research funded in the BMPA / MLA report (Peck, 2019) utilised a combination of a risk assessment and challenge test to quantify the risk presented by certain fresh, chilled meats and to determine whether it was possible to establish a safe shelf life in excess of the ten days recommended in the FSA guidance. A summary of the report and key findings is detailed below.

Existing shelf lives

48. An extensive review was conducted of the temperature and time regimes operated by the food industry for the storage of VP/MAP, fresh, chilled meat examining published guidance and industry submissions. Shelf lives of retail packed meat varied from 7 to 27 days at 3-8°C although the typical shelf lives for different red meat species were 8-13 days for beef, 8-11 days for pork and 8-11 days for lamb. It was noted that the total shelf life of many meats can be much longer than those

detailed above as it is common practice to 'deep chill' primal cuts or retail packs at temperatures below those that would support the growth of non-proteolytic *C. botulinum* i.e. <3°C. The typical shelf lives reported here are consistent with those found in a study conducted for Food Standards Scotland (Survey of Shelf Life Applied to Vacuum or Modified Air Packaged Fresh Meat at Retail and Approved Establishments in Scotland [Feb-Apr 2016]), that found typical 'non-compliant to the FSA guidance' shelf lives for retail meat to be, on average, 13 days for beef, 14 days for pork and 12 days for lamb. The study also reported maximum shelf lives of 17 days, 24 days and 13 days, respectively. It is clear that the majority of fresh, chilled meat sold at retail is given a shelf life beyond the ten days recommended by the FSA / ACMSF.

Cases of botulism from fresh chilled vacuum packed or modified atmosphere packed meat

49. The risk assessment reviewed the evidence of foodborne botulism to determine any attribution to fresh, chilled meat. The review examined outbreaks of botulism building on a 2006 (Peck et al., 2006) and 2008 (Peck et al., 2008) review of all previously published outbreaks which concluded that '*none of the outbreaks were due to correctly stored chilled foods*' and '*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*'. The latest review to August 2018 identified 26 outbreaks of botulism since 1985 implicating commercial foods intended to be stored under chilled conditions. There was no evidence found of outbreaks having been caused by VP/ MAP fresh, chilled meats within the scope of the study: beef, pork and lamb. Indeed, as reported from the previous outbreak reviews, none of the outbreaks associated with any commercial chilled food were caused by correctly stored products.

Challenge test studies

50. A literature review was conducted of challenge test studies on chilled food and food materials building on a previous comprehensive review conducted in 2011 (Stringer et al., 2011) and also including previously confidential industry data. A total of 514 studies were identified where toxin assays were used and of these 100 were positive for toxin within ten days at 8°C. However, there have been only eight studies on fresh, chilled meat and the experimental approaches used varied markedly, including inoculum size, sample size, methodology for assessing risk (growth or toxin formation), analytical methods and sensitivity of assay. The studies demonstrated variation in time to toxin formation within and between meat species and it was not possible to draw general conclusions from the studies regarding growth and toxin production of *C. botulinum* in fresh, chilled meats.

51. Consequently, a new series of challenge tests was undertaken to inform the risk assessment. The study was conducted against a newly published approach for challenge testing (Chilled Food Association, 2018) including the use of highly sensitive toxin assays (with a detection limit of 40pg toxin per g meat). Products tested included beef, lamb and pork stored at temperatures representative of those being applied in industry, including stages at 'deep chill' i.e. <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). The six products tested were selected to be representative of the UK market and included meats with both short and long maturation periods. Meats were tested in triplicate. Toxin was not detected in beef stored for up to 50 days nor lamb stored up to 35 days. One of the two fresh chilled pork products supported toxin formation at 35 days but not after 25 days. Although this challenge test has provided additional evidence regarding the production of toxin by non-proteolytic *C. botulinum* in a variety of red meats, indicating the potential to safely extend shelf lives beyond ten days, it is important to note that this only reflects the conditions in the six types of fresh meat used in this study. Further work would be beneficial to ensure this was fully representative of conditions present within and between the different meat types studied.

Exposure assessment (quantified risk)

52. In the absence of reported outbreaks of botulism associated with properly controlled fresh, chilled meats, it is possible to estimate the theoretical level of protection provided by 'normal' industry practice. 'Normal' practice would be defined as that meeting the requirements of EU Regulations for the production, storage and sale of these products. The 'protection factor' was calculated in this review by estimating the number of portions that have been sold (x) in a defined period without causing botulism and expressing the level of protection as "*1 in >10^x packs associated with botulism"*. The number of units of fresh beef, pork and lamb sold was estimated using a variety of national and international data sets (BMPA, MLA, Agriculture and Horticulture Development Board, Organisation for Economic Co-operation and Development, Food and Agriculture Organization) spanning several decades. Based on a portion size of 250g it was estimated that in the UK between 1999 and 2017 (excluding 2006) 3.1 x 10^{10}, 2.2 x 10^{10} and 8.6 x 10^9 units of beef, pork and lamb were sold giving a total of 6.2x10^{10} 250g portions. The protection factor provided by the current industry practice for fresh, chilled meat in the UK was therefore estimated at 1 in >$10^{10.8}$ packs per case of botulism. This is comparable to the protection afforded by many other processes applied to render foods 'safe' regarding a variety of bacterial pathogens. For *Listeria monocytogenes*, a six-log reduction achieved by the recommended 70°C for two minutes would provide a theoretical protection factor of 1x10^6 units, assuming 1 organism per unit. For proteolytic *C. botulinum*, 121°C for three minutes, or $F_0$3, would provide a protection factor of 1x10^8 - 1x10^9 units. It should be noted that that although 10^{12} is a recognized figure in relation to foodborne *C. botulinum* kill ($F_0$3), analysis of this in several studies has moved majority opinion to conclude that a 10^{-8}-10^{-9} probability of growth approximates to the twelve-log inactivation of proteolytic *C. botulinum* in phosphate buffer as described in the original study by Esty and Meyer (1922) and is an acceptable food safety objective.

Report summary

53. The report used a risk assessment approach to establish the level of protection afforded by current and historical industry practice with respect to non-proteolytic *C. botulinum* in fresh, chilled meat. The level of protection was estimated as >10.8 safety units or <1 reported case in over $10^{10.8}$ 250g units sold. A detailed review of

botulism outbreaks failed to identify any caused by commercially produced, fresh, chilled meat stored under correct temperature conditions. A new challenge test study on beef, pork and lamb demonstrated no (<40pg per g) toxin formation by a cocktail of type B and type E strains of *C. botulinum* when stored under typical conditions up to 25 days in pork, 35 days in lamb and 50 days in beef.

Subgroup conclusions on the BMPA report

54. The report provides new evidence regarding the safety of fresh, chilled meat with respect to non-proteolytic *C. botulinum*. It is clear that the current and historical shelf lives and storage regimes employed by the industry for the meats defined in the BMPA study, when processed and stored in accordance with EU Regulations, afford a high level of protection, even though typical shelf lives exceed the current recommended maximum of ten days. Challenge test studies demonstrate that toxin formation can take considerable time to occur in fresh, chilled meat and beyond 25 days in pork, beef and lamb, although historical challenge tests do provide differing outcomes. Whilst it does seem possible to achieve safe shelf lives in excess of ten days for chilled, fresh meats, it remains unclear as to what the controlling factors are that prevent growth and toxin formation. As such, it is not possible to provide a measurement and therefore critical limit that could be applied to assess whether the risk from fresh, chilled meat is negligible. This may pose challenges if technology used to process fresh, chilled meat leads to changes in the 'unknown' controlling factors rendering the food more vulnerable to growth and toxin production.

55. Under the current processing and storage regime, the evidence suggests that shelf lives beyond ten days do provide a high level of protection for these fresh, chilled meats (beef, lamb and pork). The subgroup therefore agrees that the maximum shelf life of these three fresh meats that have no other controlling factors in place could be extended to thirteen days, in line with the typical shelf life historically applied to the products.

56. Challenge test data does show that there is potential for the shelf life to be extended further but this would need additional evidence to encompass the potential variation between and within the meat species studied by the BMPA. It is also important to reiterate that the proposed thirteen-day shelf life does not extend to any beef, lamb or pork that is subject to further processing such as mincing, cooking or mixing with any other ingredients such as herbs, spices or curing salts.

Conclusions

57. The subgroup has reviewed three areas underpinning the current FSA guidance; thermal inactivation parameters, challenge testing and spore loading, as well as an industry funded report concerning fresh meat.

58. Drawing on a review of thermal inactivation parameters by Wachnicka *et al.*, the subgroup found evidence to recommend a change in the *z*-values within the range of 6.7-7.7C° for calculation of equivalent thermal processes below 90°C. If adopted, this would increase processing time at temperatures below 90°C.

59. Concerning challenge testing, the subgroup agreed that absence of toxin is a minimum requirement for safety and that measuring growth does provide useful additional evidence, but expert advice should be sought as growth studies need careful interpretation. It is recognised that this advice does not extend to predictive modelling, which only considers growth, therefore the subgroup advises that modelling be conducted under expert advice.

60. New evidence shows that, in principle, spore loading could contribute to risk assessment. However, it was agreed that this is a complex step, which requires a structured approach, and is not currently included in the guidelines.

61. The subgroup has reviewed the report funded by the BMPA and MLA concerning three fresh meats; beef, lamb and pork. Whilst the subgroup did not feel enough evidence was available to consider shelf lives around those demonstrated in the challenge tests, it was agreed that an increase of the shelf life of these fresh meat products from ten to thirteen days could be recommended, based on the safety record of current industrial practice.

Recommendations

Recommendations for the FSA guidance document

Ten-day rule in relation to fresh meat

62. The subgroup agreed that there is evidence from the BMPA study and the survey from Food Standards Scotland to support a change in the guidelines on the shelf life of lamb, beef and pork from ten days to thirteen. This change would apply only to lamb, beef and pork without added ingredients or further processing beyond cutting, packing, chilling, freezing and quick-freezing.

z-values

63. The subgroup agreed to present evidence from Wachnicka et al. in Table 1. The subgroup recommends that there be no change to the z-value above 90°C or to the reference D value used to define the reference process that corresponds with heating at 90°C for ten minutes. The subgroup agreed to present the options for the z-value below 90°C and the subsequent effect on the time temperature combinations.

Challenge testing

64. The subgroup recommends that detection of toxin is a minimum requirement for challenge testing, and that measuring viable counts is of merit in ensuring safety with appropriate expert advice. The subgroup recognises that current predictive models do not model toxin production and therefore recommends that all predictive modelling should be conducted following expert advice. The subgroup agreed that the mouse bioassay remains the ‘gold standard’ for BoNT detection and other detection methods should demonstrate at least equivalent specificity and sensitivity.

Upper shelf-life limit for foods with controlling factors in place

65. The FSA guidelines do not currently provide any guidance on a maximum shelf life for foods that satisfy the grounds for a longer shelf life. In view of the evidence

regarding lysozyme, the subgroup recommends that the maximum shelf-life of foods given a heat process of 90°C for ten minutes (or equivalent) should be limited to 42 days, unless it can be shown that lysozyme is absent from the food. The subgroup also recommend that expert advice should be sought if a shelf-life in excess of 42 days is desired.

Controlling factors

66. The five bullet points in the current FSA guidance detailing the suitable grounds for a longer shelf life were discussed. The subgroup recommends that the final bullet point (below) be revised.

67. "*a combination of heat and preservative factors which can be shown consistently to prevent growth and toxin production by non-proteolytic C. botulinum*"

68. The subgroup recommends that the wording be amended to appreciate that heat is not a necessary controlling factor in all cases. The subgroup therefore recommends that "*heat and preservative factors"* be amended to "controlling factors".

Other aspects that were considered

69. The following areas were discussed during the lifetime of this group, but it was agreed that either there was insufficient evidence to inform any recommendations, or that these areas were outside of the current scope of the guidance.

Nitrites

70. It was agreed that, whilst nitrites can be used to control *C. botulinum*, there was insufficient evidence available to allow any specific conclusions to be drawn on the use of nitrites as a controlling factor for *C. botulinum*.

Hyper-oxygenated foods

71. During the lifetime of the subgroup, it was queried whether hyper-oxygenated foods (packed in higher than atmospheric concentrations of oxygen) fell within the scope of the guidance as modified atmosphere foods, and whether the presence of oxygen at a level above atmospheric conditions was a control factor. The subgroup agreed that there is insufficient evidence that hyper-oxygenation can be used as a controlling factor due to the microenvironments that can form in foods.

Other bacteria possessing botulinum neurotoxin genes

72. The genes for botulinum neurotoxin have been found in bacteria other than *C. botulinum*, including other clostridia such as *C. butyricum* and *C. baratii* (Peck, 2009), as well as *Enterococcus* (Brunt et al., 2018). The subgroup agreed that consideration of other carriers of the botulinum neurotoxin genes were outside the scope of this report but should be considered in future.

Impact of the resident microflora on *C. botulinum*

73. Industry has funded some work into the impact of the resident microflora on the growth of *C. botulinum*. Issues raised during the lifetime of the subgroup include the limits of detection and the use of pH as a proxy for microflora growth. The subgroup would recommend further investigation be carried out into this area to determine whether microflora can be used a control factor.

1992 report

74. The subgroup has discussed elements of the 1992 ACMSF report throughout the lifetime of the subgroup, although it was outside of the scope of the group to review the document in full. The recommendation of the subgroup is that the ACMSF consider conducting a full review of the 1992 report.

Detection of *C. botulinum* growth

75. During the lifetime of the subgroup, concerns were raised with respect to the statistical power of the ISO standard for detecting population growth, ISO 20976-1:2019 (Challenge Testing): a change of 0.5 log in a test with three replicates. The subgroup recommends that this should be considered in future experimental work.

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Other unpublished documents considered by the subgroup

76. Food Standards Scotland. Survey of Shelf Life Applied to Vacuum or Modified Air Packaged Fresh Meat at Retail and Approved Establishments in Scotland (Feb-Apr 2016)

77. VPMAP/12. 2019. Vacuum packaged and modified atmosphere packaged meat guidance subgroup. FSA's guidance on vacuum and modified atmosphere packed chilled foods with respect to *Clostridium botulinum*: relevant scientific publications over the past year.

78. David Lindars from the BMPA attended a meeting of the subgroup to present the BMPA-funded 'Risk Assessment of Botulism from Chilled, VP/MAP (Vacuum Packed/Modified Atmosphere Packed) Fresh Meat held at 3°C to 8°C'. The BMPA also provided four unpublished reports, listed below, that were considered by the subgroup.

    - Investigation into whether or not C. botulinum growth is inhibited by the natural microflora of Lamb
    - Investigation into whether or not C. botulinum growth is inhibited by the natural microflora of Beef
    - Investigation into whether or not C. botulinum growth is inhibited by the natural microflora of vacuum packed Rind-off Pork
    - Investigation into whether or not C. botulinum growth is inhibited by the natural microflora of vacuum packed Rind-on Pork

79. Kaarin Goodburn of the CFA and Chilled Food Associates attended a meeting of the subgroup and presented a compilation of evidence, much of which is contained within references already provided, including Barker *et al.* (2016), Chilled Food Association (2006), Chilled Food Association (2018), Peck *et al.* (2006), Peck *et al.* (2008) and Wachnicka *et al.* (2016).