ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

DISCUSSION PAPER

FSA's guidance on vacuum and modified atmosphere packed chilled foods with respect to *Clostridium botulinum*: relevant scientific publications over the past 10 years

Introduction

1. At the committee's last meeting the secretariat was asked to look at what information had been published or made available over the past 10 years concerning which might be relevant to the issue of *Clostridium botulinum* and vacuum and modified atmosphere packaged foods. This paper is intended to provide an overview of what has been done in this area. It does not seek to review this information in detail or compare guidance documents produced by different organisations for different purposes. It is hoped that the information provided will assist the committee in deciding whether it is timely to revisit the scientific evidence base concerning *Clostridium botulinum* and vacuum and modified atmosphere packaged foods as this underpins the FSA's guidance on the issue.

Background

2. The ACMSF has had a longstanding interest in the issue of *Clostridium botulinum* and vacuum and modified atmosphere packaged foods dating from the time of the committee's first scientific report (ACMSF 1992). The committee has revisited this and related topics on several occasions since with extracts from the minutes relating the FSA's earlier guidelines (FSA 2008) provided at Annex 1.

3. In 2016 the committee were made aware that the FSA were reviewing the FSA guidance on the safety and shelf-life of vacuum and modified atmosphere packed chilled foods with respect to non-proteolytic *Clostridium botulinum* (ACMF 2016 papers ACM1230, 1230a*).* Committee members were invited to provide comments on the proposed guidance document via the FSA consultation prior to the final version being published on the FSA website. The main purpose of the review was to increase clarity of advice rather than re-assessing the advice itself. Following a consultation on the document the updated guidance was published by the FSA and FSS in 2017 (FSA 2017).

4. We were already aware of several documents published in this area comprising peer review publications, research reports, guidelines and other studies in the "grey literature". Although the available scientific evidence in this area is not large it was felt important to distinguish between these different types of

publications when try to summarise what work has been undertaken in this area over the past 10 years.

Peer reviewed literature

5. Literature searches were undertaken covering the 10-year period 01/01/2008-11/10/2018 using the database PubMed coupled with some additional checking using Google Scholar. The literature in this area is not large and not all of it concerns food although the search terms (MeSH – Medical Subject Heading) were kept broad to ensure good coverage of the topic and to avoid missing pertinent literature. Search terms are provided in Annex 2 together with the number of reference found in the 10-year period and without date restriction for comparison. The following are key areas of work relevant to *Clostridium botulinum* and food and brief summaries are provided under the headings of taxonomy and genomics, detection methods, growth and survival studies, heat and high pressure processing, studies on specific foods, other *Clostridium* species and risk assessment.

   Taxonomy and genomics

6. There has been significant progress in this area over the past 10 years. Most of this work has focused on improving our understanding about the genotypic characteristics of these organisms and how this relates to traditional classification based on phenotypic features. The wider application of whole genome sequencing of strains from different sources is enabling a better understanding of the genomic diversity of Groups I (proteolytic) and II (non-proteolytic) and their toxins (Peck and van Vliet 2016). Stringer *et al.* (2013) examined 43 strains of Group II *C. botulinum* which were isolated from a wide variety of environments over more than 75 years using whole genome sequencing coupled with physiological tests. The strains were found to cluster together indicating that the genetic background of Group II *C. botulinum* was stable. More recently, Weedmark *et al.* (2015) examined 163 Group II *C.botulinum* isolates from different geographic regions, outbreaks and toxins types. Using whole genome sequencing strains were found to fall into two clusters identified by multilocus sequence typing (MLST) and core single nucleotide polymorphism (SNP) analysis.

7. Analysis of the genomes has reinforced the historical Group I-VI designations and provided evidence that the genes encoding botulinum toxins can be located within the chromosome, phage or plasmids (Skarin and Segerman 2011; Carter *et al.* 2014; Hill *et al.* 2015). Carter *et al*. (2016) examined more than 150 genomes of *Clostridium botulinum* Group II toxin type E and in 6% of these the toxin-encoding genes were located on plasmids. Recently Nawrocki *et al.* (2018) showed that Group I botulinum neurotoxin-encoding plasmids could be transferred to other Group I strains and into non-toxigenic *C.sporogenes* and *C.butyricum* indicating potential from horizontal gene transfer across groups.

8. Other work has shown that botulinum toxin genes are not limited to *Clostridium botulinum*. Strains of *Clostridium butyricum* and *Clostridium baratii* can carry

botulinum toxin genes and on a few occasions have linked to outbreaks of botulism (Ghoddusi and Sherburn 2010; Tréhard *et al.,* 2016; Mazuet *et al.* 2017) and recent studies have identified homologs of botulinum toxins in the genomes of bacteria in the genera *Enterococcus, Weissella, Chryseobacterium* (Brunt *et al*. 2018; Mansfield and Doxey 2018). Clearly further work is required to understand the significance of the wider occurrence of these toxin genes and the role of horizontal gene transfer within and between groups of *Clostridium botulinum*.

Detection methods

9. Advances have been made in the detection, quantification and characterisation of *Clostridium botulinum* and botulinum toxins in clinical, food and environmental samples and botulinum neurotoxin detection methods for public health response and surveillance have recently been reviewed by Thirunavukkarasu *et al*. (2018). The approaches have mostly used conventional or quantitative PCR-based assays sometimes with pre-enrichment (De Medici *et al*. 2009; Hill *et al*. 2010; Satterfield *et al.* 2010; Takahashi *et al.* 2010; Fenicia *et al*. 2011; Malakar *et al.* 2013) but also the use of monoclonal based ELISAs (Scotcher *et al*. 2010; Singh *et al.* 2015; Simons *et al*. 2015). MALDI TOF (matrix-assisted laser desorption-ionization time-of-flight mass Spectrometry) has been used for identification and characterisation of *Clostridium botulinum* and related species and the associated toxins (Bano *et al.* 2017; Schaumann *et al*. 2018).

10. In the context of food testing, Peck *et al.* (2010) used selective enrichment culture combined with multiplex PCR to enumerate the spores of non-proteolytic *C.botulinum* in 637 food samples of 19 food materials used in producing minimally heated refrigerated pasta-based foods and in 7 complete foods. 5% (32) of samples (5 egg pastas and 27 scallops) contained spores of nonproteolytic *C. botulinum* type B or F with spore numbers in most cases being <100 spores/kg. A similar approach was used to look at the detection limit for spore of proteolytic and non-proteolytic *Clostridium botulinum* in dried mushroom samples sourced from China (Malakar *et al*., 2013).

Growth and survival studies

11. Several papers have explored factors influencing the germination of spores of non-proteolytic *Clostridium botulinum* particularly at the individual spore level including the influence of proximity to neighbouring spores (Stringer *et al.* 2009; Stringer *et al*. 2011; Webb *et al.* 2012). Spores of non-proteolytic *C.botulinum* exposed to different treatments (heated/unheated, incubation temperatures) showed variation in the stage of lag phase that was affected. A heat treatment at 80^{o}C for 20 s increased the median germination time of surviving spores by 16-fold as well as increasing the variability of spore germination times (Stringer *et al.* 2009).

12. Working with *Clostridium botulinum* is challenging from a safety and technical point of view and potential surrogates have been considered for proteolytic and non-proteolytic *C.botulinum* in challenge testing of foods (Bradshaw *et al*. 2010; Parker *et al*. 2015; Hu and Gurtler 2017; Huang 2018). Under many of the conditions studied, a two-strain cocktail of non-toxigenic *Clostridium* spp. was found to be suitable as a surrogate for non-proteolytic *Clostridium botulinum*, with the potential for use in chilled food challenge tests measuring growth. It was also suggested that non-toxigenic surrogates could also be used in studies looking at the effect of thermal processing (Parker *et al*. 2015). The use of surrogates in growth or survival studies has advantages over the use of toxigenic strains. However, care is needed in interpreting the findings of such work particularly given the heterogeneous nature of spore populations which can have impact on factors such as variability in the germination response of spores of non-proteolytic strains (Stringer *et al.* 2011). In addition, Bull *et al*. (2009) observed that using *C. sporogenes* PA3679 as a surrogate organism could overestimate inactivation of proteolytic *C. botulinum* by high pressure thermal processing.

Heat and high-pressure processing

13. Achieving a 6-log reduction on spore concentrations of non-proteolytic *Clostridium botulinum* is a key controlling step for many RTE chilled foods. Wachnicka *et al*. (2016) reviewed the available literature date on heat resistance properties for spores of non-proteolytic *Clostridium botulinum* strains. The 753 D values and 436 z values obtained revealed significant differences in the heat resistance properties of these spores. The findings support the application of a heat treatment of 90^{o}C for 10 minutes to achieve a 6-log reduction in spores providing that lysozyme is not present.

14. Recent interest in the application of high pressure processing has led to several studies looking at the effect of combined high pressure (HP) and thermal processing on inactivation of proteolytic (Bull *et al.* 2009; Skinner *et al.* 2014; Marshall *et al.* 2015) and types B, E and F of non-proteolytic (Skinner *et al.,* 2014, 2018) spores of *Clostridium botulinum.* Marshall *et al.* (2015) observed that the resistance of proteolytic *C.botulinum* spores to heat and HP processing is strain specific and it is important to assess the effect of sporulation temperature when using strains in these inactivation studies.

Studies on specific foods

15. Newell *et al*. (2012, 2015) conducted a series of challenge studies with a spore mix of six strains of non-proteolytic *Clostridium botulinum* in mussels packed under a high-oxygen (60 to 65% O_2), modified atmosphere packaging (MAP) conditions with samples stored at 4° and 12°C for 21 and 13 days, respectively. Toxin was not detected and there was no evidence that this was affected by packaging buffers or gas composition. Erickson *et al.* (2015) looked at toxin production in relation to spoilage of Atlantic Salmon pack in different gas atmospheres and with packaging films with varying oxygen permeability. In the US there have been concerns about botulinum toxin production preceding

spoilage when contaminated are held in impermeable packaging under temperature abuse conditions.

16. Keto-Timonen *et al*. (2012) examined the effect of sodium nitrite (0, 75, and 120 on the mg/kg) on growth and toxin production by non-proteolytic *Clostridium botulinum* type B using two types of sausage (Finnish wiener, Bologna) and cooked ham stored at 8°C for up to 5 weeks. The nitrite-free samples had the highest *C.botulinum* counts and those products with either 75 or 120 mg/kg nitrite showed no evidence of toxin during the study period. Hospital *et al*. (2016) also examined the impact of nitrate and nitrite on toxin production by non-proteolytic *C.botulinum* but in this case in two types of dry fermented sausages (salchichón, fuet). These were prepared with concentrations up to 150 mg/kg of sodium nitrate and 150 mg/kg of sodium nitrite. Toxin was not detected in any of the sausages even in the absence of nitrate/nitrite and was attributed to the contribution of other controlling factors (a_w, acidity, temperature). Merialdi *et al*. (2016) reported on work which included inoculating Parma ham with both proteolytic and non-proteolytic strains if *C.botulinum.*

Other *Clostridium* species

17. Ghoddusi *et al.* (2013) Examined the impact of intrinsic and extrinsic factors on the growth of neurotoxigenic strains of *Clostridium butyricum.* The minimum pH values permitting growth of toxigenic and nontoxigenic strains of *C. butyricum* was 4.1 and 4.2 when HCl was used or between 4.4 and 5.1 depending on the organic acid. The minimum water activity for growth of toxigenic strains of *C. butyricum* was 0.96. The minimum growth temperatures of the toxigenic strains of *C. butyricum* (ca 10-11°C) were somewhat higher than for non-toxigenic ones (8°C).

Risk assessment

18. Several studies have considered some of the practical challenges and knowledge gaps associated with building realistic model for growth and toxin production by *C.botulinum* in food and to inform risk assessments (Augustin 2011; Dahlsten *et al.* 2015; Ihekwaba *et al*. 2015a, b, 2016). Barker *et al.* (2016) conducted an extensive literature review to assess non-proteolytic *Clostridium botulinum* spore populations in groups of foods (meat, fish, shellfish, cereals, fresh plant material, dairy liquid, dairy nonliquid, mushroom and fungi, and dried herbs and spices) which are typically used as components of chilled minimally processed foods in the UK. A Bayesian framework was used to develop probability distributions for spore loads which can be used to inform risk assessments for non-proteolytic *Clostridium botulinum* in the production of these foods.

19. Malakar *et al*. (2011) developed a modular quantitative risk assessment model for non-proteolytic *Clostridium botulinum* in minimally processed chilled dairy-based foods and identified priority areas which would support further risk assessment work in this area. Glass *et al.* (2017) developed a predictive model for the inhibition of *Clostridium botulinum* in pasteurized cheese products with reduced sodium. The objective of this study was to create a predictive model

for proteolytic *C.botulinum* which would account for the interactive effect of moisture, pH, fat, sorbic acid, and potassium-based replacements. Dahlsten *et al*. (2015) highlighted a lack of data on genetic, stress-related mechanisms of non-proteolytic *C.botulinum* and a need to understand the effects of successive processing treatments on subsequent behaviour when subjected to further processing.

Guidelines and research reports

20. In 2017 guidance on considerations in relation to non-proteolytic and proteolytic *C. botulinum* and cheese was published by the Specialist Cheesemakers Association (SCA 2017) and Leatherhead Food Research published a white paper on controlling *Clostridium botulinum*: using challenge testing to create safe chilled foods (Wareing 2017). In 2018 a group of organisations (Quadram Institute Bioscience, Leatherhead Food Research, British Retail Consortium, Chilled Food Association, Meat Science Australia) produced guidance on the important factors to consider when determining the shelf-life of chilled foods with respect to non-proteolytic *C.botulinum* (Anon 2018). Other documents available include the Campden BRI second edition of their code of practice for the manufacture of vacuum and modified atmosphere packaged chilled foods published in 2009 (Campden BRI 2009). In the US Donnelly and Mitchell (2009) provided a standardized protocol for determining the shelf-life of refrigerated ready-to-eat foods for the Refrigerated Foods Association (RFA). In 2017 ISO published methodology for PCR detection of botulinum type A, B, E and F neurotoxin-producing clostridia (ISO 2017).

21. The Food Safety Authority of Ireland has also published guidance in this area (FSAI 2017) and the UK FSA/ACMSF approach is followed for products with a shelf-life of greater than ten days although in the case of chilled VP/MAP raw meats sold as whole joints or cuts, current industrial practice is considered acceptable (FSAI 2017).

22. The SUSSLE Process/Shelf Life is an outcome from the recently completed LINK project SUSSLE - Enhancing sustainability of chilled prepared foods (AFM266) (See https://gtr.ukri.org/projects?ref=BB%2FG010242%2F1). The project was funded by the Chilled Food Association, the Defra LINK Food Manufacturing programme, Unilever and BBSRC, with the Food Processing Faraday Partnership Ltd/Quotec managing the project to February 2012 (Defra 2012). The project was led by researchers at the former Institute of Food Research (now Quadram Institute BioScience) December 2008 - Mar 2012 (Defra 2012). The aim of this project was to identify a new thermal process (SUSSLE Process) suitable for the production of chilled food packs of up to 750 g with intermediate shelf lives (a specified value of >10 days and <42 days -the SUSSLE Shelf Life) with respect to non-proteolytic *C.botulinum*. A further project SUSSLE2 was undertaken funded by the BBSRC under the research initiative Innovate UK (TSB) from July 2013-December 2015 again led by researchers at the former IFR with work undertaken to demonstrate that the SUSSLE process and shelf-life can be safely applied to larger pack sizes (up to 1500g) and to materials that are work in progress.

https://gtr.ukri.org/projects?ref=BB%2FK021117%2F1. A paper by Barker *et al.* (2016) describes the work undertaken to develop probability distributions for spore loads which can be used to inform risk assessments for non-proteolytic *Clostridium botulinum* in the production of these foods. Further information on SUSSLE is available at https://www.chilledfood.org/2349-2/

23. A recent report reviewed the the implementation of Directive2006/52/EC concerning the use of nitrites by industry in different categories of meat products in the EU (Anon 2016). Findings from a review of the literature, a survey of manufacturers and an expert workshop suggested there was scope to review the current levels of nitrates authorised.

Recent studies concerning raw meat

24. Work has recently been undertaken by Campden BRI and QIB Extra (a subsidiary of Quadram Institute BioScience) for the meat industry to look at the potential for growth and toxin production by *Clostridium botulinum* on raw meats (beef, lamb and pork). The literature review found little evidence of published work in this area over the past 10 years.

Campden BRI

25. In 2018 a consortium of members from the British Meat Processors Association (BMPA) has funded work at Campden BRI to look at growth and toxin production of *Clostridium botulinum* on vacuum-packed red meat (beef, lamb and pork) under various chill storage temperatures. This work, which has now been completed, looked at changes in the bacterial flora on these meats characterised using 16S rRNA sequencing, the associated changes in the meat pH during storage and whether there was any growth or toxin production by non-proteolytic *Clostridium botulinum.*

QIB Extra

26. MLA (Meat and Livestock Australia) and a consortium of industry parties collated by the BMPA (British Meat Processors Association), including the IMTA (International Meat Trade Association), are presently funding a project at QIB Extra, the results of which should be available early in 2019. The aim of the project is to prepare a risk assessment setting out the level of protection, with respect to non-proteolytic *Clostridium botulinum* and foodborne botulism, for current industry practice regarding VP/MAP fresh meat for fresh chilled (raw) meat (beef, pork and lamb) distributed and/or sold to the final consumer (that do not contain known controlling factor(s)). This work includes and takes account of: (1) hazard characterisation; (2) specifies meat species/types included; (3) summarises industry practice; (4) exposure assessment (market sales data); (5) exposure assessment (spore loading); (6) reviews foodborne botulism incidents related to fresh meat; (7) summarises data on

growth/neurotoxin formation by *C. botulinum* in fresh meat, including a challenge test.

Action

27. Members are invited to:

a) comment on this summary of published information and current studies relevant to the issue of *Clostridium botulinum* and vacuum and modified atmosphere packaged foods and;

b) consider whether it would be timely for the committee to revisit the scientific evidence base in this area by establishing an ad hoc work group.

Secretariat
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Annex 1

Extracts from ACMSF minutes relating to the establishment of the FSA guidelines in 2008.

ACMSF meeting June 2006 - extract from the minutes

Vacuum-packaged and modified atmosphere packaged foods

6.1 At the Chair's invitation, Dr Kathryn Callaghan (FSA) introduced paper ACM/777*. She explained that in December 2004 the ACMSF requested that the FSA commissioned an independent review of the current scientific evidence concerning vacuum and modified atmosphere packaged foods and the risk of *Clostridium botulinum*. This followed concerns raised in response to the Agency's consultation on its guidance on the safety and shelf life of vacuum and modified atmosphere packed chilled foods regarding the proposed shelf life limitation of 5 days (based on 1995 ACMSF advice) for chilled products, and products stored above 5°C where failure to support growth of *C. botulinum* had not been established. This review, which had been carried out by the Institute of Food Research, had now been completed. A summary of the review's findings was annexed to the paper. She added that the full report of the review would be available in due course. Summing up, she explained that the views of the ACMSF were sought on whether to support the 10-day shelf life recommendation.

6.2 At the Chair's invitation, Professor Mike Peck (Institute of Food Research) presented an overview of the work carried out to review the current scientific evidence concerning vacuum and modified atmosphere packaged foods and the risk of *Clostridium botulinum*. He summarised information on sales of chilled food, current guidance and recommendations for commercial chilled foods, and recent incidence of foodborne botulism in different countries including the UK. He also outlined growth and toxin formation by non-proteolytic *C.botulinum* at or at less than 10°C. Lastly, he described the effects of other factors including unknown controlling factors, packing in air and re-packing of chilled food.

6.3 The Committee welcomed the review, and in particular, inclusion of epidemiology in the scope of the research. In discussing the proposed recommendation to increase shelf life from 5 to 10 days the Committee noted that:

- FSA needed to consider risk management issues such as costs/benefits and environmental impact of changes to the guidance;
- Levels of *C. botulinum* inoculum used per pack for challenge testing were higher than levels of toxin typically produced by *C. botulinum* in foods (and referred to previous ACMSF work on infant botulism);
- Epidemiology of *C. botulinum* over last 20 years showed that there were no reported cases of botulism linked to chilled foods. However Members noted that botulism was a difficult diagnosis to make and that under recognition of milder cases was possible.
- The 10-day shelf life recommendation for the UK was already quite restrictive and it was not applied in many other countries.

* Peck et al. (2006)

6.4 Summing up, the Chair confirmed that the Committee endorsed the recommendation to support a 10-day shelf life recommendation with the vacuum packaged Guidance document being revised from less than or equal to 5 days to less than or equal to 10 days at 8 degrees C. The Committee also identified a need for simple, summarised guidance to help enforcers and industry and agreed that a small group should be set up by the Food Standards Agency to deliver this. In addition, this guidance should be brought to the attention of other Member States via the European Commission.

The following summarises the conclusions of the small drafting group referred to above.

ACMSF meeting December 2007 - extract from the minutes

Guidance on vacuum-packaged foods (ACM/881)

8.1 At the Chair's invitation, Dr Callaghan introduced paper ACM/881. She provided a historical overview of the development of guidance on the safety and shelf-life of vacuum and modified atmosphere packed chilled foods, with respect to *Clostridium botulinum*. She reminded Members that in June 2006 ACMSF considered the findings of an independent review of *Clostridium botulinum*, which was previously requested by the Committee to support its deliberations on guidance for chilled vacuum packaged and modified atmosphere packed foods. At this meeting ACMSF recommended the 10 day shelf-life rule for vacuum-packaged and modified atmosphere packaged foods. The Committee also recommended that the FSA set up a small drafting group to revise the guidance to address issues arising from the public consultation on the Guidance on vacuum-packaged foods. Dr Callaghan outlined the scope of the drafting group and explained that the resulting guidance produced by the group was circulated to industry stakeholder for comment prior to being finalised.

8.1 The Committee discussed the scope of guidance. Members recognised that it was difficult to balance simple and complex issues in a single guidance document. They welcomed the fact that the guidance had been developed to assist EHOs carry out their enforcement duties. Members noted that the wording in the Guidance reflected that published in the 1992 ACMSF Report on vacuum packaging and associated processes and that the drafting group, where possible, had avoided overcomplicating messages. The group had also decided not to include specific examples in the text in order to provide some flexibility with regard to enforcement risk management decisions.

8.2 The Chair thanked Dr Callaghan for presenting the guidance and confirmed that ACMSF endorsed publication of the document in early 2008

Annex 2

Details of PubMed searches

Disease

("botulism"[MeSH Terms] OR "botulism"[All Fields]) AND ("2008/01/01"[PDAT] : "2018/10/11"[PDAT])
Result: 1155 references (10 years), 3953 (no date restriction) – peak year 2015 -136 references

Organism

("clostridium"[MeSH Terms] OR "clostridium"[All Fields]) AND botulinum[All Fields]
Result: 1331 references (10 years), 4825 (no date restriction) – peak year 2013 -151 references

Organism and vacuum or modified atmosphere

(("clostridium"[MeSH Terms] OR "clostridium"[All Fields]) AND ("vacuum"[MeSH Terms] OR "vacuum"[All Fields])) OR (modfied[All Fields] AND ("atmosphere"[MeSH Terms] OR "atmosphere"[All Fields])) AND ("2008/01/01"[PDAT] : "2018/10/11"[PDAT])
Result: 97 references (10 years), 206 references (no date restriction)

(("clostridium botulinum"[MeSH Terms] OR ("clostridium"[All Fields] AND "botulinum"[All Fields]) OR "clostridium botulinum"[All Fields]) AND ("vacuum"[MeSH Terms] OR "vacuum"[All Fields])) OR (modfied[All Fields] AND ("atmosphere"[MeSH Terms] OR "atmosphere"[All Fields]))
Result: 16 references (10 years), 61 references (no date restriction)

((("vacuum"[MeSH Terms] OR "vacuum"[All Fields]) OR (modified[All Fields] AND ("atmosphere"[MeSH Terms] OR "atmosphere"[All Fields]))) AND ("food"[MeSH Terms] OR "food"[All Fields])) AND ("botulism"[MeSH Terms] OR "botulism"[All Fields]) AND ("2008/01/01"[PDAT] : "2018/10/11"[PDAT])
Result: 11 references (10 years), 30 references (no date restriction)