

# ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

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

Final report

1. In June 2019 (ACM/MIN/94) the ACMSF agreed to establish a short-life subgroup to review the evidence on key aspects relating to the risk of non-proteolytic *Clostridium botulinum* and VP/MAP foods. This group, formed of six ACMSF members and one co-opted member, has since met four times and this paper forms their final report.
2. Based on evidence from the 1992 ACMSF 'Report on Vacuum Packaging and Associated Processes' and subsequent considerations by the committee, the FSA published guidelines in 2008 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*'. Subsequently updated in 2017, these guidelines currently advise that, in the absence of other controlling factors, the shelf life of these foods be set to a maximum of ten days.
3. The subgroup has reviewed the evidence underpinning the FSA guidance, focussing on thermal inactivation parameters, challenge testing and spore loading. The subgroup has also reviewed an industry funded study concerning fresh meat.

Members are invited to:

- Consider the evidence presented
- Discuss the recommendations from the subgroup
- Agree the recommendations

**Secretariat**

**January 2020**

## 1 Executive summary

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

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

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

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

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

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

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

## 38 Introduction

### 39 Non-proteolytic *C. botulinum*

40 Non-proteolytic *Clostridium botulinum* (*C. botulinum*, also known as psychrotrophic  
41 *C. botulinum*) is a spore-forming anaerobic bacterium capable of producing a  
42 neurotoxin, botulinum toxin, that is the most potent biological toxin known, with an

43 estimated median lethal dose of 1 ng per kg bodyweight. Foodborne botulism is an  
44 intoxication caused by consumption of botulinum toxin formed by *C. botulinum* in  
45 food. Foodborne botulism is a potentially fatal form of food poisoning that leads to  
46 paralysis and is fatal in approximately 10% of cases.

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

## 56 Current guidance

### 57 ACMSF involvement

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

### 69 Current guidelines

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

- 74 • a heat treatment of 90°C for ten minutes or equivalent lethality at the slowest  
75 heating point in the food
- 76 • a pH of 5.0 or less throughout the food and throughout all components of  
77 complex foods
- 78 • a minimum salt level of 3.5% in the aqueous phase throughout the food and  
79 throughout all components of complex foods
- 80 • a water activity ( $a_w$ ) of 0.97 or less throughout the food and throughout all  
81 components of complex foods
- 82 • a combination of heat and preservative factors which can be shown  
83 consistently to prevent growth and toxin production by non-proteolytic *C.*  
84 *botulinum*

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

#### 96 Terms of reference

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

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

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

## 112 Review of the FSA guidelines

### 113 Thermal inactivation parameters – z and D-values

114 Microbial thermal inactivation calculations involve two values, the D-value and the z-  
 115 value. The D-value is a measure of the heat resistance of a microorganism. It is the  
 116 time in minutes at a given temperature required to destroy one-log (90%) of the  
 117 target microorganism. The z-value is defined as the temperature change required to  
 118 change the D-value by a factor of ten. The choice of a scientifically valid z-value and  
 119 a reference D-value is essential for safe processing of chilled food stored at reduced  
 120 oxygen levels, such as VP and MAP. The FSA recommends a thermal process to  
 121 provide at least a six-log reduction in the spores of non-proteolytic *C. botulinum* in  
 122 the absence of other controlling factors.

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

125 “If heat treatment is to be used as the single controlling factor, the minimum heat  
126 treatment that should be used to manufacture a chilled VP/MAP product is 90°C for  
127 10 minutes or equivalent achieved at the slowest heating point in the product.”

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

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

146 z-values

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

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

158 In further work, Wachnicka et al. also derived an alternative z-value based on a  
159 statistical analysis of the published D-value data for temperatures of 83°C and below  
160 and concluded that “analysis of the reported D values ... indicates a z value of  
161 7.7°C”.

162 The subgroup considered this work and found that there was insufficient evidence to  
163 change the current recommended z-value of 10.0C° used for calculation of  
164 equivalent thermal processes above 90°C. However, there was sufficient information  
165 for the subgroup to consider z-values within the range of 6.7-7.7C° for calculation of

166 equivalent thermal processes below 90°C. The equivalent time temperature  
 167 combinations arising from a change in these values is included in Table 1.

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

Temp. (°C)	Equivalent Thermal Inactivation Processes (minutes, 1 d.p.)			
	FSA 2017 z=9.0C°	CFA 2006 z=7.0C°	Wachnicka A z=7.7C°	Wachnicka B z=6.7C°
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

170 D-values

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

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

182 However, in considering this proposed D-value, the subgroup was mindful of  
 183 differences in the heat resistance of spores in the different matrices used in the  
 184 different studies reviewed by Wachnicka et al. (2016). D-values derived from  
 185 inactivation studies in food tend to be higher than those derived in laboratory  
 186 medium and buffer. In certain types of food, the D-values may be higher than the  
 187 value derived in the review and are being diluted out by the larger body of data that  
 188 is derived from laboratory media and buffer. The subgroup agreed that further work  
 189 is needed before deviating from the long-established reference inactivation process  
 190 for non-proteolytic *C. botulinum* of 90°C for 10 minutes.

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

192 Lysozyme is an enzyme that is found naturally in some foods and, under certain  
193 conditions, has been shown to aid the germination of a susceptible fraction of heated  
194 spores. Wachnicka et al (2016) noted that the presence of lysozyme in the recovery  
195 media resulted in biphasic inactivation curves and this was accounted for by taking  
196 or deriving z-values for the heat-sensitive and heat-resistant spore fractions  
197 separately. The published z-values were modelled by Wachnicka et al, using a beta  
198 probability density function to represent the uncertainty and they reported a mean z-  
199 value of 9.3C° (range: 5.4C° to 14.3C°) for the heat-resistant fraction of spores  
200 recovered in the presence of lysozyme. For the heat-sensitive fraction of spores  
201 recovered in the presence of lysozyme, the mean log D-value was -0.03 ( $\sigma_{\log D} =$   
202 0.21) and for the heat-resistant fraction of spores recovered in the presence of  
203 lysozyme, the log D-value was 1.29 ( $\sigma_{\log D} = 0.20$ ). Wachnicka et al. concluded that  
204 separate advice on heat inactivation of non-proteolytic *C. botulinum* spores should  
205 be given for foods containing lysozyme.

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

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

223 Challenge testing

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

230 Predictive microbiology models are important tools for food safety management as  
231 they provide a scientific basis to underpin key aspects of HACCP-based food safety  
232 management procedures. Predictive models available include those that describe  
233 growth and growth limits of non-proteolytic *C. botulinum*. It is important to recognise

234 that models can only provide accurate information when interpreted by  
235 microbiologists with appropriate skills and experience, particularly as the models  
236 relate to growth and not toxin formation. Where a business does not have such skill  
237 and expertise it should consult an expert in food microbiology. The models are of  
238 particular benefit in providing a guide for the need for challenge testing or to enable  
239 the effective targeting of a challenge test study. Where results from predictive  
240 models and challenge testing are in conflict, the results of challenge testing should  
241 always take precedence.

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

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

261 Foodborne botulism is an intoxication caused by consumption of pre-formed  
262 botulinum neurotoxin, and as noted previously recent guidance documents for  
263 challenge test studies emphasise the importance of verifying that neurotoxin  
264 formation can be prevented (Chilled Food Association, 2018; Doyle, 1991; Health  
265 Canada, 2010; National Advisory Committee on Microbiological Criteria for Foods,  
266 2010, 1992). The clearer guidance on toxin detection has been stimulated by a  
267 number of publications that have reported that botulinum neurotoxin may be formed  
268 in some circumstances, in an absence of a measured increase in growth (Bell and  
269 Kyriakides, 2000; Brown et al., 1991; Brown and Gaze, 1990; Carlin and Peck, 1996;  
270 Hyytiä et al., 1999; Keto-Timonen et al., 2012; National Advisory Committee on  
271 Microbiological Criteria for Foods, 2010). A majority of published challenge tests  
272 have measured formation of botulinum neurotoxin rather than increase in viable  
273 count (Peck et al., 2008).

274 Various guidance documents have been produced on the conducting of challenge  
275 tests and setting of shelf-life. In June 2018, the UK food industry, in collaboration  
276 with partners, produced a guidance document on setting the shelf-life of chilled foods  
277 (Chilled Food Association, 2018). This document has been endorsed by the British

278 Retail Consortium (BRC), the European Chilled Food Federation (ECFF), Meat and  
279 Livestock Australia (MLA) and others.

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

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

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

### 302 Spore loading

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

309 The quantification included a literature review that captured 100 primary sources and  
310 1090 estimates of spore loads in foods, an extensive programme of more than 450  
311 laboratory tests to detect spores in food samples and an integrated scheme to  
312 establish the limit of detection for spores of non-proteolytic *C. botulinum* in food  
313 materials. The quantification gave a probabilistic estimate for the concentrations of  
314 spores, for non-proteolytic *C. botulinum*, in batches of food materials. The materials  
315 examined were explicitly associated with the manufacturing of minimally processed  
316 food and were categorized as Meat, Fish, Shellfish, Plant based foods, Cereals,  
317 Mushrooms and Fungi, Dairy liquids, Dairy non liquids and Herbs and Spices (with  
318 an assumption of homogeneous classes). The review did not include any information  
319 for some food materials such as eggs and honey.

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

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

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

## 349 Review of the BMPA/MLA funded risk assessment

### 350 Scope

351 The subgroup agreed to “specifically review the industry funded risk assessment of  
352 botulism from chilled, VP/MAP (Vacuum Packed/Modified Atmosphere Packed) fresh  
353 meat held at 3°C to 8°C” (Peck, 2019). This risk assessment was presented to the  
354 subgroup by the British Meat Processors Association (BMPA) and by the author of  
355 the report Prof. Mike Peck, a co-opted member of the subgroup. The report was  
356 funded by BMPA, Meat & Livestock Australia (MLA) and donor companies in the  
357 food manufacturing and retail industries and is currently undergoing independent  
358 peer review. The scope of the report focussed on fresh red meat (beef, lamb and  
359 pork) using the following definition; “meat that has not undergone any preserving  
360 process other than chilling, freezing or quick-freezing, including meat that is VP or  
361 MAP wrapped”.

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

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

#### 389 The 2019 BMPA / MLA study

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

#### 397 Existing shelf lives

398 An extensive review was conducted of the temperature and time regimes operated  
399 by the food industry for the storage of VP/MAP, fresh, chilled meat examining  
400 published guidance and industry submissions. Shelf lives of retail packed meat  
401 varied from 7 to 27 days at 3-8°C although the typical shelf lives for different red  
402 meat species were 8-13 days for beef, 8-11 days for pork and 8-11 days for lamb. It  
403 was noted that the total shelf life of many meats can be much longer than those  
404 detailed above as it is common practice to ‘deep chill’ primal cuts or retail packs at  
405 temperatures below those that would support the growth of non-proteolytic *C.*  
406 *botulinum* i.e. <3°C. The typical shelf lives reported here are consistent with those

407 found in a study conducted for Food Standards Scotland (Survey of Shelf Life  
408 Applied to Vacuum or Modified Air Packaged Fresh Meat at Retail and Approved  
409 Establishments in Scotland [Feb-Apr 2016]), that found typical 'non-compliant to the  
410 FSA guidance' shelf lives for retail meat to be, on average, 13 days for beef, 14 days  
411 for pork and 12 days for lamb. The study also reported maximum shelf lives of 17  
412 days, 24 days and 13 days, respectively. It is clear that the majority of fresh, chilled  
413 meat sold at retail is given a shelf life beyond the ten days recommended by the FSA  
414 / ACMSF.

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

417 The risk assessment reviewed the evidence of foodborne botulism to determine any  
418 attribution to fresh, chilled meat. The review examined outbreaks of botulism building  
419 on a 2006 (Peck et al., 2006) and 2008 (Peck et al., 2008) review of all previously  
420 published outbreaks which concluded that 'none of the outbreaks were due to  
421 correctly stored chilled foods' and 'illness occurred when foods were time and/or  
422 temperature abused or when pre-formed botulinum toxin was inadvertently added,  
423 via another food component, to a correctly chilled product'. The latest review to  
424 August 2018 identified 26 outbreaks of botulism since 1985 implicating commercial  
425 foods intended to be stored under chilled conditions. There was no evidence found  
426 of outbreaks having been caused by VP/ MAP fresh, chilled meats within the scope  
427 of the study: beef, pork and lamb. Indeed, as reported from the previous outbreak  
428 reviews, none of the outbreaks associated with any commercial chilled food were  
429 caused by correctly stored products.

430 Challenge test studies

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

442 Consequently, a new series of challenge tests was undertaken to inform the risk  
443 assessment. The study was conducted against a newly published approach for  
444 challenge testing (Chilled Food Association, 2018) including the use of highly  
445 sensitive toxin assays (with a detection limit of 40pg toxin per g meat). Products  
446 tested included beef, lamb and pork stored at temperatures representative of those  
447 being applied in industry, including stages at 'deep chill' i.e. <3°C for 1 day, then at  
448 5°C for 1 day, 22°C for 2 hours (to simulate potential abuse during consumer  
449 purchase and transportation), and then at 8°C for the remaining incubation period (to  
450 reflect domestic storage). The six products tested were selected to be representative  
451 of the UK market and included meats with both short and long maturation periods.

452 Meats were tested in triplicate. Toxin was not detected in beef stored for up to 50  
453 days nor lamb stored up to 35 days. One of the two fresh chilled pork products  
454 supported toxin formation at 35 days but not after 25 days. Although this challenge  
455 test has provided additional evidence regarding the production of toxin by non-  
456 proteolytic *C. botulinum* in a variety of red meats, indicating the potential to safely  
457 extend shelf lives beyond ten days, it is important to note that this only reflects the  
458 conditions in the six types of fresh meat used in this study. Further work would be  
459 beneficial to ensure this was fully representative of conditions present within and  
460 between the different meat types studied.

#### 461 Exposure assessment (quantified risk)

462 In the absence of reported outbreaks of botulism associated with properly controlled  
463 fresh, chilled meats, it is possible to estimate the theoretical level of protection  
464 provided by 'normal' industry practice. 'Normal' practice would be defined as that  
465 meeting the requirements of EU Regulations for the production, storage and sale of  
466 these products. The 'protection factor' was calculated in this review by estimating the  
467 number of portions that have been sold ( $x$ ) in a defined period without causing  
468 botulism and expressing the level of protection as "1 in  $>10^x$  packs associated with  
469 botulism". The number of units of fresh beef, pork and lamb sold was estimated  
470 using a variety of national and international data sets (BMPA, MLA, Agriculture and  
471 Horticulture Development Board, Organisation for Economic Co-operation and  
472 Development, Food and Agriculture Organization) spanning several decades. Based  
473 on a portion size of 250g it was estimated that in the UK between 1999 and 2017  
474 (excluding 2006)  $3.1 \times 10^{10}$ ,  $2.2 \times 10^{10}$  and  $8.6 \times 10^9$  units of beef, pork and lamb  
475 were sold giving a total of  $6.2 \times 10^{10}$  250g portions. The protection factor provided by  
476 the current industry practice for fresh, chilled meat in the UK was therefore estimated  
477 at 1 in  $>10^{10.8}$  packs per case of botulism. This is comparable to the protection  
478 afforded by many other processes applied to render foods 'safe' regarding a variety  
479 of bacterial pathogens. For *Listeria monocytogenes*, a six-log reduction achieved by  
480 the recommended 70°C for two minutes would provide a theoretical protection factor  
481 of  $1 \times 10^6$  units, assuming 1 organism per unit. For proteolytic *C. botulinum*, 121°C for  
482 three minutes, or  $F_03$ , would provide a protection factor of  $1 \times 10^8$  -  $1 \times 10^9$  units. It  
483 should be noted that although  $10^{12}$  is a recognized figure in relation to foodborne  
484 *C. botulinum* kill ( $F_03$ ), analysis of this in several studies has moved majority opinion  
485 to conclude that a  $10^{-8}$ - $10^{-9}$  probability of growth approximates to the twelve-log  
486 inactivation of proteolytic *C. botulinum* in phosphate buffer as described in the  
487 original study by Esty and Meyer (1922) and is an acceptable food safety objective.

#### 488 Report summary

489 The report used a risk assessment approach to establish the level of protection  
490 afforded by current and historical industry practice with respect to non-proteolytic *C.*  
491 *botulinum* in fresh, chilled meat. The level of protection was estimated as  $>10.8$   
492 safety units or  $<1$  reported case in over  $10^{10.8}$  250g units sold. A detailed review of  
493 botulism outbreaks failed to identify any caused by commercially produced, fresh,  
494 chilled meat stored under correct temperature conditions. A new challenge test study  
495 on beef, pork and lamb demonstrated no ( $<40$ pg per g) toxin formation by a cocktail

496 of type B and type E strains of *C. botulinum* when stored under typical conditions up  
497 to 25 days in pork, 35 days in lamb and 50 days in beef.

#### 498 Subgroup conclusions on the BMPA report

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

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

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

## 526 Conclusions

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

530 Drawing on a review of thermal inactivation parameters by Wachnicka et al., the  
531 subgroup found evidence to recommend a change in the z-values within the range of  
532 6.7-7.7°C for calculation of equivalent thermal processes below 90°C. If adopted, this  
533 would increase processing time at temperatures below 90°C.

534 Concerning challenge testing, the subgroup agreed that absence of toxin is a  
535 minimum requirement for safety and that measuring growth does provide useful  
536 additional evidence, but expert advice should be sought as growth studies need  
537 careful interpretation. It is recognised that this advice does not extend to predictive

538 modelling, which only considers growth, therefore the subgroup advises that  
539 modelling be conducted under expert advice.

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

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

## 549 Recommendations

### 550 Recommendations for the FSA guidance document

#### 551 Ten-day rule in relation to fresh meat

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

#### 557 z-values

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

#### 564 Challenge testing

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

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

573 The FSA guidelines do not currently provide any guidance on a maximum shelf life  
574 for foods that satisfy the grounds for a longer shelf life. In view of the evidence  
575 regarding lysozyme, the subgroup recommends that the maximum shelf-life of foods  
576 given a heat process of 90°C for ten minutes (or equivalent) should be limited to 42  
577 days, unless it can be shown that lysozyme is absent from the food. The subgroup  
578 also recommend that expert advice should be sought if a shelf-life in excess of 42  
579 days is desired.

## 580 Controlling factors

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

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

586 The subgroup recommends that the wording be amended to appreciate that heat is  
587 not a necessary controlling factor in all cases. The subgroup therefore recommends  
588 that “heat and preservative factors” be amended to “controlling factors”.

## 589 Other aspects that were considered

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

### 593 Nitrites

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

### 597 Hyper-oxygenated foods

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

### 604 Other bacteria possessing botulinum neurotoxin genes

605 The genes for botulinum neurotoxin have been found in bacteria other than *C.*  
606 *botulinum*, including other clostridia such as *C. butyricum* and *C. baratii* (Peck,  
607 2009), as well as *Enterococcus* (Brunt et al., 2018). The subgroup agreed that  
608 consideration of other carriers of the botulinum neurotoxin genes were outside the  
609 scope of this report but should be considered in future.

### 610 Impact of the resident microflora on *C. botulinum*

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

### 616 1992 report

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

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

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- 725 Wachnicka, E., Stringer, S.C., Barker, G.C., Peck, M.W., 2016. Systematic  
 726 Assessment of Nonproteolytic *Clostridium botulinum* Spores for Heat  
 727 Resistance. *Applied and Environmental Microbiology* 82, 6019–6029.  
 728 <https://doi.org/10.1128/AEM.01737-16>  
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- 730 Other unpublished documents considered by the subgroup
- 731 Food Standards Scotland. Survey of Shelf Life Applied to Vacuum or Modified Air  
 732 Packaged Fresh Meat at Retail and Approved Establishments in Scotland (Feb-Apr  
 733 2016)
- 734 VPMAP/12. 2019. Vacuum packaged and modified atmosphere packaged meat  
 735 guidance subgroup. FSA’s guidance on vacuum and modified atmosphere packed  
 736 chilled foods with respect to *Clostridium botulinum*: relevant scientific publications  
 737 over the past year.
- 738 David Lindars from the BMPA attended a meeting of the subgroup to present the  
 739 BMPA-funded ‘Risk Assessment of Botulism from Chilled, VP/MAP (Vacuum  
 740 Packed/Modified Atmosphere Packed) Fresh Meat held at 3°C to 8°C’. The BMPA  
 741 also provided four unpublished reports, listed below, that were considered by the  
 742 subgroup.
- 743 • Investigation into whether or not *C. botulinum* growth is inhibited by the  
 744 natural microflora of Lamb
  - 745 • Investigation into whether or not *C. botulinum* growth is inhibited by the  
 746 natural microflora of Beef
  - 747 • Investigation into whether or not *C. botulinum* growth is inhibited by the  
 748 natural microflora of vacuum packed Rind-off Pork
  - 749 • Investigation into whether or not *C. botulinum* growth is inhibited by the  
 750 natural microflora of vacuum packed Rind-on Pork
- 751 Kaarin Goodburn of the CFA and Chilled Food Associates attended a meeting of the  
 752 subgroup and presented a compilation of evidence, much of which is contained  
 753 within references already provided, including Barker et al. (2016), Chilled Food  
 754 Association (2006), Chilled Food Association (2018), Peck et al. (2006), Peck et al.  
 755 (2008) and Wachnicka et al. (2016).