Clostridium botulinum in vacuum packed (VP) and modified atmosphere packed (MAP) chilled foods

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PART ONE – EXECUTIVE SUMMARY

1. Summary

1. A substantial quantity of chilled foods has been sold in the UK and overseas in the last two decades, and when correctly stored has not been associated with foodborne botulism. Current practice would therefore appear to have a high degree of safety.

2. The majority of commercially produced pre-packaged chilled foods have a shelf life greater than 5 days, and some have a shelf life greater than 10 days without receiving any of the control measured specified by the ACMSF (1992). The ACMSF (1995) recommendation of 10 days at 5°C/5 days at 10°C is not adhered to any significant extent in the UK or elsewhere. The 10 day rule at 8°C specified in the 1996 industry code of practice is only adhered to by major producers in the UK and Benelux market. In some countries (e.g. France, Finland), chilled products have been safely produced, over several decades, with shelf lives greater than 10 days. Many of these products will not have received a 6 log non-proteolytic *C. botulinum* process or any of the other control measured specified by the ACMSF (1992) and their shelf lives take account of lower temperature storage than 8°C.

3. It is not easy to determine the maximum shelf-life of chilled foods at 8°C (where other controlling factors are not known) on only the data from 1307 independent challenge tests of toxin formation by inoculated non-proteolytic *C. botulinum*. It is clear that, given the correct circumstances, if present, non-proteolytic *C. botulinum* is able to form toxin in 10 days or less at 8°C. Also, predictive models indicate that toxin formation can occur in 10 days or less at 8°C (the model in ComBase Predictor estimates toxin formation in 6 days at 8°C). That toxin formation has not occurred in correctly stored short shelf-life chilled foods sold in the UK (and internationally) must be due to presence of one or more “unknown controlling factors”. The difficulty is that the magnitude, variability, and nature of these “unknown controlling factors” is not known, and it is suspected that the magnitude, variability, and nature are not the same for all chilled foods. The position is therefore that while short shelf-life foods have been produced safely in the UK (and internationally) for more than two decades, it is not known why they are safe with respect to foodborne botulism, or what the safety margins are.

4. Based on the extensive sales of chilled foods without any incidence of foodborne botulism (when correctly stored), current industrial practice (application of GMP, GHP and HACCP principles) would appear to provide a good level of protection. It would seem reasonable, therefore, that current industrial practice be allowed to continue. It is noted that in the UK the majority of commercially produced pre-packaged chilled foods have a shelf life greater than 5 days, and some have a shelf life greater than 10 days without receiving any of the control measured specified by the ACMSF (1992). Consideration should therefore be given to the FSA including “storage at ≤8°C and a shelf-life of ≤10 days” in their document, rather than “storage at ≤5°C and a shelf-life of ≤10 days or storage at 5°-8°C and a shelf-life of ≤5 days”. It is cautioned, however, that if present, non-proteolytic *C. botulinum* can form toxin in 10 days and less at 8°C, and there is insufficient clear information as to what the safety margins are in foods as sold, particularly when attempting to take into account the temperature performance of the complete chill chain throughout foods’ shelf lives. It is therefore strongly recommended that extreme caution be used when modifying current industrial practice (e.g. extending the shelf-life of chilled foods over that currently used), and in the development of new products. Since, although current industrial practice appears safe, it is possible that chilled foods could be produced for which a 10 day shelf life at 8°C would not be suitable. It would seem logical to apply this approach to all chilled food sold in the UK.

5. It was noted in several studies that toxin formation by non-proteolytic *C. botulinum* was as rapid (or in some circumstances more rapid) in foods packed in air as under VP or low-oxygen MAP. This is presumably because there is no oxygen in the food, i.e. the food is reduced. Packaging under air or a similar oxygen-containing atmosphere is therefore not a guarantee that toxin formation by non-proteolytic *C. botulinum* will be prevented.

6. It has been brought to our attention that some chilled VP/MAP foods such as meat may be given a “rolling 10 day shelf-life”. That is, the product is opened during the initial 10 day shelf-life, some is used, and then the remainder is repacked and given a further 10 day shelf-life. Thus, the shelf-life is extended beyond 10 days without the identification of other factors that control toxin formation by non-proteolytic *C. botulinum*. While we are not aware of this practice leading to outbreaks of botulism, this represents a significant divergence from the guidance and would appear to be a high risk practice. It is therefore proposed that for foods where no other controlling factor can be identified, the maximum shelf-life is 10 days, and that this commences once the product is first vacuum or modified atmosphere packed. The shelf-life must not be restarted if the product is subject to a further packing under vacuum or modified atmosphere, unless other controlling factors (as described by the ACMSF) are applied.

7. Improvements in temperature control throughout the chill chain could make a significant contribution to microbiological food safety with respect to foodborne pathogens that are able to grow at chilled temperatures, such as non-proteolytic *C. botulinum* and *Listeria monocytogenes*. It is noted that maintenance at a temperature of 5°C was recommended by Richmond in 1991. The chill chain of major UK multiples is targeted at 5°C or below throughout distribution and retail display. In practice, available surveys of all types of UK chilled food outlets (including major multiples, farmers markets, small stores and other outlets) indicated that the average temperature at retail was 4°C-6°C, with 6% of samples at >8°C. In the UK, domestic refrigerators are replaced on average every 8 years, thus the last domestic refrigerator survey that was carried out in 1990 is of date, and needs to be re-run in order to ensure the UK has up to date information on domestic refrigerator temperature performance. The 1990 UK survey found that there was variation in performance between domestic fridges and within each refrigerator over time. Also, different temperatures were recorded in different parts of single refrigerators. The overall mean temperature was 6.6°C, with approximately 9% of the time spent at >9°C. Temperature control in the chill chain appears to be similar in other developed countries.

2. Introduction

Foodborne botulism is a severe disease. It is an intoxication resulting from consumption of pre-formed botulinum neurotoxin in food, with as little as 30 ng of neurotoxin sufficient to cause illness and even death. The consumption of as little as 0.1g of food in which *Clostridium botulinum* has grown can result in botulism. Foodborne botulism is primarily associated with two physiologically and genetically distinct clostridia, proteolytic *C. botulinum* and non-proteolytic *C. botulinum*. Proteolytic *C. botulinum* is a mesophile, with a minimum growth temperature of 10°C-12°C, while non-proteolytic *C. botulinum* is a psychrotroph that grows and forms toxin at 3.0°C (Table 1).

Table 1 Characteristics of the two physiologically and genetically distinct clostridia most frequently associated with foodborne botulism

	Proteolytic *C. botulinum* (mesophile)	Non-proteolytic *C. botulinum* (psychrotroph)
neurotoxins formed	A, B, F	B, E, F
minimum growth pH	4.6	5.0
minimum growth temperature	10-12°C	3.0°C
maximum growth NaCl	10%	5%
spore heat resistance ($D_{100°C}$)	>15 min	<0.1 min

In view of the severity of botulism, regulators and industry work hard to ensure that the risk of it occurring is very low. This has led to the production of various guidelines, recommendations and codes of practice with respect to the control of *C. botulinum* in foods, including VP (vacuum packed) and MAP (modified atmosphere packed) chilled foods. There has been a substantial increase in sales of VP/MAP chilled foods over the last two decades. These foods address consumer demand in being of high quality and requiring little preparation time. The principal microbiological safety hazard is foodborne botulism, as presented by non-proteolytic *C. botulinum*.

In 1992, the ACMSF published a report that made recommendations on the safe production of VP/MAP chilled foods with respect to *C. botulinum* and the associated foodborne botulism hazard. These were:

(1) storage at <3.0°C*
(2) storage at ≤10°C and a shelf-life of ≤10 days (the “10 day rule”)
(3) A heat treatment of 90°C for 10 min or equivalent lethality (e.g. 80°C for 129 min, 85°C for 36 min) combined with storage at chill temperature (designed to give a 6D process for non-proteolytic *C. botulinum*)** .
(4) A ≤pH 5.0 throughout the food, combined with storage at chilled temperature
(5) A salt concentration ≥3.5% throughout the food, combined with storage at chilled temperature
(6) An ≤a_w 0.97 throughout the food, combined with storage at chilled temperature
(7) Combinations of heat treatment and other preservative factors which can be shown consistently to prevent growth and toxin production by *C. botulinum,* combined with storage at chilled temperature

Notes:
* originally 3.3°C, but growth has now been demonstrated at 3.0°C
** chill temperature is specified as 8°C in England, Wales and Northern Ireland. In Scotland the requirement is to keep chilled food in “a refrigerator, or refrigerating chamber, or a cool ventilated place”.

All of these recommendations are still in place, except for the second one. The second recommendation deals with the maximum shelf-life/storage temperature for chilled foods for which no other controlling factors could be demonstrated, and is known as the “10 day rule”.

In response to questions raised by a team drawing up an industry code of practice (at CCFRA), in 1995 the ACMSF revised this recommendation on the basis of a review of 31 references from the literature on the production of toxin by non-proteolytic *C. botulinum* within 10 days at ≤10°C in foods or food materials. The ACMSF also took account of predictions from a PC based version of Food MicroModel and unpublished data on *C. botulinum* (both provided by Dr. Baird-Parker, Unilever Research). In 1995, the ACMSF revised the second recommendation (10 day rule) to "storage at ≤5°C and a shelf-life of ≤10 days, or storage at 5°-10°C and a shelf-life of ≤5 days".

The group involved in drawing up an industry code of practice at CCFRA, considered the recommendations of the ACMSF (made in 1992 and in 1995), and replaced the second recommendation (10 day rule) with "storage at ≤8°C and a shelf-life of ≤10 days". The temperature of 8°C was selected as it is the legal upper limit for chilled food storage in England, Wales and Northern Ireland, rather than there being any scientific evidence regarding unsuitability of storage at 10°C. This code of practice was developed in conjunction with representatives of MAFF/Department of Health, and probably reflects much current industrial practice as it stands today, where specific controlling factors (e.g. 6 log reduction process, Aw, pH controls, other specific controls) are not present.

In response to a request from the ACMSF, in 2003 the FSA produced a draft small concise guidance document on the safe production of VP/MAP chilled foods with respect to *C. botulinum*. The recommendations included in this document are similar to those made by the ACMSF in 1995, and the industry code of practice in 1996, with an important difference for foods with a short shelf-life where other specific controlling factors cannot be demonstrated (10 day rule) (Table 2). At the ACMSF meeting held on 18th September 2003, the ACMSF agreed that the FSA concise document should go out to full public consultation. The consultation period ended in August 2004. The most significant issue raised by the consultation surrounded a perceived change in the "10 day rule", and highlighted differences between the ACMSF recommendations and the industry code of practice. The different recommendations are summarised in Table 2.

Table 2 Recommendations on ensuring the safety of short shelf life chilled foods when other specific controlling factors cannot be demonstrated

Recommendation from	Maximum storage temperature and shelf life recommended
ACMSF (1992)	storage at ≤10°C and a shelf-life of ≤10 days
ACMSF (1995)	storage at ≤5°C and a shelf-life of ≤10 days, or storage at 5°-10°C and a shelf-life of ≤5 days
Industry Code of Practice (CCFRA, 1996)	storage at ≤8°C and a shelf-life of ≤10 days
FSA draft concise guidance document (FSA, 2003)	storage at ≤5°C and a shelf-life of ≤10 days, or storage at 5°-8°C and a shelf-life of ≤5 days

At its meeting in December 2004, the ACMSF concluded that in the light of the concerns that were raised in response to the consultation, it needed to examine recent scientific evidence before advising on the FSA concise guidance document with respect to recommendations for foods with a short shelf-life where other specific controlling factors cannot be demonstrated (10 day rule). The ACMSF proposed that the FSA commission an independent review of the current scientific evidence, with the findings to be presented at a future ACMSF meeting. The purpose of this document is to provide the ACMSF with all the necessary up to date information to enable them to judge recommendations included in the FSA document on "foods with a short shelf-life where other specific controlling factors cannot be demonstrated".

3. Production and sales of chilled VP/MAP foods

The range of chilled foods sold in the UK and internationally is extremely varied, and the market is large. It is estimated that approximately 6 x 10^9 packs of chilled food are sold in the UK each year, and it is likely that more than 10^{11} packs have been sold during the two decades (Table 3). These chilled foods are packed under MAP, VP, air and other atmospheres containing oxygen. Even when air/oxygen is present above the food, there may be little oxygen within the food (i.e. the food is reduced), and these foods should be considered to present a similar botulism risk as MAP or VP foods.

Table 3 Details of chilled foods sold in the UK

Product	Packing (VP/MAP/Air)	Mean number of packs sold per annum (million)*	Shelf life	UK legal storage temperature (°C)**	Notes
Raw red meat	VP (primals, retail)	1,153	≤ 6m (primals) 13d (retail)	7	High O_2 (70%)
	MAP (retail)		≤10d		
Meat preparations	MAP	912	minced meat: 8d major multiple, 14-21d butcher	4	
Poultry preparations	MAP	327	10d	4	Low O_2
Poultry/products	MAP	256	10d (uncured) 28-35d (cured)	4 (whole) 8(products)	Low O_2
Sliced cooked meat and alternatives	MAP	1,128	10-15d (uncured) 15 to >30d (cured)	8	Nitrite (cured)
Fish and seafood	MAP	700	5-7d (MAP fresh fish) 8-9d (MAP cooked prawns) 21-28d (VP seafood sticks)	On melting ice (unpacked) 8	High O_2 (30%) (fresh fish/seafood and smoked trout)
Smoked fish	VP	15	6-16d	8	Smoked trout not generally VP in UK, NaCl is a key CCP
Mussels	VP (cooked) MAP (live)	2	10d to >21d (VP, cooked, not retorted) 6-9d (MAP, live)	On melting ice (unpacked) 8	
Bagged salads	MAP	71	4-7d	8	Iceberg lettuce
Bagged salads	Air	286	4-7d	8	Excl. iceberg. Film permeability regulates pack atmosphere
Fresh pasta and gnocchi	MAP	86	≤35d (longer for imports)	8	Low a_w (not NaCl), chill
Cooked chilled ready meals	Air – low O_2 in meal	1,185	≤10d, longer for imports	8	10 day rule applied (voluntary)
Total mean number of packs sold per annum (million)		**6,121**			

* Typically based on sales over the last five years

** chill temperature is specified as 8°C in England, Wales and Northern Ireland. In Scotland the requirement is to keep chilled food in "a refrigerator, or refrigerating chamber, or a cool ventilated place".

While the chilled food market in the UK is one of the largest in the world, other countries also have significant chilled food markets. It may be that global sales are an order of magnitude higher than those in the UK. It estimated that more than 1.5×10^{10} pre-packed chilled ready meals have been consumed in the EU in the last 20 years. In 2004, 1.0×10^{9} pre-packed chilled ready meals were consumed in the UK, 4.5×10^{8} meals in France, 1.8×10^{8} meals in Germany, 1.2×10^{8} meals in Finland, and 7.5×10^{7} meals in Italy. All of these products had a shelf life greater than 5 days, and many had a shelf life greater than 10 days without receiving a 6 log non-proteolytic *C. botulinum* process or any of the other control measures specified by the ACMSF (1992). The ACMSF (1995) rule of 10 day at 5°C/5 day at 10°C is not adhered to any significant extent in the UK or elsewhere. The 10 day rule at 8°C (CCFRA, 1996) is only adhered to by major producers in the UK and Benelux market. In some other countries (e.g. France, Finland), chilled products have been produced, over several decades, with shelf lives greater than 10 days. Many of these products will not have received a 6 log non-proteolytic *C. botulinum* process or any of the other control measured specified by the ACMSF (1992).

4. The position in the UK, other European countries and internationally with respect to guidance on control of non-proteolytic *C. botulinum* in chilled VP/MAP foods

The only legal requirements in the UK in relation to VP/MAP chilled foods are that they should be produced using HACCP principles, as required under European hygiene law, and that they should be stored at a maximum of 8°C (as defined in law in England, Wales and Northern Ireland), or a refrigerator, refrigerating chamber, or a cool ventilated place (as specified in regulations in Scotland). As discussed above, recommendations have been made by the ACMSF (1992 and 1995), and there is guidance in an industry code (CCFRA, 1996). The code recommendations were taken up widely by professional food manufacturers and retailers in the UK and were taken forward into various other guidance documents. Both in the UK and internationally, a variety of approaches are described, and most allow for flexibility of control measures on the basis of safety being demonstrable by the manufacturer on the basis of HACCP.

THERMAL PROCESSING

Many documents refer to 6-log non-proteolytic *C. botulinum* processes for long shelf life products (90°C/10 min or equivalent), which are based on the ACMSF 1992 approach. However, different z values are referred to, resulting in a range of processes that are intended to be equivalent, but which are not in reality. The appropriate choice of z value is a matter that needs to be addressed by research.

The use of heat treatments less than 90°C/10 min (or equivalents) can be effectively combined with storage temperature and shelf-life to prevent toxin formation from 10^{6} spores of non-proteolytic *C. botulinum* (i.e. provide a 6-log non-proteolytic *C. botulinum* process). For example, heating at 80°C for 11 min prevented toxin formation at 8°C in 20 days, while heating at 80°C for 98 min prevented toxin formation at 8°C in 40 days.

In France, legislation has permitted the use of heat treatments that deliver less than a 6-log non-proteolytic *C. botulinum* process. For example, 1988 French legislation enabled ready meals receiving a process equivalent of 70°C for 100 min (core temperature of 65°C; and equivalent to only 90°C for 1 min) to have a shelf life of up to 21 days, although the precise shelf life was to be determined by the manufacturer.

The French retail sous vide industry includes in its current approach the use of less than a 6-log non-proteolytic *C. botulinum* process. Heat treatments less than 90°C/10 min (or equivalents), such as 80°C for 93 min, combined with chilled storage regimes have been shown to provide for shelf lives of up to 30 days.

All approaches either leave the selection of thermal processes to the manufacturer to determine using HACCP, or provide example thermal equivalents, with flexibility for equivalents or otherwise demonstrably effective processes to be used.

HURDLES AND INTRINSIC FACTORS

Examples of single hurdles targeted at control of non-proteolytic *C. botulinum* are included in many documents, such as those produced by the ACMSF (1992, 1995), and the Industry Code of Practice (CCFRA, 1996). Examples of the hurdles include, pH<5 throughout a product and its components, $a_w \leq 0.97$ throughout a product and its components, and NaCl 3.5% (aq) throughout a product and its components.

Some other documents appear to be targeted at control of other pathogens such as *Listeria monocytogenes* and proteolytic *C. botulinum* (e.g. CFSAN, 2005), rather than non-proteolytic *C. botulinum*.

The importance of HACCP is stressed, and allow for the manufacturer to select appropriate hurdles (e.g. CFA, ECFF and SYNAFAP guidelines).

CHILLED TEMPERATURE

Storage at temperatures of less than 3°C are generally recognised as being a means of preventing growth and toxin formation by non-proteolytic *C. botulinum*.

In all approaches the main emphasis is on low temperature storage (not necessarily with specific limitation of shelf life). However, the specified temperatures vary. For example the Canadian and French approaches refer to 4°C as the national legal maximum, while in England, Wales and Northern Ireland this is 8°C. Further details are also given below.

SHELF LIFE

A wide range of approaches exist, but the emphasis is on demonstrable safety and HACCP.

In many cases the shelf-life relies on a controlling factor in addition to storage at chill temperature alone. For example, draft French industry guidance reflecting longstanding practice for sous vide foods allows for a shelf life of 21 days and potentially up to 30 days for products subjected to less than 6-log thermal reduction of non-proteolytic *C. botulinum*, provided that GHP is respected, and the product is distributed under controlled chill chain conditions.

Other advice relies on storage at 3°C or below. For example, Finnish Government advice in relation to cold smoked and gravad fish is for shelf life to be a maximum of 3 weeks at 3°C (note that this is based on control of *Listeria monocytogenes*).

A general feature of many guidance documents is that a shelf life of 10 days is permitted under chilled storage conditions, and that if a shelf life of greater than 10 days is required then the manufacturer is required to make available appropriate data demonstrating safety. The importance of HACCP is stressed.

5. Chilled storage and handling of foods

The maximum temperature specified in legislation for retail of chilled food is 8°C in England, Wales and Northern Ireland. The Food Hygiene (Scotland) Regulations specify chilled food to be stored in a refrigerator, refrigerating chamber, or a cool ventilated place. Neither is there a harmonised approach to legislated temperature rules within the EU, with temperatures of 0°C to 8°C specified in different countries. There are also different requirements for different food groups.

Within the UK, when held and distributed by the manufacturer, it is likely that chilled food is maintained at no more 5°C, and probably lower. Indeed, agreed retailer own label chilled prepared food temperature on delivery to retailers' Regional Distribution Centres is commonly set at 5°C maximum, through commercial agreements.

In practice, surveys of all chilled food outlets (including major multiples, farmers markets, small stores and other outlets) indicated that in the UK, the average temperature at retail was 4°C-6°C, with 6% of samples at >8°C. The position appears similar in many other European countries.

In the UK, a 1990 study showed that transportation of food from the point of purchase to the domestic refrigerator took an average of 43 min, with most achieved in 60 min. The majority of people (87%) did not chill food during transport, and in some cases the food reached temperatures in excess of 20°C, albeit for a short period of time. It took several hours for the food to cool to below 7°C. The increased use of insulation bags or boxes would help consumers maintain the chill chain.

Chilled food purchased through mail order is exempt from legislation in England, Wales and Northern Ireland, although the temperature should be maintained at a "safe level". A MAFF study in 1991 reported that mail order chilled foods spent 70% of their time at 8°C or higher, and that the average temperature on receipt was 15°C. Since 15 years have elapsed, there would be merit in repeating the survey of the temperature control of chilled mail order foods. MOFFA (Mail Order Fine Foods Association) state that "if the temperature is likely to rise in transit above 8°C, the main order operator should be confident that it is safe by reference to supporting technical or other data".

Domestic refrigerators are present in >99% of households in the UK, and on average are replaced every 8 years. Refrigerators provide a key food safety device within the domestic kitchen, their correct operation will reduce the risks of the growth of food poisoning organisms in foods stored within them. Unfortunately there is a lack of recent published data on the temperatures of domestic refrigerators and the last UK domestic refrigerator survey was carried out in 1990. In order to ensure the UK has up to date information on domestic refrigerator temperatures, a new survey is required.

The 1990 UK survey found that the mean domestic fridge temperature ranged from -1°C to 11°C over a 7 day period, and that the overall mean temperature was 6.6°C, with 65-70% of fridges at more than 5°C. There was variation in performance between fridges, and within each fridge over time. Different temperatures were also recorded in different parts of single fridges. Overall, for all domestic fridges the time spent at various temperatures were as follows; 28% of the time at <5.0°C, 35% of the time at 5.0-6.9°C, 28% of the time at 7.0-8.9°C, and 9% of the time at >9°C. The position appears similar in other countries, and an average temperature of 6.64°C has been reported for European fridges. It was recommended by Richmond in 1991 that the maximum temperature of domestic fridges in the UK should not exceed 5°C.

A survey of consumer behaviour in France established that for short shelf life chilled products, approximately 60% of the shelf life was spent in commercial refrigeration, and 40% in domestic refrigeration. The general applicability of this to other countries is not known, given different practices in various countries.

UK consumer understanding of the "use by date" or "best before date" is poor, and UK consumer handling of chilled foods in practice needs to be more widely studied. Recent FSA data indicate that 27-34% of consumers believe that food past the "use by date" or "best before date" should be thrown away, 24-31% of consumers believe that such food might be past its best but not necessarily unsafe to eat, and 36-37% believe that the action would depend on the food. An earlier survey found that 26% of consumers would eat products after the expiry of their use by date.

It is suggested that the UK continues to strive for better temperature control throughout the chill chain (including domestic storage), and that 5°C is adopted as a target for best practice. This could be aided by new domestic refrigerators including chill compartments and a temperature measuring device to assist consumers in assuring the appropriate chilled storage of foods. This proposal is made in order to further extend the margins of safety of chilled foods with respect to psychrotrophic foodborne pathogens, rather than on the basis of any specific problems.

6. Recent incidents of foodborne botulism

Foodborne botulism is a severe and deadly disease, with most outbreaks associated with home-made foods where known control measures have not been implemented. More rarely, outbreaks of foodborne botulism have been associated with commercial foods and with restaurants. Very large costs are associated with botulism outbreaks involving commercial foods, and are orders of magnitude greater than those associated with other foodborne pathogens (e.g. *Salmonella*, *Listeria*).

Foodborne botulism is most frequently associated with proteolytic *C. botulinum* or with non-proteolytic *C. botulinum*, than with other neurotoxin-forming clostridia. Proteolytic *C. botulinum* is a mesophile, has a minimum growth temperature of 10°C-12°C, and forms toxins of types A, B and F. Non-proteolytic *C. botulinum* is a psychrotroph that is able to grow and form toxin at 3.0°C, and forms toxins of types B, E and F. In view of its ability to grow and form toxin at 3.0°C, non-proteolytic *C. botulinum* is a larger concern in chilled foods.

In order to collect literature data on toxin formation in chilled foods/food materials, an extensive literature search was carried out. In Europe, more than 2,500 cases of foodborne botulism were reported in 1999/2000. In the UK, 62 cases of foodborne botulism were reported between 1922 and 2005 (Table 4). Twenty of these cases were fatal. In the last twenty years there have been 34 cases of foodborne botulism in the UK, three of which have been fatal. Non-proteolytic *C. botulinum* has been associated with one outbreak of botulism in the UK (four cases and two deaths) that involved post-process contamination of ambient stable canned salmon in 1978. Non-proteolytic *C. botulinum* has not been associated with foodborne botulism in correctly stored chilled foods in the UK.

Table 4 Foodborne botulism incidents reported in the UK

Year	Food vehicle	Home prepared food	Number of cases (deaths)	Toxin type
1922	Duck paste	No	8 (8)	A^P
1932	Rabbit and pigeon broth	Yes	2 (1)	?
1934	Jugged hare	Yes	1 (0)	?
1935	Vegetarian nut brawn	Yes	5 (4)?	A^P
1935	Minced meat pie	Yes	1 (1)	B
1947	Macaroni cheese	Yes	5 (1)	?
1955	Pickled fish (from Mauritius)	?	2 (0)	A^P
1978	Canned salmon (from USA)	No	4 (2)	E^n
1987	Rice and vegetables (Kosher airline meal)	No	1 (0)	A^P
1989	Commercial hazelnut yoghurt	No	27 (1)	B^P
1998	Home bottled mushrooms in oil (from Italy)	Yes	2 (1)	B^P
2002	Homemade sausage (from Poland)	Yes	1 (1)	B
2004	Commercial chilled organic hummus	No	1 (0)	*
2005	Travel from Georgia	?	1 (0)	A^P
2005	Home preserved pork (from Poland)	Yes	1 (0)	B

^{P} = proteolytic *C. botulinum*, ^{n} = non-proteolytic *C. botulinum*

* Case is suspected and not laboratory confirmed

Outbreaks of foodborne botulism have only been associated with commercially produced chilled foods when they have been time/temperature abused, and also when botulinum toxin has been inadvertently added (with a food component) to a correctly chilled food product. There have been outbreaks associated with each of these scenarios in the UK, and in other countries. Twelve examples are given in Table 5.

Table 5 Examples of foodborne botulism involving commercial chilled foods

Country (year)	Product	Organism and toxin type	Cases (deaths)	Factors contributing to outbreak
Canada (1985)	Commercial garlic-in-oil	Proteolytic *C. botulinum* B	36	temperature abuse
UK (1989)	Commercial hazelnut yoghurt	Proteolytic *C. botulinum* B	27(1)	Toxin added with hazelnut conserve to correctly chilled yoghurt
USA (1990)	Barbequed [fresh] surgeon fish (palani)	*C. botulinum* B*	3	temperature abuse
USA (1993)	Restaurant, commercial cheese sauce	Proteolytic *C. botulinum* A	8 (1)	Recontamination and temperature abuse
USA (1994)	Restaurant; potato dip ("skordalia") and aubergine dip ("meligianoslata")	Proteolytic *C. botulinum* A	30	Toxin added with potatoes to correctly chilled yoghurt
USA (1994)	Commercial clam chowder	Proteolytic *C. botulinum* A	2	temperature abuse
USA (1994)	Commercial black bean dip	Proteolytic *C. botulinum* A	1	temperature abuse
Italy (1996)	Commercial mascarpone cheese	Proteolytic *C. botulinum* A	8(1)	temperature abuse
Germany (1997)	Commercial hot-smoked vacuum-packed fish ("Raucherfisch")	Non-proteolytic *C. botulinum* E	2	Suspected temperature abuse
France (1999)	Commercial chilled fish soup	Proteolytic *C. botulinum* A	1	temperature abuse
Germany (2004)	Commercial vacuum-packed smoked salmon	Non-proteolytic *C. botulinum* E	1	Consumed after "use-by date"
UK (2004)	Commercial chilled organic hummus	Not known	1	Time/temperature abuse

* Not clear whether proteolytic *C. botulinum* or non-proteolytic *C. botulinum*

No cases of foodborne botulism can be attributed to non-proteolytic *C. botulinum* and correctly stored commercial chilled foods in the UK or overseas. There is, however, speculation that a recent change in aetiology of foodborne botulism in France may be associated with refrigerated vacuum-packed foods, although a link with commercial chill foods has not been established.

7. Summary and discussion of data on growth and toxin formation by non-proteolytic *C. botulinum* at ≤10°C

SUMMARY OF DATA

Several predictive models have been developed for growth of non-proteolytic *C. botulinum*. Each model is based on a different dataset and gives a slightly different prediction but, in general, the models are constructed to deliver a fail-safe prediction of time to toxin formation when other factors are not limiting. Four predictive models have been considered:

1. Combase Predictor. This model was developed and validated by Graham *et al.* (1996), and is based on growth curves done in a microbiological broth medium. Most of the growth curves were at ≤10°C, and the model is most robust in this region. This model is freely available in ComBase Predictor (www.combase.cc). In this report, predicted time to a 1000-fold increase in viable count is taken to be time to toxin formation.
2. PMP (Pathogen Modeling Program). A probability model developed by Whiting and Oriente (1997) is freely available in PMP (www.arserrc.gov/mfs/PMP6_CurMod.htm). This probability model is also based on tests done in a microbiological broth medium, but time to turbidity is used as the measure of toxin formation. In this report, time to toxin formation is taken as the time to turbidity from a starting concentration of 10^4 spores/ml.
3. Baker/Genigeorgis model. This model is based on tests carried out in more than 17,000 raw fish homogenates (Baker and Genigeorgis, 1992). It is a lag time model, where lag time is taken as the last time that all replicate samples were negative for toxin. This model is freely available in the PMP website (www.arserrc.gov/mfs/PMP6_CurMod.htm).
4. Skinner/Larkin. The Skinner/Larkin model is a more conservative version of the Baker/Genigeorgis model, modified to take account of observations of growth in other experiments (Skinner and Larkin, 1998).

All four models predict that toxin formation will occur in less than 10 days at 8°C (Fig. 1, Table 6). The different predictions of time to toxin formation reflect the different datasets on which the models were based. The model in ComBase Predictor is designed to be most robust at ≤10°C, and may give more reliable predictions in this region than the other two original models. The Skinner/Larkin model is designed to be an ultimate failsafe model. It may be that the prediction of toxin formation in 5-6 days at 8°C is the most reasonable fail-safe prediction from the models, and the prediction of toxin formation in 4 days at 8°C is the most conservative failsafe prediction. It is important to recognise, however, that these models are designed to represent various worst-case scenarios, and the issue that must be addressed is how closely predictions from these models relate to toxin production in actual chilled foods sold in the UK and elsewhere.

Just as models developed using microbiological broth media predict toxin formation in less than 10 days at 8°C (Table 6), tests in microbiological broth media have also reported growth/toxin formation in 10 days or less at 8°C. In this report it is accepted that in microbiological broth media, growth and toxin formation can occur in 10 days or less at 8°C, and no further data have been collected on tests carried out in microbiological broth media.

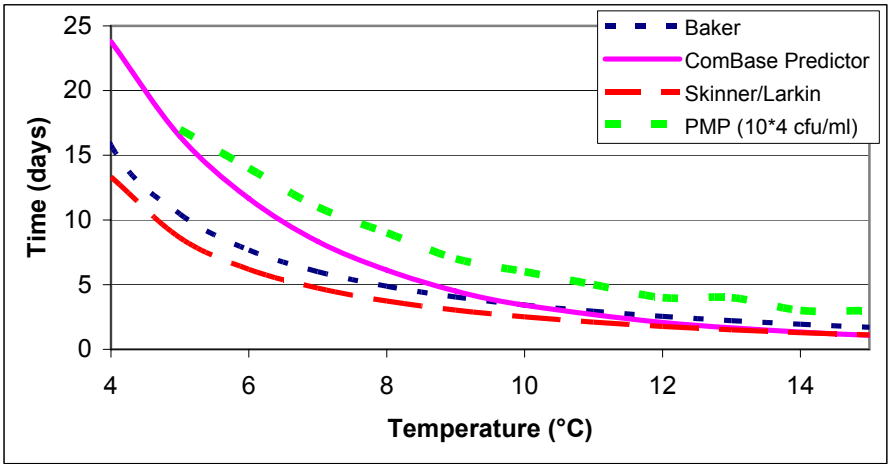

Fig. 1 Effect of incubation temperature on the time to toxin formation by non-proteolytic *Clostridium botulinum*, as predicted by four mathematical models

Table 6 Example predictions of time to toxin formation by non-proteolytic *Clostridium botulinum* at 4°C-12°C from four predictive models

Model	Predicted time to toxin formation (d) at specified temperature								
	4°C	5°C	6°C	7°C	8°C	9°C	10°C	11°C	12°C
ComBase Predictor	24	16	12	8	6	5	3	3	2
PMP	--	17	14	11	9	7	6	5	4
Baker/Genigeorgis	16	10	8	6	5	4	3	3	3
Skinner/Larkin	13	9	6	5	4	3	3	2	2

In order to collect literature data on toxin formation in chilled foods/food materials, an extensive literature search was carried out in January 2006, and combined with articles held in the personal libraries of the authors of this report. Data extracted from 61 literature publications yielded 887 independent tests of time to toxin formation. Additionally, 27 confidential datasets that contained 420 independent tests of time to toxin formation were kindly donated by members of the food industry. This gave a total of 1307 independent tests. One independent test would typically be one product, inoculated with a defined number of spores of non-proteolytic *C. botulinum* (a mixture of strains), incubated at one temperature, and sampled a number of times. Replicate samples would be removed and tested for toxin at various time points (often in duplicate or triplicate), and the last time when all the replicate samples were negative, and the first time that one of the replicate samples was positive noted. Toxin would typically be detected using a mouse test, although some data were based on growth tests. All the data are therefore from "challenge tests", where spores of non-proteolytic *C. botulinum* have been added to foods/food materials.

It should be noted that most of the data included in this assessment had not been generated for the purpose of evaluating the potential for growth and toxin production by non-proteolytic *C. botulinum* in chilled foods sold in the UK within 10 days or less at 10°C or below. The relevance of these data to short shelf-life chilled foods sold in the UK is in some cases, therefore, limited. Also, the number and proportion of positive tests is to some extent a reflection of the experimental design in the tests that have been carried out. For example, some tests have been carried out in sterile raw materials where conditions are very favourable for growth and toxin formation, while other tests have been carried in conditions not at all conducive to growth and toxin formation (e.g. preservatives added). The proportion of positive tests is a reflection of the balance between these extremes (and all intermediate positions). It is not easy to relate the number and proportion of positive tests to the safety of short shelf life chilled foods sold in the UK.

The results from 1307 independent challenge tests do, however, demonstrate that non-proteolytic *C. botulinum,* if present, is in some circumstances able to form toxin in foods and food materials at ≤10°C within 10 days. In total, 237 individual tests were positive for toxin formation by day 10 (19%). At 10°C, 132 of the tests were positive at day 10 (36%); at 8°C, 100 of the tests were positive at day 10 (19%); and at 4°C-7°C, five of the tests were positive (1%) (Table 7).

Since the current recommended storage temperature for chilled foods in England, Wales and Northern Ireland is ≤8°C, much effort has been dedicated to analysing data at 8°C. A total of 527 independent challenge test datasets with storage at 8°C were considered, and 100 of these were positive for toxin at day 10. Of the 100 positive tests, 56 were with raw or smoked fish, 41 were with sterile or pre-cooked food, two with sous-vide foods, and one with salted ham (Table 8). Based on these data there is a possibility that if contaminated with spores of non-proteolytic *C. botulinum*, raw or smoked fish could become toxic within 10 days at 8°C. Many of these positive tests provide a fail safe indication of the time to toxin formation in chilled foods, however in view of the large variety of chilled foods sold in the UK, it is possible that these observations of

toxin formation within 10 days at 8°C may be relevant to some chilled foods. The difficult issue is to identify which chilled foods. It should be noted that two sous-vide foods that were inoculated with spores prior to the heat treatment became toxic in nine days at 8°C, with eleven other sous-vide foods becoming toxic at day 11/12.

Table 7 Effect of storage conditions on toxin formation by non-proteolytic *C. botulinum* in challenge test experiments

Storage conditions		Number of samples (percentage) negative/positive for toxin under specified storage conditions	
Temperature	time	Negative for toxin	Positive for toxin
10°C	≤5 days	319 (93%)	24 (7%)
	≤10 days	238 (64%)	132 (36%)
	≤15 days	166 (50%)	166 (50%)
8°C	≤5 days	500 (98%)	12 (2%)
	≤10 days	414 (81%)	100 (19%)
	≤15 days	360 (72%)	142 (28%)
4-7°C	≤5 days	389 (100%)	0 (0%)
	≤10 days	382 (99%)	5 (1%)
	≤15 days	360 (94%)	22 (6%)
TOTAL (4-10°C)	≤5 days	1208 (97%)	36 (3%)
	≤10 days	1034 (81%)	237 (19%)
	≤15 days	886 (73%)	330 (27%)

Table 8 Summary of different food types in which toxin formation has been reported by non-proteolytic *C. botulinum* in 10 days at 8°C in challenge test experiments

Food type	Total number of positive tests (total number of all tests)	Number of positive tests at each indicated day							
		3d	4d	5d	6d	7d	8d	9d	10d
Raw/smoked fish	56 (169)	-	5	-	1	24	2	0	24
Sterile minced beef	25 (97)	-	2	5	3	5	2	7	1
Cooked turkey breast	13 (75)	-	-	-	-	-	7	6	-
Pre-cooked sous-vide foods	2 (15)	-	-	-	-	-	2	-	-
Sous-vide foods	2 (110)	-	-	-	-	-	-	2	-
Other foods	2 (61)	-	-	-	-	-	-	-	2
Total	100 (527)	-	7	5	4	29	13	15	27

All the data for toxin production within 25 days or less are summarised in Fig. 2, along with the prediction of time to toxin formation from ComBase Predictor. In 38 independent tests, time to toxin formation was more rapid than predicted by this model. These tests were from ten different publications, and the foods/food materials involved were raw fish (in 21 tests), cooked turkey breast (1 test), pre-cooked sous-vide beef with gravy (2 tests), cooked minced beef (12 tests), and sterile chicken skin and exudate (2 tests).

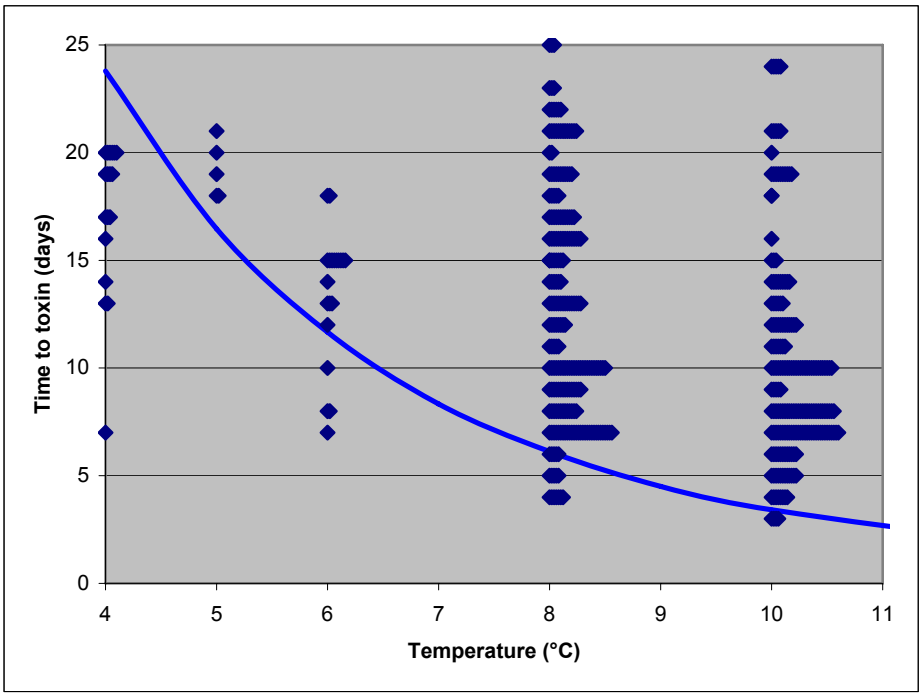

Fig. 2 Effect of incubation temperature on time to toxin formation by non-proteolytic *C. botulinum*. The curve is predicted time to toxin from ComBase Predictor. Observations of time to toxin formation in foods and food materials at 4°, 5°, 6°, 8° and 10°C are shown. Where there is more than one observation at each temperature/time, successive observations are plotted to the right (and give the "bars"). Many tests were negative for toxin formation at 25 days (especially at 4°C-7°C) and are not shown.

It was noted in several studies that toxin formation can be as rapid (or in some circumstances more rapid) in foods packed in air as under VP or low-oxygen MAP (presumably because there is no oxygen in the food, i.e. the food is reduced [in some cases possibly by aerobic organisms]). For example, in one study at 12°C flounder became toxic in 11 days in air, in 10-14 days in various anaerobic modified atmospheres, and in 15 days in VP. Packaging under air or a similar oxygen-containing atmosphere is therefore not a guarantee that toxin formation by non-proteolytic *C. botulinum* will be prevented.

In considering the effect of new processing technologies, it is apparent that there is very little information on the effect of these technologies compared to the wealth of data on the effect of heat. This results in a major issue when it comes to using a new process, while some of its effects may be predictable, the confidence in the prediction will be low, and the need for extensive validation is high. The effects of four "new" processes (high hydrostatic pressure (HPP), pulsed electric field, irradiation pasteurisation and pulsed light) on non-proteolytic *C. botulinum* have been considered, and the effectiveness is dependent on the type of process applied, some do have a killing effect if properly applied, whilst others have a very limited effect. Thus, any use of new process technology to inactivate non-proteolytic *C. botulinum* will have to be extensively validated. The validation would need to take into account strain variation, the effects of the food type, composition and storage regime, and any effects on other food microflora. In establishing safe processes, it is important that at the very least, 'equivalence' with established safe technologies must be assured.

DISCUSSION OF THE DATA

At a first glance, the reported ability of non-proteolytic *C. botulinum* to form toxin at 8°C and below within 10 days would appear to be in conflict with the observation of safe production and

sale of large quantities of correctly stored commercial chilled food in the UK and overseas without incidence of botulism. For example, approximately 4 x 10^9 commercial chilled prepared meals have been produced following 8°C/10 days (shelf-life often 7-8d) since 1990 in the UK. That there have been no botulism outbreaks associated with correctly stored chilled foods is presumably a reflection that one or both of the following controls has ensured safety;

(i) the foods contain no spores or only a low number of spores of non-proteolytic *C. botulinum*,
(ii) the foods do not support growth and toxin formation by non-proteolytic *C. botulinum* within the time and temperature of storage.

Short shelf-life chilled foods that have been sold in the UK would therefore appear to have "unknown controlling factors (unknown hurdles)" that have prevented growth and toxin formation by non-proteolytic *C. botulinum*. These "unknown controlling factors" might include:

a. low spore contamination
b. a heat process that damages or decreases the number of spores
c. an inhibitory background microflora
d. a reduced pH, low water activity, high NaCl concentration, preservatives, inhibitory modified atmosphere, an effect of food structure, or a combination of these
e. storage at less than 8°C through part or all of the chill chain
f. consumption of the food before the end of shelf-life
g. the food is heated before consumption to inactivate any toxin formed

It is important to note however, that:

(i) different "unknown controlling factors" are likely to be important in different chilled foods
(ii) the magnitude and variability of these "unknown controlling factors" is not known and is likely to be different for different foods, consequently the safety margin is not known and will vary from chilled food to chilled food, and may also vary from pack to pack for each food.

Considering the large range of short shelf life chilled foods sold in the UK, and the above comments, it is likely that some chilled foods are a bigger risk than others. It may be possible to categorise the foods as a high, medium or low risk, and for these different risk categories to have a different maximum shelf-life at 8°C. For example, raw fish is a high risk product, with numerous challenge tests indicating the possibility of toxin formation in 10 days at 8°C. A difficult matter is to decide which foods fit into which category, and from the unpublished work of Baker on sous-vide foods, it is clear that this will not be straightforward. The designation of low, medium or high risk should be made only on the basis of sound scientific evidence.

In conclusion, it is not easy to base a determination of the maximum shelf-life of chilled foods at 8°C (where other controlling factors are not known) on only the data from 1307 independent tests of toxin formation by non-proteolytic *C. botulinum*. It is clear that, given the correct circumstances, if present, non-proteolytic *C. botulinum* can form toxin in 10 days or less at 8°C. That this has not happened with short shelf-life chilled foods sold in the UK (or internationally) must be due to presence of one or more "unknown controlling factors". The difficulty is that the magnitude, variability and nature of these "unknown controlling factors" is not known, and it is suspected that they are not the same for all chilled foods. The position is therefore that although short shelf-life foods have been produced safely in the UK and internationally for more than two decades, it is not known precisely what the safety margins are with respect to foodborne botulism. Research is needed to identify the magnitude, variability and nature of these "unknown controlling factors". This will aid the continued safe development of chilled foods in the UK and internationally.

This study noted a dramatic effect of storage temperature on toxin formation by non-proteolytic *C. botulinum*. For example from the model in ComBase Predictor, time to toxin is predicted as 3 days at 10°C, 6 days at 8°C, 12 days at 6°C, 16 days at 5°C, and 24 days at 4°C. Thus, if all chilled food could be maintained at 4°C/5°C (for example) throughout the chill chain (including in

the home), the safety margin would be extended further. It is likely that there will also be a benefit with other psychrotrophic pathogens and shelf-life extension may also be possible. In order to further extend the margins of safety of chilled foods with respect to psychrotrophic foodborne pathogens, it is suggested the UK continues to strive for better temperature control throughout the chill chain (including domestic storage), and that 5°C is adopted as a target for best practice. It should be noted that this comment is not based on any particular outbreak of food poisoning. This suggestion re-iterates various recommendations made by Richmond in 1991.

8. Re-packing VP/MAP chilled foods during the 10 day shelf-life

It has been brought to our attention that some chilled VP/MAP foods such as meat may be given a “rolling 10 day shelf-life”. That is, the product is opened during the initial 10 day shelf-life, some is used, and then the remainder is repacked and given a further 10 day shelf-life. Thus, the shelf-life is extended beyond 10 days without the identification of other factors that control toxin formation by non-proteolytic *C. botulinum*. While we are not aware of this practice leading to outbreaks of botulism, this represents a significant divergence from the guidance and would appear to be a high risk practice.

For foods where no other controlling factor can be identified, the maximum shelf-life is 10 days, and this should commence once the product is first vacuum or modified atmosphere packed. The shelf-life must not be restarted if the product is subject to a further packing under vacuum or modified atmosphere, unless other controlling factors (as described by the ACMSF) are applied.

9. Risk assessments

Risk assessment is a frequently used term that can have many definitions. Formal Codex type risk assessments are well defined, require considerable data and are formally recorded, in order to provide a transparent assessment of the risks associated with the production of particular products. In this review, formal risk assessments of growth and toxin formation by non-proteolytic *C. botulinum* in a cooked sliced meat and in gnocchi, have been considered. Both assessments used slightly different approaches, but came to the conclusion that the products (which were not given a 90°C/10 min process and did not have pH or Aw controls), could be stored for longer than 10 days at 8°C, and remain safe with respect to the risks from non-proteolytic *C. botulinum*. In the case of the gnocchi the results from the risk assessment were confirmed in a challenge test.

The term risk assessment is also often used to describe less formal determinations of product risk, however these less formal and structured assessments provide useful information in specific product categories. Work done on fresh produce packed in MAP, suggests that whilst there may be risks of growth from *C. botulinum*, this may only occur in temperature abused product (>12°C) and will usually be preceded by gross product spoilage that would render that product organoleptically unacceptable to the consumer. Assessment of various fish products has considered that unless a sporicidal heat process is given, the presence of non-proteolytic *C. botulinum* spores should be assumed, and suitable controls (pH or Aw) put in place. These assessments have not however indicated any shelf life requirements (times or temperatures) if the controls are not in place. In these situations where no specific controls are recognised, recommendations to employ predictive microbiology or challenge testing are often given.

One issue noted while considering the risks presented by non-proteolytic *C. botulinum* in chilled MAP/VP products, is that while challenge test data may indicate the potential for growth and toxin formation, it is known that many thousands of millions of packs of product of this type have been sold around the world, with no evidence of botulism having occurred (except very rarely, when a product has been temperature abused). The use of data from this large commercial production enables an assessment of the risks arising from these products. This has been done

previously for proteolytic *C. botulinum* in canned meats, and this type of approach adds value to an assessment of non-proteolytic *C. botulinum* in chilled MAP/VP products. Some very preliminary data assessment indicates that the safety units for cooked chilled foods, sliced cooked meats and raw red meats are all >9.8 (i.e. 1 in $>10^{9.8}$ packs are associated with botulism) while for smoked fish it is estimated that the safety unit is >8.0 (i.e. 1 in $>10^{8.0}$ packs are associated with botulism). These of the same order as for canned meats.

10. Conclusions

1. It is proposed that for short shelf life foods where other controlling factors are not identified, the FSA should include in their document, "storage at ≤8°C and a shelf-life of ≤10 days", rather than "storage at ≤5°C and a shelf-life of ≤10 days or storage at 5°-8°C and a shelf-life of ≤5 days". This proposal is made on the basis of extensive sales of chilled foods with this 10 days shelf-life without any incidence of foodborne botulinum (when correctly stored). It is cautioned, however, that if present, non-proteolytic *C. botulinum* can form toxin in 10 days and less at 8°C, and there is insufficient clear information as to what the safety margins are in foods as sold, particularly when attempting to take into account the temperature performance of the complete chill chain throughout foods' shelf lives. Great care must be used when modifying current industrial practice (e.g. extending the shelf-life of chilled foods over that currently used), and in the development of new products. Since, although current industrial practice appears safe, it is possible that chilled foods could be produced for which a 10 day shelf life at 8°C would not be suitable. It would seem logical to apply this approach to all chilled food sold in the UK.

2. The safety of short shelf life commercial chilled foods in the UK (and internationally) relies on the presence of one or more "unknown controlling factors", the magnitudes, variabilities or natures of which are not known. Research is needed to improve understanding of these "unknown controlling factors", in order to aid the continued safe development of chilled foods in the UK (and internationally).

3. It is proposed that for foods where no specific controlling factor can be identified, the maximum shelf-life is 10 days, and that this commences once the product is first vacuum or modified atmosphere packed. The shelf-life should not be restarted if the product is subject to a further packing under vacuum or modified atmosphere, unless other controlling factors (as described by the ACMSF (1992)) are applied.

4. Software tools and predictive models should be developed to contribute to the continued safe development of chilled foods. Software tools could be built using existing data and models, for example to describe the effect of time/storage temperature during manufacture, distribution, retail storage and domestic storage on time to toxin formation by non-proteolytic *C. botulinum*. There would be merit in including distributions of time and temperature. Software packages could also be developed, using existing data, to describe the effect of heating time and temperature and incubation temperature (and also mild preservative factors) on time to toxin formation from 10^6 spores of non-proteolytic *C. botulinum*. Predictive models are not available that describe the effect of nitrite on growth of non-proteolytic *C. botulinum* and further work is needed in this area (e.g. the current model in ComBase Predictor could be extended to a four factor model, with nitrite added).

5. Many documents refer to 6-log non-proteolytic *C. botulinum* processes for long shelf life products (90°C/10 min or equivalent), which are based on the ACMSF 1992 approach. However, different z values are referred to, resulting in a range of processes that are intended to be equivalent, but which are not in reality. The appropriate choice of z value is a matter that needs to be addressed by research.

6. The last surveys of UK domestic refrigerator temperatures and the temperature of UK mail order chilled food during distribution were carried out in the early 1990s. In view of the elapsed

time, there would be merit in repeating these surveys. This would provide up to date information on current practice.

7. It is proposed the UK continues to strive for better temperature control throughout the chill chain (including domestic storage), and that 5°C is adopted as a target for best practice. This could be aided by new domestic refrigerators that include chill compartments and a temperature measuring device to assist consumers in assuring the appropriate chilled storage of foods. This proposal is made in order to further extend the margins of safety of chilled foods with respect to psychrotrophic foodborne pathogens (since their growth is slower at 5°C than 8°C), rather than on the basis of any specific problems.

PART TWO – MAIN REPORT

Chapter one - Introduction

There has been a substantial increase in sales of vacuum packed (VP) and modified atmosphere packed (MAP) chilled foods over the last decade. These foods address consumer demand in being of high quality and requiring little preparation time. The microbiological safety of these foods commonly depends on one or a combination of factors that may include heat, refrigerated storage, pH control, control of water activity and a restricted shelf-life. The principal microbiological safety hazard for these foods is foodborne botulism, as presented by non-proteolytic *Clostridium botulinum* (Peck, 1997; Peck and Stringer, 2005; Peck, 2006).

Foodborne botulism is primarily associated with proteolytic *C. botulinum* and with non-proteolytic *C. botulinum* (and very occasionally with neurotoxigenic strains of *C. butyricum* and *C. baratii*). Proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are physiologically and genetically distinct organisms. Proteolytic *C. botulinum* is a mesophile, with a minimum growth temperature of 10°C-12°C, while non-proteolytic *C. botulinum* is psychrotrophic and is able to grow and form toxin at 3.0°C (Table 1.1). The distinct physiological characteristics of proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are summarised in Table 1.1. This report is primarily concerned with the risk presented to the safety of chilled foods by non-proteolytic *C. botulinum*.

Table 1.1 Characteristics of the two physiologically and genetically distinct clostridia most frequently associated with foodborne botulism

	Proteolytic *C. botulinum* (mesophilic)	Non-proteolytic *C. botulinum* (psychrotrophic)
neurotoxins formed	A, B, F	B, E, F
minimum growth pH	4.6	5.0
minimum growth temperature	10-12°C	3.0°C
maximum growth NaCl	10%	5%
spore heat resistance ($D_{100°C}$)	>15 min	<0.1 min

Foodborne botulism is a severe but rare disease. It is an intoxication resulting from consumption of foods in which *C. botulinum* has grown and produced botulinum neurotoxin. As little as 30 ng of neurotoxin is sufficient to cause illness and even death (Peck and Stringer, 2005). The consumption of as little as 0.1g of food in which *C. botulinum* has grown can result in botulism (Lund and Peck, 2000). In view of the severity of botulism, regulators and industry work hard to ensure that it remains rare. This has led to the production of various guidelines, recommendations and codes of practice with respect to the control of non-proteolytic (psychrotrophic) *C. botulinum*, in foods generally and more specifically for the safe production of vacuum and MAP chilled foods.

In 1992, the ACMSF published a report that included recommendations on the safe production of vacuum and MAP chilled foods with respect to non-proteolytic *C. botulinum* and the associated foodborne botulism hazard (ACMSF, 1992). These were:

(1) storage at <3.0°C*
(2) storage at ≤10°C and a shelf-life of ≤10 days (the "10 day rule")
(3) A heat treatment of 90°C for 10 min or equivalent lethality (e.g. 80°C for 129 min, 85°C for 36 min) combined with storage at chill temperature** .
(4) A ≤pH 5.0 throughout the food, combined with storage at chilled temperature
(5) A salt concentration ≥3.5% throughout the food, combined with storage at chilled temperature
(6) An ≤a_w 0.97 throughout the food, combined with storage at chilled temperature

(7) Combinations of heat treatment and other preservative factors which can be shown consistently to prevent growth and toxin production by *C. botulinum,* combined with storage at chilled temperature

Notes:
* originally 3.3°C, but growth has now been demonstrated at 3.0°C (Graham *et al*., 1997)
** chill temperature is specified as 8°C in law in England, Wales and Northern Ireland. In Scotland the requirement is to keep chilled food in "a refrigerator, or refrigerating chamber, or a cool ventilated place".

Later, in response to questions raised by the team drawing up an industry code of practice (at CCFRA), one of the key ACMSF recommendations were revised (ACMSF, 1995). This recommendation was revised on the basis of a review of 31 references from the literature on the production of toxin by non-proteolytic *C. botulinum* within 10 days at ≤10°C. Most of these were challenge tests carried out in food materials. The group also took account of predictions from a PC based version of Food MicroModel and unpublished data on *C. botulinum* (both provided by Dr. Baird-Parker, Unilever Research). The second recommendation of "storage at ≤10°C and a shelf-life of ≤10 days (the 10 day rule)" included in the 1992 report (ACMSF, 1992) was replaced with "storage at ≤5°C and a shelf-life of ≤10 days, or storage at 5°-10°C and a shelf-life of ≤5 days" (ACMSF, 1995).

A number of other recommendations, guidelines and a code of practice have been targeted at ensuring the safe production of these foods by preventing growth and toxin production by *C. botulinum* (e.g. ECFF, 1996; Betts, 1996; CFA, 1997; Martens, 1997; Martens, 1999; Gould, 1999; FSA, 2003). In particular, attention is drawn to an industry code of practice developed at CCFRA (Betts, 1996). The group involved in the drawing up of this industry code of practice considered the recommendations of the ACMSF (1992 and 1995). The recommendations included in this code of practice are similar to those made by the ACMSF (1992), except that recommendation (2) was replaced by a recommendation of "storage at ≤8°C and a shelf-life of ≤10 days". The temperature of 8°C was selected as it is the legal upper limit for chilled food storage in England, Wales and Northern Ireland, rather than there being any scientific evidence regarding unsuitability of storage at 10°C (Betts, 1996). This code of practice was developed in conjunction with representatives of MAFF/Department of Health, and probably reflects much current industrial practice as it stands today.

In response to a request from the ACMSF, the FSA have produced a draft small concise guidance document on the safe production of VP and MAP chilled foods with respect to *C. botulinum* (FSA, 2003). The recommendations included in this document are similar to those made by the ACMSF (ACMSF, 1995) and the industry code of practice (Betts, 1996), except for an important difference for foods with a short shelf-life where other specific controlling factors cannot be demonstrated (10 day rule) (Table 1.2). At the ACMSF meeting held on 18th September 2003, the ACMSF agreed that the FSA concise document should go out to full public consultation. The consultation period ended in August 2004. The most significant issue raised by the consultation surrounded a perceived change in the "10 day rule", and highlighted differences between the ACMSF recommendations and the industry code of practice (Table 1.2).

Table 1.2 Recommendations on ensuring the safety of short shelf life chilled foods when other specific controlling factors cannot be demonstrated

Body making recommendation	**Maximum storage temperature and shelf life recommended**
ACMSF (1992)	storage at ≤10°C and a shelf-life of ≤10 days
ACMSF (1995)	storage at ≤5°C and a shelf-life of ≤10 days storage at 5°-10°C and a shelf-life of ≤5 days
Industry Code of Practice (CCFRA, 1996)	storage at ≤8°C and a shelf-life of ≤10 days
FSA draft concise guidance document (FSA, 2003)	storage at ≤5°C and a shelf-life of ≤10 days storage at 5°-8°C and a shelf-life of ≤5 days

At its meeting in December 2004, the ACMSF concluded that in the light of the concerns that were raised in response to the consultation, it needed to examine recent scientific evidence before advising on the FSA concise guidance document with respect to recommendations for foods with a short shelf-life where other specific controlling factors cannot be demonstrated (10 day rule). The ACMSF proposed that the FSA commission an independent review of the current scientific evidence, with the findings to be presented at a future ACMSF meeting.

This review aims to provide the ACMSF with all the necessary up to date information to enable them to judge recommendations included in the FSA concise guidance document on “foods with a short shelf-life where other specific controlling factors cannot be demonstrated”. This independent review summarises: (a) sales and properties of chilled VP/MAP foods in UK and overseas; (b) the position in other European countries and internationally with respect to guidance on control of *C. botulinum* in chilled VP/MAP foods; (c) storage and handling of chilled foods; (d) recent outbreaks of foodborne botulism; (e) growth and toxin formation by *C. botulinum* at ≤10°C; (f) re-packaging of chilled VP/MAP foods; (g) risk assessments on *C. botulin*um in chilled VP/MAP foods.

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Chapter two - Practice and Market: VP & MAP Equipment and Chilled Foods Sold in the UK and Overseas

In the UK there are 6.8 $x10^{10}$ eating occasions annually, 79% of which are in the home (MLC/TNS, 2004). The vast majority of home eating occasions are based on food retailed to the consumer, either pre-packed or provided through temperature controlled supply chains, or foodservice (e.g. restaurants, takeaways). According to the 1997 National Food Survey (MAFF, 1998), more than 10^{11} food packages were sold in the UK.

More than 50% of food in developed countries is retailed under refrigerated conditions (Billiard, 2002). A wide range of chilled foods are available in the UK. Many of these are packed under MAP or VP, with others packed under air or an oxygen-containing atmosphere. Even when air is present, some of these foods may contain limited amounts of oxygen within the foods (i.e. the foods are highly reduced), so they may present a risk of foodborne botulism similar to MAP or VP foods. This chapter deals with the manufacture and market of chilled MAP and VP foods, including those packed under air or an oxygen-containing atmosphere

2.1 Equipment for MAP and Vacuum Packaging

There are numerous, well-established manufacturers of VP and MAP equipment, serving different segments including suppliers of primals (VP), large scale manufacturers (MAP and VP), small scale manufacturers and catering butchers (VP primarily), and even domestic users in the home (VP only).

In 2002 there were 7,520 food and drink manufacturing enterprises in the UK, employing 65 people on average, compared with the EU average of 16. The EU average number of employees at meat product companies is 21 (Eurostat, 2006). These figures indicate that on average, UK food manufacturing companies are well-resourced compared with those in other Member States.

In 2004 there were 7,300 independent butchers in the UK, declining at a rate of ~4% per annum (MLC, 2004). Eurostat reports that in 2002 there were 8,221 meat and meat products specialist stores in the UK out of 127,000 in the EU in total (Eurostat, 2006).

The majority of independent butchers in the UK are believed, by equipment suppliers and proprietors contacted during this project, to have VP equipment. This market picture applies internationally, with the exception of artisan producers. For example, in France, 0.3% and 0.7% of artisan producers are reported to use VP and MAP, respectively (Conseil National de Consommation, 2000).

In comparison, sales of MAP equipment are largely restricted to industrial-scale manufacturers in all countries owing to relatively high capital requirements; VP equipment is reportedly available new for *ca.* £1,000, compared with MAP equipment *ca.* £10,000. Total MAP equipment unit sales are reported to be in the order of 3,000 in the UK and there are approximately10 major international MAP equipment producers (Air Products, Personal communication).

From a survey of VP/MAP equipment sales literature and websites, *C. botulinum* is rarely mentioned and VP/MAP equipment manufacturers/suppliers do not as a rule offer comments or guidance to their customers with respect to specific control of this organism. This applies internationally to equipment designed for industrial and small scale manufacture, catering and domestic usage. However, larger industrial VP equipment suppliers and MAP equipment suppliers provide recommendations on gas mixes and example attainable shelf lives under chilled storage and major gas suppliers indicate that *C. botulinum* is a hazard that needs to be controlled.

Larger equipment suppliers also generally offer customers training on the operation and maintenance of their packaging systems. In addition, they offer guidance on controls and disciplines beyond the actual packaging, including hygiene standards and temperature control which would have a potential direct impact on *C. botulinum* and other pathogens.

2.2 Overall chilled market structure

2.2.1 UK

The UK chilled prepared foods market is dominated by a few multiple retailers with their own brands. Generally for retailer own label chilled prepared foods, distribution of products to their Regional Distribution Centres (RDCs) will be done at a temperature no greater than 5°C. These RDCs then supply product directly to Retail Stores.

UK multicomponent chilled prepared foods tend not to contain preservatives except as incidental ingredients in components. In the UK, chilled prepared products are minimally heat processed to retain quality and are given short shelf lives owing to the absence of preservatives. This approach contrasts most starkly with the USA, and to a lesser extent with other European countries, where longer shelf lives are the norm.

In the UK, multiple retailers are also important outlets for raw meat, accounting for 79% of raw meat sales overall and 83% of red meat sales (MLC, 2004). However, in 2002 that there were some 10,000 meat, meat products, fish and seafood retailers in the UK (Eurostat, 2006).

In terms of foodservice, in 2002 there were 1,195,817 restaurants, bars and canteens in the EU, of which 107,739 were in the UK (Eurostat, 2006).

The Food Standards Agency estimates that up to 20,000 new, small catering ventures are started up every year in England and Wales (FSA, 2003).

The UK foodservice sector additionally includes

- More than 10,000 publicly listed and independent hotels with more than 300,000 rooms
- Foodservice management companies with a combined turnover of more than £3x10^9

VP and MAP are also used widely in the UK foodservice sector.

2.2.2 Non-UK

Retail chilled prepared food markets are comparatively undeveloped compared with the UK in terms of variety, volumes and values, but there is longstanding widespread use of VP and MAP, particularly in the protein sectors.

Non-UK chilled food markets are dominated by non-retailer brands and chill chains are largely operated by suppliers (manufacturers).

In 2002 there were 282,668 food manufacturing enterprises in the EU (Eurostat, 2006). In 2002 there were ~120,000 retail outlets for meat, meat products, fish and seafood products in the EU excluding the UK. About 60% of these were in Italy and Spain and a further 14% were in France.

The pattern of foodservice markets internationally mirrors that in the UK.

Retailed chilled meals in the USA are prepared in either a commercial facility such as a factory, a supermarket backroom kitchen, a fast food kitchen (commissary) that may supply several retail outlets or even supplied by nearby restaurants. The lines between retail and foodservice can therefore be blurred. About 60% of US supermarkets were reported to have ‘backroom kitchens’

in 1998 (Brody, 1998). However, such in-store production presents challenges since food scientists/microbiologists are not present, stores are not equipped for high-volume production, and product quality varies from day to day and site to site. Such foods are generally intended for same/next day sale and use.

VP and MAP are widely used internationally. For example, in 2005 an estimated 30,000 (out of approximately 30,600) retail grocery stores in the USA sold VP meat. This included (Food From Britain, personal communication):

- 25,000 stores sold case-ready poultry
- 20,000 stores sold case-ready added-value products
- 10,000 stores sold at least some case-ready ground beef
- 6,000 stores sold case-ready pork
- 1,000 stores sold both beef and pork in a fully case-ready format

The USA meat, poultry and seafood packaging industry amounts to £3.2×10^9 annually. Demand for meat, poultry and seafood packaging is projected to grow by 4% annually through 2009 based on smaller package sizes, more processed cuts and an emphasis on value-added, case-ready packaging. Major increases in sales are expected in “flexible packaging”, “ready to eat foods” and “poultry” (Food From Britain, personal communication).

In the USA chilled prepared food is not equated with "fresh" as it is in the UK/Europe, but instead it is valued for the competitive advantage that can result from an extension of shelf life. US pre-packed chilled foods are invariably long shelf life stabilised products (e.g. 60-90 days is common across the complete range of chilled prepared foods). This is achieved by addition of, for example, preservatives and acidulants. Irradiation is also permitted.

2.3 Main chilled market segments using VP or MAP

The following market segments use VP and/or MAP internationally:

- Meat
    - Raw meat (to be cooked): VP primals, MAP (fresh) and VP (fresh and frozen) retail packs
    - Ready to eat/delicatessen meats: VP and MAP
- Fish and seafood
    - Fresh and cooked fish: mainly MAP
    - Smoked fish: mainly VP (except trout, which is MAP)
- Produce
    - Bagged salads/prepared produce: MAP (UK: iceberg, USA: general)
- Fresh pasta and gnocchi:
    - MAP (low a_w owing to partial drying, not NaCl content)
- Dairy products:
    - Low a_w/pH (matured) cheese: MAP
    - Paneer: VP
- Chilled ready meals:
    - VP/sous vide (continental Europe)

Since packing under an atmosphere containing oxygen (such as air) cannot be relied on to prevent growth and toxin formation by non-proteolytic *C. botulinum*, there are other product types for which non-proteolytic *C. botulinum* can be considered a potential hazard unless effective CCPs are in place:

- Cooked Chilled Bakery Products
    - Chilled bread including those with fillings
    - Chilled dough
- Herbs/vegetables in oil

- Tofu
- Chilled cooked products with extended shelf life

2.4 Recommended generic shelf lives of chilled VP products

International equipment manufacturers' recommended shelf lives for chilled VP foods range from 10 days to 8 weeks, dependent on the product type (Table 2.1).

Table 2.1 International equipment manufacturers' recommended shelf lives for consumer VP foods

VP product	Generic shelf lives
Smoked fish	to 6 weeks, dependent on NaCl/a_w
Fresh red meat	to 20 days
Cooked, cured meat	3-8 weeks
Ready meals	10-30 days

Source: Cryovac (Personal communication)

2.5 Recommended generic shelf lives and gas mixtures of chilled MAP products

International MAP gas suppliers recommended shelf lives for chilled MAP foods range from 4 days to 12 weeks, dependent on the product type (Table 2.2).

- International MAP gas suppliers recommended gas mixtures range from high oxygen to no oxygen, dependent on the product type and whether packs are bulk or retail (Table 2.2).

Table 2.2 Generic international equipment manufacturers' recommended shelf lives, temperatures and gas mixtures for MAP foods

MAP product	Generic shelf lives	Temperature (°C)	Generic gas mixtures
Fresh fish	4-6 days	-1°C to +2°C	**Low fat:** Retail: 30% O_2, 40% CO_2, 30% N_2 Bulk: 70% CO_2, 30% N_2 **High fat/Oily:** Retail: 40% CO_2, 60% N_2 Bulk: 70% CO_2, 30% N_2
Smoked fish	1-3 weeks	0°C to +3°C	Retail: 30% CO_2, 70% N_2 Bulk: 70% CO_2, 30% N_2
Fresh red meat	5-8 days	-1°C to +2°C	Retail: 80% O_2, 20% CO_2 Bulk: 65% O_2, 35% CO_2 (except pork (80% O_2, 20% N_2), venison, wild boar: 80% O_2, 20% CO_2) Primal: 50% CO_2, 50% N_2 (except pork: 80% O_2, 20% CO_2)
Raw offal	4-8 days	-1°C to +2°C	Retail or bulk: 80% O_2, 20% CO_2
Fresh poultry & game	10-21 days	-1°C to +2°C	Retail: 30% CO_2, 70% N_2 Bulk: 100% CO_2
Cooked, cured meat	3-7 weeks Poultry 1-3 weeks Salami etc 4-8m*	0°C to +3°C	Retail: 40% CO_2, 60% N_2 Bulk: 50% CO_2, 50% N_2
Dairy products	2-12 weeks	0°C to +5°C	Hard cheeses, except mould-ripened: 100% CO_2 Grated and soft cheeses except mould-ripened: 30% CO_2, 70% N_2 Aerosol creams (UHT): 100% N_2O Other dairy products: 100% N_2
Fresh pasta	3-4 weeks	0°C to +5°C	Retail: 50% CO_2, 50% N_2
Combination products	3-21 days	0°C to +3°C	Retail: 50-100% CO_2, 0-50% N_2 Bulk: 50% CO_2, 50% N_2
Bakery products	4-12 weeks*	0°C to +5°C	Retail: 50-100% CO_2, 0-50% N_2
Bagged salads	5-35 days, but in practice limited organoleptically	0°C to +3°C	Retail and bulk: 5% O_2, 5% CO_2, 90% N_2
Cooked and dressed vegetable products	1-3 weeks	0°C to +3°C	Retail: 30-50% CO_2, 50-70% N_2
Ready meals	5-10 days	0°C to +3°C	Retail: 30-50% CO_2, 50-70% N_2

Source: Air Products (2006) * may not always be chilled

2.6 UK sales of chilled MAP/VP/low oxygen foods

In the UK more than $6x10^9$ packs of VP/MAP/low oxygen chilled foods are consumed yearly (Table 2.3) and 10^{11} packs are estimated to have been consumed in the UK over the past 25 years. Virtually none are believed to be in compliance with the 1995 ACMSF guidance (5 days at 10°C/10 days at 5°C. This is evidenced by many products having a shelf-life of 10 days or greater without including specific control measures for non-proteolytic *C. botulinum*, and the assumed storage temperature being greater than 5°C for at least part of the chill chain (see Chapter 4). In addition 10°C is higher than the England, Wales and NI maximum temperature (8°C) for chilled foods (Food Law 2006).

Table 2.3 UK Chilled MAP/VP/Low Oxygen Foods - Characteristics and Annual Average Sales

Product	Packing (VP/MAP/Air)	Mean number of packs sold per annum (million)*	Shelf life	UK legal storage temperature (°C)**	Notes
Raw red meat	VP (primals, retail)	1,153	≤ 6m (primals) 13d (retail)	7	High O_2 (70%)
	MAP (retail)		≤10d		
Meat preparations	MAP	912	minced meat: 8d major multiple, 14-21d butcher	4	
Poultry preparations	MAP	327	10d	4	Low O_2
Poultry/products	MAP	256	10d (uncured) 28-35d (cured)	4 (whole) 8(products)	Low O_2
Sliced cooked meat and alternatives	MAP	1,128	10-15d (uncured) 15 to >30d (cured)	8	Nitrite (cured)
Fish and seafood	MAP	700	5-7d (MAP fresh fish) 8-9d (MAP cooked prawns) 21-28d (VP seafood sticks)	On melting ice (unpacked) 8	High O_2 (30%) (fresh fish/seafood and smoked trout)
Smoked fish	VP	15	6-16d	8	Smoked trout not generally VP in UK, NaCl is a key CCP
Mussels	VP (cooked) MAP (live)	2	10d to >21d (VP, cooked, not retorted) 6-9d (MAP, live)	On melting ice (unpacked) 8	
Bagged salads	MAP	71	4-7d	8	Iceberg lettuce
Bagged salads	Air	286	4-7d	8	Excl. iceberg. Film permeability regulates pack atmosphere
Fresh pasta and gnocchi	MAP	86	≤35d (longer for imports)	8	Low a_w (not NaCl), chill
Cooked chilled ready meals	Air – low O_2 in meal	1,185	≤10d, longer for imports	8	10 day rule applied (voluntary)
Total mean number of packs sold per annum (million)		**6,121**			

* Typically based on sales over the last five years

** chill temperature is specified as 8°C in England, Wales and Northern Ireland. In Scotland the requirement is to keep chilled food in “a refrigerator, or refrigerating chamber, or a cool ventilated place”.

Source: Various tables in Appendix one

2.7 International sales of chilled MAP/VP/low oxygen foods

Drawing together information from various sources it is clear that more than 10^{10} packs are consumed per annum outside the UK (Table 2.4). It should be noted that the size of the market and the complexity of the available data makes this figure only a best estimate.

Table 2.4 Examples of international annual average sales of various chilled MAP/VP/low oxygen foods

Product	**VP/MAP/Air**	**Mean number of packs sold per annum (million)**	**Key CCPs**	**Notes**
Raw red meat	VP (primals, retail), MAP (retail)	10 (NZ lamb to EU) 1600 (Germany)	Chill	High O_2 (70% O_2)
Meat/poultry preparations /products	VP/MAP	12 (MAP - Hungary) 36 (VP - Hungary) 4500 (VP - USA)	Chill	Low O_2
Sliced cooked meat	MAP	2500 (USA)	Chill	Nitrite (cured)
Smoked fish	VP	120 (international)	Chill, NaCl	
Mussels	VP (cooked) MAP (live)	2 (EU)	Chill	
Bagged salads	MAP	120 (international)	Chill	
Fresh pasta & gnocchi	MAP	270 (France, Italy)	Low a_w (not NaCl), chill	
Cooked chilled ready meals	VP, Air	750 (EU)	Chill	VP (France, Belgium), Air (low internal O_2)

Source: Industry data (see Appendix one)

2.8 Details of sales and production of raw meat (to be cooked)

- The pre-packed chilled meats sector has existed for some 40 years in industrialised countries. The vast majority of chilled pre-packed meat products are MAP or VP.

- Raw meat primals tend to be VP throughout the world. VP coupled with deep chill, is the basis of the meat export industry.

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

- A typical packing cycle for a non-cured meat product (e.g. pork escalopes) is as follows:
  i) Slaughter (carcasses held overnight at 4°C) – day 1
  ii) Primal cutting and butchery (not more than 10°C) – day 2
  iii) Portions held at 0°C overnight – day 2
  iv) MAP, check weighing and labelling – day 3
  v) Finished packs stored at transported at 0°C

2.8.1 Raw meat in the UK

Annual UK total fresh and frozen red meat (beef, lamb, pork) sales were £2.8x10^9 in 2004, showing annual value growth of 1% (MLC/TNS, Personal communication). Raw red meat pack sales totalled 8x10^9 in the period 1999-2005 (Appendix 1 Table 2).

VP is commonly used in red meat maturation, not necessarily under chilled conditions.

90% of fresh (raw) red meat sold in retail packs are either high oxygen MAP or VP (lamb), with retail shelf lives of 5-7 days (MLC/TNS, personal communication). This equates to 2 x10^6 tonnes or, assuming all is pre-packed, some 8 x10^9 MAP and VP retail packs between 1999-2005 (Appendix 1 Tables 2 and 3).

- In 2004 UK sales of bacon (all VP or MAP) were worth £1.0x10^9 (2x10^5 tonnes), equating to some 8x10^8 retail packs @250g. The annual volume growth rate is 1% (MLC/TNS, personal communication).

Meat preparations (i.e. raw) sold pre-packed are generally MAP, red meat-based being in high oxygen. Some 6 x10^9 packs of meat preparations were sold in the UK from 1999 to 2005, of which low oxygen MAP packs numbered an estimated 2 x10^9 (Appendix 1 Table 4).

Some 1.5x10^9 packs of poultry products were consumed in the UK from 1999 to 2005, comprising MAP (low oxygen) and air (Appendix 1 Tables 4 and 5).

- Shelf lives of raw uncured pre-packed meat sold through UK multiples are generally up to 10 days, being reliant on chilled storage as the key CCP once packed (Appendix 1 Table 6).

- UK butchers use VP to supply other outlets and to a lesser extent for retail packs for direct sale to consumers.

- Shelf lives given by butchers to uncured meat/products/preparations are reported to range from 10 days to 6 weeks or longer under deep chill (Appendix 1 Table 7).

- Meat may be displayed (VP and packed in air) outside butchers shops on tables that may or may not have refrigerated bases.

2.8.2 Raw meat outside the UK

The pattern of VP widespread usage for primals, joints and cured meat is largely as per the UK, with particular emphasis on small producers for VP end products

- Shelf lives are reported to be similar to those in the UK.

- EU imports of chilled VP New Zealand lamb totalled 8x10^4 tonnes in 2004/2005 (Meat & Wool New Zealand, 10/2/06). Shelf lives can be up to 6 months under deep chill. These imports equates to an estimated 10^7 packs per annum.

- South American VP red meat primals are imported into the UK with 3-4 months shelf life, under deep chill.

- In Germany more than 1.6x10^9 packs of fresh red meat were sold in 2003/2004, being MAP or VP (Appendix 1 Table 8).

- As in the UK, MAP is gaining wider usage by larger manufacturers despite the relatively high capital investment since there can be shelf life and quality advantages.

- There were some 127,000 meat and meat product specialist retail stores in Europe in 2002 (Eurostat, 2006). Many of these can be expected to be using VP equipment or selling VP products (Appendix 1 Table 9).

- Some $2.7x10^6$ tonnes of fresh and chilled chicken were consumed in 18 European countries in 2004 (Eurostat, 2006). Approximately 20% of this tonnage was consumed in the UK (Appendix 1 Table 10).

- The USA barcode-labelled packaged meat market was worth £$5.8x10^9$ in the 52 weeks to 16 April 2005. The majority of these products are VP or MAP (IDDBA/ACNielsen, personal communication). USA barcode-labelled packaged meat excluding chilled sliced lunchmeat was £$3.8x10^9$ in the 52 weeks to 16 April 2005 (IDDBA/ACNielsen, personal communication). Using an average value of £2500-5000/tonne retail value, this equates to some 1.6-$3.2x10^6$ tonnes or 3-6 $x10^9$ packs of barcode-labelled packaged meat (based on an average pack size of 500g), either MAP or VP consumed in the USA in 52 weeks to 16 April 2005.

2.9 Ready to eat/delicatessen meats

- In the UK pre-packed sliced cooked meat is MAP (70% market) or VP (30%) (BRC, personal communication)

In the period 1999-2005 more than $7.2x10^9$ packs of sliced cooked meat were sold in the UK. Annual volume growth rate is 3% (Appendix 1 Table 11).

- Some 10 million packs of chilled vegetarian sliced cooked meat alternatives, all reported to be MAP or VP, were consumed in the UK between 1999-2005 (Appendix 1 Table 11).

- Figures for Hungary show consumption of $3x10^5$ tonnes of meat products in 2005, 30% of which were VP and 10% MAP (CCFRA, personal communication). Based on 250g average pack size, this equates to $3.6x10^8$ VP packs and $1.2x10^8$ MAP packs.

- The USA barcode-labelled packaged chilled sliced lunchmeat comprised £$2.1x10^9$ in the 52 weeks to 16 April 2005 (IDDBA/ACNielsen, personal communication). Again using an average value of £2500-5000/tonne retail value, this equates to some 4.3-$8.5x10^5$ tonnes or 1.7-3.4 $x10^9$ chilled sliced lunchmeat packs (based on an average pack size of 250g), either MAP or VP in the 52 weeks to 16 April 2005.

- The ready to eat chilled delicatessen food market is estimated to have had a value of £$1.3x10^{11}$ in 2003 (Europe, Asia-Pacific and the Americas). Chilled delicatessen products were defined as chilled bakery products, chilled meat products, chilled ready meals (including chilled pizza and soup), delicatessen food, fresh fish, seafood and salads, sandwiches and fresh pasta (www.researchmarkets.com). MAP and VP are the preferred packaging technologies for the vast majority of these products by volume, i.e. chilled meat products, chilled fish and seafood (not MAP in USA) and fresh pasta.

- In general, sliced cooked meats sold by major UK multiples have received a heat process of at least 70°C for 2 min, or calculated equivalent (BRC, personal communication) (Appendix 1 Tables 12 and 13).

- Sliced cooked meats' salt contents vary, owing to longstanding recipe variability and more recently to salt reduction activity (Appendix 1 Table 12).

- Delicatessen meat shelf lives (Appendix 1 Table 12) sold through UK major multiples range from 10-15 days (uncured) to 15->30 days (cured).

- Non-UK delicatessen meat shelf lives (Appendix 1 Table 13) range from 2-4 weeks (uncured) to 2-8 weeks (cured).

2.10 Fish and Seafood

- MAP of fish was first reported in the 1930s.

- In 2002, there were 3435 European fish products enterprises (Appendix 1 Table 14).

Trout produced by major UK manufacturers is generally packed with the presence of oxygen (i.e. not VP) in order to control the potential for *C. botulinum* growth and toxin formation. This is not necessarily the case in other countries including elsewhere in the EU.

- Salmon is either dry salted or brined before being cold smoked, the time of salting varying with the size of salmon being cured. Trout and mackerel are brined and then hot smoked, either as gutted whole fish or as fillets.

- Cold smoking processes are dependent on the kiln and smoke type used. Antimicrobial compounds are claimed to be present in smoke.

- Hot smoking forms a pellicle, which provides antimicrobial shielding. The pellicle is removed when the product is sliced, therefore introducing the potential for post-process contamination, which is controlled in practice by high hygiene standards.

- Since neither the smoking nor the drying parts of production processes are particularly severe, non-proteolytic *C. botulinum* can survive and potentially grow in the finished product, thus is it essential that shelf-life is restricted and an appropriate chilled storage temperature maintained (according to a defined HACCP procedure).

- Public taste has changed over recent years, as have methods of curing and NaCl levels used. Salt levels vary by product type, given shelf life and country (Table 2.5). UK-sold cold smoked salmon salt contents were found in this survey to range from 2.2-3.5%, and shelf lives from 10-16 days (Table 2.5). The MAFF (1991) study of 'The Microbiological Status of Some Mail Order Foods' reported salt levels ranging from 3.29-8.11% and shelf lives from 11-20 days.

- While it is notable that many seafood products' gastrointestinal tracts are often consumed (e.g. prawns, shellfish) these are not, with certain exceptions, eaten raw, therefore minimising risk of exposure to pathogens and their toxins through this particular route, depending on the heat process used.

Table 2.5 Details of seafood products sold in the UK

Product	VP/MAP	NaCl	Shelf life (chilled)	Process	Notes
Cold smoked salmon	VP	Aqueous >3.5% from top to bottom of salmon side	16 days	22-30°C, 12-24h	UK major multiple
		unknown	1-6 weeks		International (range)
	VP or MAP	3%	10 days	22-30°C, 12-24h	UK major multiple
Cold smoked salmon side	VP	2.2%	≥14 days	22-30°C, 12-24h	UK: Sold on eBay. 'Despatch overnight by express carrier'
Hot smoked salmon	VP	salt + sugar added: not shelf life critical	9 days	≥74°C centre	UK major multiple
Hot smoked mackerel	MAP	1.5-2.5% aqueous	6-9 days	72°C/2 mins or 66°C/10 mins	UK. Shelf life limited to control scombrotoxin
	VP	1.75%	13 days		
Cold smoked trout	MAP (10% O_2, 50% N_2, 40% CO_2)	Aqueous >3.5% from top to bottom of salmon side	16 days	22-30°C, 12-24h	UK. Shelf life limited in practice by organoleptic quality
Cooked prawns	MAP (30:70 CO_2:N_2 or 40:60 CO_2:N_2)	1%	8 days	Equiv to 70°C/2 mins	UK. Alternatively use MAP with up to 10% O_2 to prevent syneresis
Cooked prawns (shell-on or peeled)	MAP	1.5%	9 days	Equiv to 70°C/2 mins	UK major multiple
Live mussels	MAP	none added	6-7 days 8-9 days	None	Export from NL Canada
Cooked mussels	VP (cooked in bag)	1.2%	10 days	Equiv to 70°C/2 mins	UK: Bought frozen by brand owner, sold on defrost
			≥14 days	Equiv to 70°C/2 mins	UK major multiple: NL import.
			≥21 days	Equiv to 70°C/2 mins	UK retail: EU imported.
			1 year	Retort process	New Zealand
Seafood sticks	VP	1%	21-28 days	90°C/10 mins	Bought frozen by brand owner, sold on defrost

Source: Industry data

Chilled fish accounts for some 40% of the value of the UK's £2x10^9 fish market. Pre-packaged chilled fish accounts for 71% of all fish, representing some 7x10^8 packs annually (SFIA, 2006).

- All chilled pre-packaged unprocessed fish sold in the UK is MAP, whereas that sold processed is primarily VP (except trout) (SFIA, 2006).

2.10.1 Smoked Fish - UK

More than 2.5x10^8 packs of chilled VP and MAP seafood and smoked fish were consumed in the UK in 2003-2005. Of these, slightly less than half were VP smoked fish (Appendix 1 Table 15).

- The Scottish smoked salmon industry is characterised by a large number of small smoke houses and only a small number of major producers. In 2002, the 10 largest producers accounted for 88% of Scottish production while the smallest 30 smoke houses (half the total number of producers) collectively accounted for only 1% of the total Scottish output. (Source: Industry-commissioned research)

- In 2002, hot smoked salmon accounted for only 4% of sales of Scottish salmon by value, a variety of cures made up 3% and the remainder was cold smoked in various formats (Appendix 1 Table 16).

- 69% of Scottish smoked salmon sales were in the UK in 2002. The majority of these sales were as VP retail packs (industry data)

- Sales of Scottish smoked salmon in the UK are predominantly via major multiples (78% in 2002) (Appendix 1 Table 17).

- Sales of mail order Scottish smoked salmon in the UK between 1995 and 2002 were between 2-3% of the total sold by value, amounting to approximately £3x10^6 in 2002 (Appendix 1 Table 17).

2.10.2 Smoked Fish - Non-UK

Since 1983 an estimated 2.8 x10^9 packs of smoked salmon (VP) have been consumed globally (Appendix 1 Table 18).

- In 2004 in EU Member States some 2.3x10^9 packs of smoked salmon (VP) were consumed (Appendix 1 Table 19).

- Reported shelf lives within the EU (non-UK) for chilled VP ready to eat salmon and pre-packaged trout range from 14-50 days, with reported salt levels ranging from 2.0-3.3%, sometimes with added sugar (unspecified amounts). Cold smoked salmon shelf lives outside the UK are reported up to 6 weeks (Appendix 1 Table 20). Details of shelf lives of hot- and cold-smoked and gravad fish in Sweden are given in Appendix 1 Tables 21 and 22.

- Salmon is not necessarily the most consumed cold-smoked fish in every country. For example, in Finland, annual VP cold smoked rainbow trout volumes are comparable with those of cold smoked salmon in the UK despite the population being less than 10% of that of the UK.

2.10.3 Mussels

Prepared mussel imports into the EU in 2002-4 totalled €2.5x10^8, equating to more than 5.6x10^8 packs assuming 500g pack sizes (Appendix 1 Table 23).

- VP cooked mussels on retail sale in the UK were found to have salt contents of 1.2-3.5% NaCl and shelf lives ranging from 10 to >21 days using heat processes that do not give a calculated 6-log reduction of spores of non-proteolytic *C. botulinum* (Table 2.5).

- 83% of mussels consumed in the EU were from the 25 Member States, and the remainder from Chile (9.4%) and New Zealand (4.4%) (Eurostat, 2005).

- VP Mussel consumption in the UK in 2003-5 totalled 1.6x10^7 packs (Appendix 1 Table 24).

- The Netherlands is a major exporter of live MAP mussels, the shelf life of which is 6-7 days. Dutch exports are in the order of 5x10^4 tonnes per year, equivalent to 1x10^8 packs (500g MAP) per year (Appendix 1 Table 25).

2.11 Bagged leafy salad and prepared produce

Since 1990 some 2.6 x10^9 and 1.8x10^{10} packs of bagged leafy salads have been consumed in the UK and globally, respectively (Appendix 1 Table 26).

- Bagged prepared lettuce sales at retail began in the USA between 1990 and 1993. In the USA average pack size increased from ~1 kg in 1984 to ~1.8 kg in 1990 as foodservice uptake increased. Since 1990 average pack size has reduced to 1.6 kg as consumer packs become increasingly popular. Sales in the USA increased significantly between 1984 and 1993 (Appendix 1 Table 27).

- Industry figures for sales of bagged salads in 1999 and 2004 show an average 75% growth in consumption in seven major international markets (Appendix 1 Table 28).

- In Hungary (2005), fresh cut vegetables (potato, tomato, green pepper, mixed vegetables for soups, carrot, white carrot and onion) totalled 9x10^5 tonnes, with an average pack size of 1 kg, approximately 1 million packs. Half of these were MAP, the remainder being packed in air (CCFRA, personal communication).

- Approximately 20% of UK bagged salads are MAP (iceberg lettuce), the remainder respire to produce reduced O_2 modified atmospheres over their shelf life. Non-MAP packs use packaging material allowing transfer of gases arising from the respiration of the packaged produce, resulting in a naturally modified atmosphere. Such packaging material permeability is a factor affecting shelf life, which is limited in practice by organoleptic quality rather than food safety.

- UK and EU bagged salad shelf lives are 4-7 days, whereas in the USA shelf lives are commonly in the order of 3 weeks (industry data).

- In the USA there is widespread usage of MAP for fresh-cut produce, not only for iceberg.

- Low oxygen MAP is recommended by US industry guidance for fresh-cut produce, but no shelf life maxima are specified (Appendix 1 Table 29).

2.12 Fresh Pasta and Gnocchi

More than 6.6 x10^9 packs of fresh MAP pasta have been consumed in the UK, France and Italy since 1989 (Appendix 1 Table 30).

- Some 4.8 x10^9 packs of MAP fresh pasta and gnocchi were consumed in Italy alone from 1991 to 2004 (Appendix 1 Table 31).

- Residual oxygen in MAP packs is often >1%

- Preservatives cannot be added to plain fresh pasta under EU additives legislation (95/2/EC).

- Sorbate salts (calcium or potassium) or sorbic acid can only be used in the filling of the fresh pasta, at a maximum concentration of 1,000 mg/kg. However, its usage has almost disappeared from the Italian market in fresh pasta, being replaced with pasteurisation in pack or use of hurdle technologies. Gnocchi can contain sorbate.

- Hurdle technology is often used in fresh pasta. However, predictive models do not take account of a_w reduction resulting from partial drying, but only from NaCl levels (and thus predictions from existing models are very failsafe).

There are various national guidance/rules regarding the composition of fresh pasta, with a LACOTS agreement from 1996 limiting shelf life in the UK (Appendix 1 Table 32).

2.13 Dairy products

- MAP is used widely in hard cheese packaging. For example, cheddar has a 3 month shelf life, 1.75% NaCl, pH control through maturation, resulting in *C. botulinum* control

- VP is used with paneer. This is produced by the acidification and heating of milk, traditionally produced on the day of consumption. The UK's largest industrial producer combines a heat treatment of 95°C/10 min plus acidification to achieve a shelf life of 56 days in VP. There are reported significant volumes arising from localised manufacture and distribution through small shops. There are also sales of paneer over the internet (eBay), with a quoted shelf life of 4 days.

2.14 Retailed chilled ready meals

- Retailed chilled ready meals are produced using a wide range of processes, packaging technologies and shelf lives. There are thousands of different recipes on sale in the UK. Most of the chilled ready meals sold in the UK are packed under air, but may have a low O_2 concentration within the food, and the risk presented by non-proteolytic *C. botulinum* should be considered similar to that in MAP/VP foods.

More than 1.5x10^{10} pre-packaged chilled ready meals have been consumed in the EU in the last 20 years, all of which had shelf lives >5 days (Appendix 1 Table 33).

- The chilled ready meals produced in France are principally long shelf life VP (sous vide) with approximately 4.4 x10^9 packs sold between 1990 and 2005 (Appendix 1 Table 33). Details of the French chilled ready meal segmentation in 2004 are shown in Appendix 1 Table 34.

Some 6 x10^9 chilled ready meals (45%) sold over the last 20 years or so had shelf lives >10 days but had not necessarily been subjected to a 6 log non-proteolytic *C. botulinum* spore heat process (non-UK) (Appendix 1 Table 33).

- In 2004 alone, some 1.9x10^9 chilled ready meals were consumed in eight major markets in the EU, of which the UK is the largest (Appendix 1 Table 35).

2.14.1 Chilled ready meals in the UK

- Typical production timeframe for a UK own label chilled ready meal:
  i) Assembly of ingredients/production (not more than 5°C): day 1
  ii) Pre-distribution storage (not more than 5°C): day 1
  iii) Despatch to retailer Regional Distribution Centre (not more than 5°C): day 1-2
  iv) Distribution to individual retail store (5°C): day 2-3
  v) Merchandised in retail store <8°C: day 2-3

- The UK is the largest chilled ready meal market in the world, with more than 8 x10^9 chilled ready meals consumed in the last 20 years.

- MAP or VP is rarely used.

- Ready meals are primarily pre-cooked and then either cold or hot filled and sealed (non-hermetic) containers.

- The 10 day rule at 8°C (CCFRA, 1996) is largely adhered to by major multiples for their own brand products (in view of the low oxygen content of foods), but it may not be adhered to by others (e.g. discount retailers).

- Generally 1% NaCl maximum.

- There are imports of ready meals and other unpreserved/minimally processed chilled foods from continental Europe with longer shelf lives and no apparent food safety hurdle other than chilled storage, e.g.
  - pizzas 15 days (Germany) (air)
  - pancakes 30 days (Netherlands) (MAP)

2.14.2 Chilled ready meals in Belgium

- An estimated 14 million chilled ready meals were sold in 2004

- Two approaches are used by major producers, accounting for a reported 20% of production companies:
  - Post-pack pasteurised calculated equivalent to 90°C for 10 min. Shelf life 3 weeks at 3°C followed by 3 weeks based on 5°C, i.e. 6 weeks total.
  - Components are cooked to 90°C for 10 min, then cold assembled and lidded. No products have >10 days shelf life unless there are additional hurdles.

2.14.3 Chilled ready meals in Finland

- Finland is the biggest Scandinavian ready meal market. Pre-packed chilled ready meals have been available since the 1950s. An estimated 2.4 x10^9 chilled ready meals have been consumed since 1984.

- Finnish chilled ready meals are either cooked in (open, unlidded) packs or cold filled. Those not having undergone a 6 log non-proteolytic *C. botulinum* process (i.e. 90°C for 10 mins or calculated equivalent) typically have 14 days shelf life, based on 2-3 days at 2°C and 11-12 days at 6°C.

2.14.4 Chilled ready meals in France

- Chilled ready meals in France are as a rule sous vide products that are packaged under vacuum and cooked in pack by the producer, and given a shelf life of several weeks (Appendix 1 Table 34).

4.4 $x10^9$ sous vide chilled ready meals have been consumed in France since 1990.

- From the mid-1970s to late 1980s calculated 6-log non-proteolytic *C. botulinum* reduction heat processes were not used even for long shelf life products.

An estimated $5x10^8$ chilled (sous vide) ready meals since 1990 were fish-based, which are reported to still not be subjected to a 90°C/10 mins heat process.

- The approach used by industry to produce chilled ready meals has evolved since 1977 (CCFRA, 1992):
  - 1977-88 industry approach (21-42 days shelf life)
    - Post-pack treatment with Pv100 (equivalent to 90°C for 1 min, i.e. calculated 0.6 log reduction in non-proteolytic *C. botulinum*) and core temperature 57-65°C, or
    - Post-pack treatment with Pv100-1000 (equivalent to 90°C for 1-10 min, i.e. calculated 0.6-6 log reduction in non-proteolytic *C. botulinum*)
    - ≤42 days allowed following storage trials at 3°C, and 3°C for 2/3 of the shelf life followed by 8°C for the remaining one third of shelf life and microbiological and organoleptic analyses.

  - 1988-2000+ industry approach (SYNAFAP, personal communication):
    - ≤21 days shelf life using post-pack treatment with Pv>100 (equivalent to >90°C for 1 min, i.e. calculated 0.6 log reduction in non-proteolytic *C. botulinum*) and core temperature >65°C
    - 21 to 42 days shelf life using post-pack treatment with Pv>1000 (equivalent to >90°C for 10 min, i.e. calculated 6 log reduction in non-proteolytic *C. botulinum*) and core temperature >70°C

  - 2005 industry approach (SYNAFAP, personal communication):
    - No specific shelf life rules – it is for the manufacturer to ensure safety for the shelf life given
    - Shelf lives are several weeks.
    - A French industry survey of practices and CCP controls used is underway at the time of writing.

- Chilled product examples:
  - Fish-based ready meals are reported by the industry not to comply with the 90°C/10 mins approach, while meat-based meals do (SYNAFAP, personal communication).
  - Ready meal shelf lives up to 44 days (sous vide) using Pv ≥3000 (at least 90°C for 30 mins)
  - MAP savoury filled pancakes: heated 73-80°C centre temperature. Shelf life 30 days
  - VP soft poached eggs: 31 days at <4°C. For the albumen to be cooked and the yolk to remain runny requires a heat process of between 62-65°C. To achieve a 6 log reduction in non-proteolytic *C. botulinum* would require the heating at these temperatures for some

18 days. The now-revoked UK Egg Products Regulations 1993 required 64.4°C for a minimum of 2.5 minutes or another time/temperature combination to achieve the same degree of destruction of vegetative pathogens, and then cooled as quickly as possible to below 4°C. New EU legislation (853/2004) requires processing as quickly as possible to eliminate microbiological hazards or to reduce them to an acceptable level, followed by storage at no more than 4°C. No specific mention is made of sporeformers as a hazard.

2.14.5 Chilled ready meals in Germany

- The German chilled ready meal market was worth 1.5M DM (£0.5M) in 1994, having shown 70% growth between 1989 and 1994. (Source: CMA)

- In 2002-2003 some 17 million complete chilled ready meals were consumed (Appendix 1 Table 36).

- Ready meals' shelf lives are 1-3 weeks, using in-pack pasteurisation.

2.14.6 Chilled ready meals in Hungary

- Chilled ready meals production in 2005 was $3x10^3$ tonnes, of which 5% was MAP (equivalent to 450,000 packs), the remainder being in packed in air (CCFRA, personal communication).

2.14.7 Chilled ready meals in Netherlands

- Three approaches are used by major producers:
  - Post pack pasteurised calculated equivalent to 90°C for 10 min. Shelf life 3 weeks at 3°C followed by 3 weeks based on 5°C, i.e. 6 weeks total
  - Ready meal shelf lives are 4 weeks when using a thermal process calculated to be equivalent to 90°C for 10 min. Longer shelf lives are given for specific chill chains.
  - Components are cooked to 90°C for 10 min (or equivalent), then cold assembled and lidded. No products have >10 days shelf life unless there are additional hurdles.
- Chilled MAP pancakes (plain, ~2mm thick) have a 30 days shelf life

2.14.8 Chilled ready meals in Australia

- Retailed ready meals (cold filled and lidded) are on sale with 14 days shelf life through major multiples reportedly without including a non-proteolytic *C. botulinum* control measure.

2.14.9 Chilled ready meals in USA

- US industrially-produced chilled prepared foods are invariably long shelf life stabilised products, with 60-90 days common across the complete range of chilled prepared foods
- Safety is dependent on the addition of preservatives, acidulants and by other means.
- The US pre-packed chilled ready meal market is relatively undeveloped, but
  - Showed growth of 39% from 1999 to 2003
  - Was estimated to be worth some £570 million in 2004, equating to approximately 250 million packs annually.
  - Factors contributing to the relative lack of development of the US chilled ready meal market include the greater frequency of eating out of the home, the large transportation distances involved and the relative lack of investment in the chill chain.

2.15 Other chilled foods

2.15.1 Chilled Bread (packed in air)

- UK chilled bread products:
  - £108M market (2004), equivalent to ~60 million packs
  - Garlic butter and other fillings are applied after baking.
  - Example products/shelf lives (February 2006):
    - 12 days garlic baguette 0.75% NaCl
    - 13 days garlic baguette 1.25% NaCl
    - 6 days garlic ciabatta 1.25% NaCl
    - 5 days garlic bread slices 1.5% NaCl
  - Measured pH and a_w (March 2000):
    - Garlic baguette: pH 5.87, a_w 0.95 (1 sample)
    - Garlic bread: pH 5.65, a_w 0.94 (mean of 3 samples)
    - Italian style garlic ciabatta: pH 5.45, a_w 0.94 (mean of 3 samples)

- Australia chilled bread:
  - Garlic bread 16 days
  - Herb bread 16 days

2.15.2 Chilled Dough (packed in air)

- No preservatives. Chilled storage is the key CCP once packed.

- Example compositional parameters (UK):
  - pH 4.9-8.0
  - 0.7-1.2% NaCl
  - 3.5-4.0% NaCl (aqueous)
  - 19-28% moisture
  - a_w 0.93-0.94,
- On average some $1.3x10^9$ packs of chilled dough are sold annually in the major international markets, with shelf lives ranging from 18-90 days
  - UK. £108M market (2004), representing ~$1x10^8$ packs. 18-20 days shelf life, 1% NaCl as sold to the consumer.
  - France. Some $1.4x10^9$ packs of chilled dough are estimated to have been sold between 1990 and 2004 (Appendix 1 Table 37).
  - Germany. 32 million packs of chilled dough estimated to have been sold in 2002 and 2003
  - Italy. $3.5x10^8$ packs of chilled dough (including pizza bases) were sold between 1995 and 1997.
  - USA. $1x10^9$ market (2004), equivalent to ~$1x10^9$ packs. Shelf life 90 days.

2.15.3 Tofu

- Chilled tofu is available pre-packed in water in major UK retailers, with a shelf life of >40 days. NaCl is present only at trace amounts and the safety of the product is reliant on the production process, which for a major producer includes a 90°C/10 mins heat process. Pack labelling states “Can be kept unopened until the use by date at 4°C in the refrigerator. Once opened can be kept in a refrigerator for 4 days in chilled water, changed daily”.

- Pre-packed chilled tofu is also widely available in specialist UK stores, e.g. oriental supermarkets, but process details are not available.

- In Australia, pre-packed tofu is sold by major retailers with 2 months chilled shelf life

2.15.4 Herbs/Vegetables in Oil

- Major UK multiples selling these products require the use of a control measure such as low a_w (dried herbs), pH control or the addition of preservative.

- Precise information is not available regarding the production of herbs/vegetables in oil not sold through major UK multiples. However, one SME now producing mixtures of pure essential oil and olive oil reported that he had discontinued sale of herb/vegetable in oil products since restaurants made their own (often apparently stored ambient) and did not wish to buy these products ready made.

- During the period of this study a range of recipes for these products were found on websites targeting consumers, few referring to the need to chill the product and to limit shelf life.

- One UK recipe, now removed from the internet at the request of the author, was as follows:
  - Stuff sprigs of your chosen herb (or a combination) into a jar and fill with the oil.
  - Let it sit on the window sill for several days to allow the flavour to transfer.
  - Drain and keep the herb-flavoured oil in a clean jar, replacing the old herbs with a fresh sprig for an attractive look.
  - Store the oil in a dark cupboard instead of in the sunlight.
  - You can also add peeled, whole garlic cloves and or chillies to add to the herb flavours.

  Use the herb-scented oil to make salad dressings or drizzle directly into soups, stews, sauces and marinades.

- Articles on the production of oil infusions also appear in magazines. For example instructions on an easy way to add a taste of the Mediterranean to salads and roasted vegetables appeared in a recent edition of a magazine. The article was accompanied by a photograph of the final product (rosemary in oil), and made no mention of the risk presented by *C. botulinum*. Instructions comprised:
  - Warm some extra virgin olive oil in a pan with freshly picked, clean rosemary stalks.
  - Wash bottles or jars in warm, soapy water to sterilise, then place in a moderate oven for five minutes.
  - Leave oil until completely cool, then decant into the sterilised bottles.
  - Makes a lovely gift for friends and family and will last for a number of weeks. Choose the prettiest glass containers you can find to make the most of your present.

- The FSA notes that recipes for flavoured oils can be found in cookery books, magazines and websites, and recognises these might not have considered the risk of botulism. The FSA go on to state that if you make your own flavoured oil, the safest option is to make a small quantity and use it on the day you have made it. If you have some oil left over, put it in the fridge straight away and use it within a week (http://www.eatwell.gov.uk/asksam/keepingfoodsafe/asksamstoringpreparing/).

- Following outbreaks of botulism in USA and Canada in relation to a garlic in oil product that was labelled 'keep refrigerated' but which had been kept at room temperature, guidance was developed in the USA (FDA, 1993) and Canada (Health Canada, undated) targeting domestic and commercial production. This is reproduced widely/referred to on North American-hosted websites, e.g. limiting chilled shelf life to 1-2 weeks, discarding after 2 hours at room temperature.

2.16 Internet and Mail Order Foods

- Estimated sales of food and drink over the internet in 2002 were £7.7×10^8, which was less than 1% of all food and drink sold in the UK (FSA Board Paper Note 03/11/03, 2003).

- Sales data are not available for other distance selling methods such as standard mail order, which is exempt from legislated specified temperature requirements.

2.17 Farm Market Sales of VP/MAP foods

- VP is used widely by small scale business operators including those selling through farmers' markets. However, market data for VP/MAP product sales via farmers markets are not available. The National Farmers' Retail & Markets Association (FARMA, 2005a) estimates that the turnover of UK farmers' markets was ~£$2x10^8$ in 2004 and could grow threefold within an unspecified timeframe.

- FARMA also report (FARMA, 2005b) that there were more than 500 farmers' markets in 2005, 'of which around half are Certified, or in the process of being Certified, as genuine farmers' markets by FARMA'. In 2005 the number of farm shops was reported by FARMA as being around 3,500, and box-schemes 300.

2.18 Domestic VP

- Home VP equipment is widely available e.g. over the internet and through TV advertisements.

- From a survey of web-based advertising information there is as a rule no mention of *C. botulinum* as being a particular hazard with VP foods.

- According to Foodsafe (2001), 'FDA alerted the Consumer Product Safety Commission (the responsible Federal Agency) that these small appliances, if used incorrectly, would surely lead to botulism deaths among those vacuum packaging things from their gardens. CPSC then insisted that the instructions accompanying the equipment must contain appropriate warnings and guidance to prevent food poisonings.'

- A non-UK company specialising in small scale VP equipment, particularly for domestic use claims that 'vacuum packaged foods last 3-5 times longer than normal' but does not state under what conditions. On querying this, additional but unclear information was given by email:
  "For short term storage (under 7 days, depending on the use by date on the package), refrigerated vacuum packaged meats will last 3-5 times longer than when using a normal storage method. However, to store meats for a longer length of time we recommend freezing and storing the VP item in your freezer until ready to defrost for use. Fresh fish should be cooked or frozen within 24-48 hours, fresh meat 3-4 days if refrigerated. It is difficult to predict how long foods will retain their top quality flavour, appearance or texture because it depends on age and condition of the food on the day it was vacuum packaged. Vacuum packaging does not replace refrigeration or freezing of perishable items."

- No information is available regarding the level of uptake of this equipment beyond reference on the website to various celebrity users. However there are no restrictions on the sale of this equipment nor provision of guidance by the company prior to purchase.

2.19 Conclusions

Approximately 6 x 10^9 packs of chilled food are sold in the UK each year, and it is likely that more than 10^{11} packs have been sold over the last two decades. The range of chilled foods is extensive, and includes raw meat and poultry, ready-to-eat/delicatessen meats, fresh and

cooked fish, smoked fish, bagged salads and other prepared produce, fresh pasta and gnocchi, dairy products (including paneer), chilled ready meals, and cooked/part-cooked bakery products and dough. These chilled foods are packed under MAP, VP, air and other atmospheres containing oxygen. Even when air/oxygen is present above the food, there may be little oxygen within the food, and these foods should be considered to present a similar botulism risk as MAP or VP foods.

In the UK, chilled prepared foods are dominated by a few multiple retailers with their own brands. In order to maintain fresh-like quality, preservatives are rarely added.

The ACMSF (1995) rule of 10 day at 5°C/5 day at 10°C is not adhered to any significant extent in the UK or elsewhere. The 10 day rule at 8°C (CCFRA, 1996) is only adhered to by major producers in the UK and Benelux markets. In some other countries (e.g. France, Finland), chilled products have been produced, over several decades, with shelf lives greater than 10 days. Many of these products will not have received a 6 log non-proteolytic *C. botulinum* process or any of the other control measured specified by the ACMSF (1992).

It is estimated that more than 1.5×10^{10} pre-packed chilled ready meals have been consumed in the EU in the last 20 years. All of these products had a shelf life greater than 5 days, and many had a shelf life greater than 10 days without receiving a 6 log non-proteolytic *C. botulinum* process or any of the other control measured specified by the ACMSF (1992). The UK chilled ready meal market is the largest in the world. In 2004, 1.0×10^{9} packs were consumed in the UK, 4.5×10^{8} in France, 1.8×10^{8} in Germany, 1.2×10^{8} in Finland, and 7.5×10^{7} in Italy.

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2.21 Appendix 1 – Supplementary information for Chapter two (Practice and Market: VP & MAP Equipment and Chilled Foods Sold in the UK and Overseas)
(For references see section 2.20)

Table 1 (Appendix 1): UK Consumption of VP/MAP/low Oxygen Chilled Foods

Product	VP/MAP/Air	Period	Packs (million)	Key CCPs	Notes
Raw red meat	VP (primals, retail) MAP (retail)	1999-2005	7,939	Chill	High O_2 (70% O_2)
Meat/poultry preparations	MAP	1999-2005	6,387 2,292	Chill	Total Low O_2
Poultry/products	MAP	1999-2005	1,576	Chill	Low O_2
Sliced cooked meat + alternatives	MAP	1999-2005	7,232	Chill	Nitrite (cured)
Fish and seafood	MAP	1999-2005	4,900	Chill	High O_2 (30% O_2) (fresh fish/seafood and smoked trout)
Smoked fish	VP	2003-2005	99	Chill, NaCl	Smoked trout not VP
Mussels	VP (cooked) MAP (live)	2003-2005	16	Chill	
Bagged salads	MAP	1985-2005	500	Chill	Iceberg lettuce
Bagged salads	Air	1985-2005	2,000	Chill	Excl. iceberg. Film permeability regulates pack atmosphere
Fresh pasta & gnocchi	MAP	1989-2005	600	Low a_w (not NaCl), chill	
Cooked chilled ready meals	Air – low O_2 internally	1986-2005	8,293	Chill	10 day rule applied
Total number of packs			**41,483**		

Source: composite data

Table 2 (Appendix 1): UK Raw red meat sales (1999-2005)

Raw red meat	Total tonnes	Total number of packs
Beef	$5.6x10^5$	$4.2x10^9$
Lamb	$2.1x10^5$	$1.2x10^9$
Pork	$1.2x10^6$	$2.5x10^9$
Veal	$7.0x10^2$	$2.9x10^6$
TOTAL	$2.0x10^6$	$7.9x10^9$

Source: Derived from MLC/TNS (2006)

Table 3 (Appendix 1): Beef and Pork – VP/MAP usage prevalence and markets (UK)

Meat	VP/MAP split (%)	Market split		
		Further processing	**Retail**	**'Corner shops'**
Beef	Overall: 30% VP, 70% MAP Self service/case ready 80% MAP	Retail/further processing: 80% Catering: 19% (mainly VP frozen)		20% (not MAP)
Pork	Overall: 80% VP, 20% MAP	Industry: 60% Catering: 8% (mainly VP frozen)	23%, of which 92% (MAP) Self service/ case ready: 5% (20% inc back of store prep) Over the counter sales (no MAP): 3%	8% (not MAP)

Source: Derived from Cryovac (Personal communication)

Table 4 (Appendix 1): UK Sales of Meat Preparations 1999-2005

Meat preparation	**Total number of packs**
Fresh beef preparations	$1.0x10^9$
Fresh chicken preparations	$1.6x10^9$
Fresh Lamb preparations	$2.7x10^8$
Fresh Mixed meat preparations	$8.5x10^8$
Fresh Pork preparations	$2.0x10^9$
Fresh Turkey preparations	$3.9x10^7$
Fresh Other Poultry preparations	$3.1x10^8$
Fresh Veal preparations	$4.2x10^6$
Fresh Other Meat preparations	$3.1x10^8$
Totals	**$6.4x10^9$**
Red meat-based (High oxygen MAP)	$4.1x10^9$
Other (low oxygen/VP)	**$2.3x10^9$**

Source: Derived from MLC/TNS (2006)

Table 5 (Appendix 1): Fresh Poultry Products Consumption in UK (1999-2005)

Poultry product	Number of packs consumed per year (million)							
	1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**1999-2005**
Fresh Chicken:								
Chilled Bitesize Snacks	16.9	24.5	24.7	27.5	29.4	37.8	37.4	**198.2**
Chilled Main Meal Accompaniments	74.8	86.4	96.1	110.3	118.8	135.3	125.5	**747.2**
P/P Meat & Pastry	42.0	42.8	41.9	42.3	45.2	46.1	47.8	**308.1**
Processed Poultry	17.5	18.6	20.2	22.8	23.4	21.9	22.2	**146.6**
Fresh Turkey:								
Chilled Bitesize Snacks	0.2	0.4	0.7	0.8	0.8	1.0	1.6	**5.5**
Chilled Main Meal Accompaniments	25.9	19.9	15.7	17.6	16	13.5	12.2	**120.8**
P/P Meat & Pastry	0	0	0	0	0	1.8	2.7	**4.5**
Processed Poultry	2.6	2.0	2.1	1.4	1.9	1.2	1.1	**12.3**
Fresh Other Poultry:								
Chilled Bitesize Snacks	0.6	0.4	0.4	0.3	0.4	0.7	0.7	**3.5**
Chilled Main Meal Accompaniments	1.8	1.6	2.2	2.7	2.8	3.4	4.1	**18.6**
P/P Meat & Pastry	2. 4	2.5	1.8	0.8	1.1	0.2	0.8	**7.2**
Processed Poultry	0.2	0.1	0.1	0.1	0.6	0.3	0.1	**1.5**
TOTALS	**182.5**	**199.2**	**205.9**	**226.6**	**240.4**	**263.2**	**256.2**	**1574.0**
% Growth		9	3	10	6	9	-3	

Source: Derived from MLC/TNS (2006)

Table 6 (Appendix 1): VP/MAP Usage and Example Raw Pre-packed Meat Shelf Lives and CCPs (Major UK Multiples)

Raw Meat Type	**Packaging**	**Shelf life**	**Key CCP**
Fresh chicken (raw)	Air or MAP	10 days	Chilled storage
Fresh poultry meat	majority MAP, remainder air	10 days	Chilled storage
Poultry preparations (par-cooked, breaded)	MAP	10 days	Chilled storage
Minced meat	MAP	8 days	Chilled storage
Cured	MAP VP	28 days 35 days	Nitrite and chilled storage

Source: Industry data

Table 7 (Appendix 1): Characteristics of Meat Sold by UK Butchers

Product	VP/MAP	Shelf life	Key CCPs
Primals	VP	3-4 m (South American beef) Up to 6m (NZ lamb)	Deep chill (≤3°C)
Fresh raw meat	VP	Up to 6 weeks	Chill
Red meat preparations	VP	14-21 days	Chill
Cooked joints	VP	10-21 days	Chill
Cured meat	VP	15-25 days	Nitrite, chill
Cured cooked meats	VP	Up to 6 weeks	Nitrite, chill

Source: Industry data

Table 8 (Appendix 1): German Meat/Products Market 2003-2004

Product type	Weight (k tonnes)		% change	Packs (million)*		
	2003	2004		2003	2004	Total
Meat products inc sausage	**1,277**	**1,269**	**-0.6**	**2,554**	**2,538**	**5,092**
Fresh meat	**1,049**	**1,005**	**-4.4**	**818**	**784**	**1,602**
Beef	177	179	0.8	138	140	278
Pork	725	671	-7.4	566	523	1,089
Beef/pork mixed	118	128	8.0	92	100	192
Veal	12	12	0	9	9	19
Lamb/mutton	17	15	-9.5	13	12	25
Poultry	**372**	**381**	**2.3**	**290**	**297**	**587**
Total	**2,698**	**2,655**	**-1.6**	**3,662**	**3,619**	**7,281**

Source: GfK-Haushaltspanel in Auftrag der ZMP/CMA (2005)
* pack nos. based on 500g average for fresh meat, and taking into account (Food From Britain, personal communication) information that 39% of red meat is sold pre-packed in Germany. 39% pre-pack level also used in poultry figures.

Table 9 (Appendix 1): European Meat and Meat Products Specialist Retail Stores (2002)

Country	No. retail Stores	Country	No. retail Stores
Austria	337	Luxembourg	109
Belgium	4,743	Hungary	1,605
Cyprus	621	Malta	360
Denmark	736	Netherlands	3,095
Estonia	21	Poland	7,191
Finland	80	Portugal	6,792
France	18,027	Slovenia	192
Germany	4,377	Slovak Republic	29
Ireland	1,153	Spain	33,819
Italy	35,186	Sweden	149
Latvia	39	UK	8,221
Lithuania	47	**Total**	**126,929**

Source: Eurostat (2006)

Table 10 (Appendix 1): European Fresh and Chilled Chicken Consumption (2004)

Country	**Tonnes**
Belgium	281,874
Czech Republic	47,561
Denmark	24,591
Estonia	216
Finland	16,742
France	263,677
Germany	167,666
Greece	168
Hungary	83,655
Italy	255,485
Latvia	19
Lithuania	1,188
Netherlands	374,334
Poland	248,982
Portugal	17,082
Slovak Republic	16,536
Spain	297,014
Sweden	8,335
UK	529,759
Total	**2,651,516**

Source: Eurostat (2006)

Table 11 (Appendix 1): UK Sliced Cooked Meat (& Alternatives) Sales 1999-2005

Sliced Cooked Meat	**Total tonnes**	**Total number of packs (million)**
Beef	159,974	800
Chicken	129,982	650
Pork	907,006	4,535
Turkey	207,426	1,037
Other meat	19,928	100
Chilled vegetarian alternatives	1,961	10
TOTAL	**1,457,277**	**7,232**

Source: Derived from MLC/TNS (2006)

Table 12 (Appendix 1): Examples of UK Pre-packed Multiple Retailer Deli Meat (NaCl, Shelf Life, CCPs)

Product	VP/MAP	% Salt	Shelf Life (days)	Heat Process	Preservative
Cooked chicken breast pieces	MAP	0.5	10	>70°C/2 min	
Cooked chicken joints	MAP	1.0-1.3	15	>70°C/2 min	
Cooked turkey breast	MAP	1.8-2.0	15	>70°C/2 min	
Par-cooked breaded chicken (fried)	MAP	0.75	10		
Honey cured ham	MAP + O_2 scavenger	1.0	15-25	>70°C/2 min	Sodium nitrite
Smoked ham	MAP + O_2 scavenger	2.3	15-25	>70°C/2 min	Sodium nitrite
Cooked ham	VP	2.1	28	>72°C/2 min	Sodium nitrite
Turkey ham	MAP	1.0	15-25	>70°C/2 min	Sodium nitrite
Cured sliced meat	MAP	2.3	21	72°C/2 min	Sodium nitrite
Cured cooked sliced meat	VP	2.3	23	>72°C/2 min	Sodium nitrite
Cured raw meat	MAP	2.0	28		Sodium nitrite
Bacon	MAP	3.5	26		Sodium nitrite
Bacon	VP	3.1	>30		Sodium nitrite
Sausage	MAP	1.5	>13		Sulphur Dioxide

Source: Industry data

Table 13 (Appendix 1): Examples of Non-UK Pre-packed Multiple Retailer Deli Meat (NaCl, Shelf life, Processes)

Product	% Salt	Shelf Life	Heat process	Preservative	Country
MAP honey roast ham		4 weeks		Sodium nitrite	Australia
MAP hickory smoked ham		4 weeks		Sodium nitrite	USA
Cured sliced meat MAP	2.1	4-8 weeks		Sodium nitrite	Italy
Cured sliced meat VP	2.0	2-3 weeks	>70°C/2 min	Sodium nitrite	Finland
MAP pancetta		3 weeks		Sodium nitrite	Australia
Uncured sliced meat MAP		2-4 weeks	>70°C/2 min		Italy
Hot smoked game VP	1.5-1.6	14 days	>70°C/2 min		Finland
MAP cooked meat		75-84 days			USA
VP frankfurters		2 months		Sodium nitrite	USA
VP cooked pork shoulder		6 weeks		Sodium nitrite	USA
MAP Cooked chicken		4 weeks			Australia
MAP Cooked turkey		5 weeks			Australia
VP Cooked chicken		>3 weeks			Spain
VP Cooked chicken wieners		46 days			USA
VP jalapeno beef log		1 year			USA
Sausages		18-30 days	>70°C/2 min	Sodium nitrite	Ireland

Source: Industry data

Table 14 (Appendix 1): European Fish Products Enterprises (2002)

Country	No. enterprises
Austria	6
Belgium	55
Bulgaria	34
Czech Republic	21
Denmark	126
Estonia	91
Finland	154
France	482
Germany	178
Ireland	87
Italy	455
Latvia	116
Lithuania	91
Luxembourg	0
Netherlands	135
Portugal	95
Slovak Republic	11
Romania	49
Spain	683
Sweden	173
UK	393
Total	**3,435**

Source: Eurostat (2006)

Table 15 (Appendix 1): UK Sales of VP and MAP Fish and Seafood 2003-2005

	Weight (tonnes)				Packs (million)			
	2003	**2004**	**2005**	**2003-5**	**2003**	**2004**	**2005**	**2003-5**
VP Mackerel	5,660	5,477	5,520	**16,657**	12	12	12	**36**
VP Salmon	4,077	4,467	5,485	**14,029**	9	10	12	**31**
VP Kippers	4,374	4,523	4,571	**13,468**	10	10	10	**30**
MAP Trout	280	304	255	**839**	<1	<1	<1	**2**
VP/MAP Smoked fish	**14,391**	**14,771**	**15,831**	**44,993**	**32**	**33**	**35**	**99**
MAP Selection	0	24	72	**96**	<1	<1	<1	**<1**
VP Mussels	2,316	2,686	2,298	**7,300**	5	6	5	**16**
MAP prawns total	13,527	16,112	18,062	**47,701**	39	46	514	**137**
MAP cold water prawns	9,596	10,827	11,319	**31,742**	29	33	34	**96**
MAP warm water prawns	3,919	5,280	6,729	**15,928**	10	14	17	**40**
MAP other prawns	12	5	14	**31**	<1	<1	<1	**<1**
TOTAL VP/MAP	**30,234**	**33,593**	**36,263**	**100,090**	**76**	**85**	**91**	**252**
Wet/smoked fish	107,596	110,076	117,143	**334,815**	323	330	351	**1,004**
Total wet fish	77,362	76,483	80,880	**234,725**	247	246	261	**753**

Source: SFIA (2006), MLC/TNS (2006)

Table 16 (Appendix 1): Smoked Salmon Products Produced in Scotland (2002)

Products	**Value (£million)**	**% Sales**	**Pack type**
Retail packs of slices	80.4	67	VP
Sliced sides	11.8	10	VP
Cocktail pieces/trimmings	6.4	5	VP/MAP/air
A variety of cures	4.2	3	VP
Whole sides	4.9	4	VP
Hot smoked salmon	4.5	4	VP
Pates	3.4	3	VP/MAP/air
Fillet Royale	1.7	1	MAP
Loose wrapped	<0.1	<1	Air
Other	2.5	2	Unknown
TOTAL	**119.9**	**100**	

Source: Industry-commissioned research

Table 17 (Appendix 1): UK Customer Types for Scottish Smoked Salmon

Customer Type	**% of total UK sales**					**2002 sales (£million)**
	1995	**1998**	**2000**	**2001**	**2002**	
Consumers direct	1.0	0.9	1.4	1.2	0.9	**1.08**
Mail order	1.8	2.7	2.5	2.6	2.4	**2.88**
Major multiples	77.3	67.3	70.8	79.5	77.8	**93.36**
Independent retailers	4.0	3.8	7.4	1.9	2.2	**2.64**
Retail wholesalers	7.6	7.7	3.2	3.9	4.8	**5.76**
Catering wholesalers	4.1	6.1	4.4	4.6	4.4	**5.28**
Caterers	2.0	6.2	5.0	3.1	4.8	**5.76**
Processors/manufacturers	2.2	4.7	5.1	3.2	2.6	**3.12**
Other	0	0.6	0.2	0	0.1	**0.12**
TOTAL	**100**	**100**	**100**	**100**	**100**	**120.00**

Source: Industry-commissioned research

Table 18 (Appendix 1): Global Sales of Smoked Salmon 1983-2005

Year	Weight (k tonnes)	Number of packs (million)*	% Annual growth
1983	4	12	
1984	4	12	0.0
1985	6	18	50.0
1986	8	24	33.3
1987	10	30	25.0
1988	13	39	30.0
1989	15	45	15.4
1990	16	48	6.7
1991	17	51	6.3
1992	19	57	11.8
1993	17	51	-10.5
1994	17	51	0.0
1995	20	60	17.6
1996	25	75	25.0
1997	30	90	20.0
1998	37	111	23.3
1999	50	150	35.1
2000	65	195	30.0
2001	75	225	15.4
2002	90	270	20.0
2003	110	330	22.2
2004	130	390	18.2
2005	145	435	11.5
Total	**923**	**2,769**	

Source: FAO (1983-97), Gram (2002), Eurostat (2006), Industry data [*assumes 3 packs per kg]

Table 19 (Appendix 1): Smoked Salmon Consumption in Certain EU States (2004)

Country	Weight (tonnes)	*Number of packs (million)
Belgium	1,877	5.6
Denmark	11,389	34.2
Estonia	216	0.7
Finland	427	1.3
France	23,145	69.4
Germany	17,794	53.4
Italy	1,320	4.0
Latvia	1,188	3. 6
Lithuania	19	0.1
Poland	6,850	20.6
Spain	6,235	18.7
Sweden	1,638	4.9
UK	4,467	12.1
Total	**101,565**	**228.6**

Source: Eurostat (2006), SFIA (2006). * Based on 3 packs/kg (except UK)

Table 20 (Appendix 1): Examples of Non-UK VP Fish (NaCl, Shelf life, Process details)

Product	% NaCl	Shelf life	Notes
Cold smoked rainbow trout		25 days at 0-3°C	Smoked at 20°C
Cold smoked rainbow trout	2.0 2.9	14 days 14 days	Finland. National Food Agency recommends 10-14d. If whole chain controlled (0-3°C) 21 days possible.
Hot smoked rainbow trout	 2.3	25 days at 0-3°C 10 days	Smoked at 80°C Imported from France & Spain
Cold smoked salmon		>5 months	USA
Cold smoked salmon	3.3	14 days	Finland
Cold smoked salmon		21-50 days	Denmark
Cold smoked salmon		4-6 weeks	Australia
Cold smoked salmon		6 weeks	New Zealand
Hot smoked salmon	1.6	14 days	Finland
Raw (gravad) salmon, sliced	2.9	14 days	Not smoked. Includes unspecified amount of sugar

Source: FSAI (2004), Industry data, Vehmaan Savut OY (2006)

Table 21 (Appendix 1): Swedish Smoked/Gravad Salmon Shelf Lives

Shelf life	% of salmon with indicated shelf life
≤1 week	6
2 weeks	4
3 weeks	48
4 weeks	11
5 weeks	29
6 weeks	1

Source: Rosengren and Lindblad (2003)

Table 22 (Appendix 1): Swedish Smoked/Gravad VP Salmon Shelf Lives

Shelf life (days)	Type	No. samples with indicated shelf-life
21	cold smoked	1
21	gravad	2
22	cold smoked	9
22	hot smoked	1
22	gravad	8
25	gravad	1
26	gravad	1
29	cold smoked	1
31	cold smoked	4
31	gravad	5
36	cold smoked	6

Source: Mandorf (2003)

Table 23 (Appendix 1): European mussel imports 2002-2004

	Number of packs (millions)			
	2002	**2003**	**2004**	**Total**
Live, fresh or chilled	92.8	104.8	134.0	**331.6**
Frozen marinated	32.0	29.6	32.0	**93.6**
Prepared	47.2	44.8	46.4	**138.4**
Totals	**172.0**	**179.2**	**212.4**	**563.6**

Source: Eurostat (2006)

Table 24 (Appendix 1): UK Consumption of VP Mussels 2003-2005

Weight (tonnes) of VP mussels consumed per year				Number of packs (million) consumed per year			
2003	**2004**	**2005**	**2003-5**	**2003**	**2004**	**2005**	**2003-5**
2,316	2,686	2,298	**7,300**	4.94	5.77	4.98	**15.69**

Source: SFIA (2006)

Table 25 (Appendix 1): Dutch Live MAP Mussels Export Volumes (2003-2004)

Destination (country)	Weight (tonnes)		
	2003	**2004**	**2003&2004**
France	3,570	3,550	**7,120**
Germany	210	660	**870**
Other	48,420	47,990	**104,400**

Source: Eurostat (2006), Industry estimates

Table 26 (Appendix 1): International Sales of Bagged Salads (1990-2004)

Country	Number of packs sold per year (million)															
	90	**91**	**92**	**93**	**94**	**95**	**96**	**97**	**98**	**99**	**00**	**01**	**02**	**03**	**04**	**Total**
UK	30	40	45	53	60	75	95	115	137	150	165	180	200	220	240	**2,570**
France		10	20	30	40	50	60	70	80	90	110	130	150	165	180	**1,698**
Portugal & Spain										10	14	22	30	38	48	**276**
Italy						10	15	20	30	40	60	80	105	125	145	**985**
Germany						10	14	16	18	20	22	24	26	28	30	**305**
Benelux			10	17	25	30	45	50	55	60	58	55	60	45	40	**740**
USA	163	210	265	316	365	415	460	500	550	600	660	730	810	900	1,000	**11,144**
TOTAL	**193**	**260**	**340**	**416**	**490**	**590**	**689**	**771**	**870**	**970**	**1,089**	**1,221**	**1,381**	**1,521**	**1,683**	**17,718**

Source: IFPA (2003), industry estimates

Table 27 (Appendix 1): US Sales of Fresh-Cut Produce 1984, 1990, 1993

	Number of packs sold per year (million)		
	1984	**1990**	**1993**
Foodservice - lettuce		163	184
Foodservice - total	110	214	303
Retail - lettuce			132
TOTAL cut	33	97	207
Total lettuce		**163**	**316**
Grand total	143	311	510

Source: IFPA (2003)

Table 28 (Appendix 1): International Sales of Bagged Salads in 1999 and 2004

Country	**Number of packs sold per year (million)**		
	1999	**2004**	**% change**
UK	150	250	67
France	90	180	100
Portugal & Spain	10	48	380
Italy	40	145	263
Germany	20	30	50
Benelux	60	40	-33
USA	600	1000	67
TOTAL	**970**	**1693**	**75**

Sources: IFPA (2003), Industry estimates

Table 29 (Appendix 1): US Industry Fresh-cut Produce Generic Recommended Modified Atmospheres

Vegetable	**Temperature (°C)**		**Atmosphere**	
	Optimum	**Range**	**% O_2**	**% CO_2**
Cabbage	0	0-5	2-3	3-6
Chinese cabbage	0	0-5	1-2	0-5
Herbs*	1	0-5	5-10	4-6
Lettuce (crisphead)	0	0-5	1-3	0
Lettuce (cut or shredded)	0	0-5	1-5	5-20
Lettuce (leaf)	0	0-5	1-3	0
Parsley	0	0-5	8-10	8-10
Spinach	0	0-5	7-10	5-10

Source: IFPA (2003)

* Herbs: chervil, chives, coriander, dill, sorrel, watercress

Table 30 (Appendix 1): Sales of Fresh Pasta and Gnocchi in UK, France and Italy (1989-2005)

Year	Number of packs sold per year (million)			
	UK	France	Italy	TOTAL
1989		50		**50**
1990		62		**62**
1991		69	245	**314**
1992		76	272	**348**
1993		81	297	**378**
1994		87	313	**400**
1995		90	330	**420**
1996		95	341	**436**
1997		101	351	**452**
1998		108	350	**458**
1999	37	116	357	**510**
2000	43	122	366	**531**
2001	49	128	374	**551**
2002	62	134	380	**577**
2003	66	149	386	**601**
2004	69	158	393	**619**
Total	**326**	**1,575**	**4,755**	**6,656**

Sources: Industry estimates
Note: These calculations assume 250g/pack. The total includes available data for certain countries only and is therefore an underestimate of the true total.

Table 31 (Appendix 1): Sales of MAP Fresh Pasta and Gnocchi in Italy (1991-2004)

	Number of packs sold per year (million)					
	Filled fresh pasta	Plain fresh egg pasta	Semolina pasta	Fresh pasta (total)	Gnocchi	Grand Total
1991	140	20	15	175	70	**245**
1992	154	23	17	194	78	**272**
1993	169	25	19	213	84	**297**
1994	175	27	21	223	90	**313**
1995	182	30	23	235	95	**330**
1996	188	32	25	245	96	**341**
1997	190	35	28	253	98	**351**
1998	185	36	31	252	98	**350**
1999	186	38	34	258	99	**357**
2000	187	41	37	266	100	**366**
2001	189	44	40	273	101	**374**
2002	189	45	44	278	102	**380**
2003	189	46	48	283	103	**386**
2004	188	48	53	289	104	**393**
TOTAL	**2511**	**490**	**435**	**3437**	**1318**	**4755**

Source: Industry data. Note: Assumes pack size = 250g.

Table 32 (Appendix 1): Fresh Pasta – Legal and other requirements in various European countries

Country	Source	Moisture	NaCl %	Shelf life	Notes
France	Decree n° 55-1175 of 31 August 1955	Only fresh pasta can contain >12.5%			
Italy	Presidential Decree N° 187, dated 9/2/01 , pursuant to Article 50 of Law N° 146, dated 22/2/94.	Not less than 24% if pre-packaged	a_w between 0.92 and 0.97	No national rules: shelf determined by manufacturers. In practice 50-60 days	Must have undergone a heat treatment at least equivalent to pasteurisation. Must be stored between manufacture and sale, at 4°C +2°C.
Switzerland	Art. 152 to 154 of Ordonnance sur les denrées alimentaires	If <13% it is called "dry pasta". Therefore, 13% or more water is inferred to be "fresh pasta".			Must be kept at max 5°C.
UK	LACOTS agreement (14 96 7)	Minimum 25%	Not specified. In practice >1% (filled)	Maximum 5 weeks, being reliant on chilled storage	

Source: ECFF, personal communication

Table 33 (Appendix 1): Chilled Ready Meal Sales 1984-2005 in UK, France and Finland

Year	Number of packs sold per year (million)			
	UK	France	Finland	TOTAL
1984			99	**99**
1985			100	**100**
1986	75		100	**175**
1987	98		102	**200**
1988	121		102	**223**
1989	143		103	**246**
1990	168	150	105	**423**
1991	185	175	104	**464**
1992	212	188	103	**503**
1993	248	196	101	**545**
1994	280	208	102	**590**
1995	320	220	105	**645**
1996	372	232	104	**708**
1997	416	240	106	**762**
1998	462	252	108	**822**
1999	505	268	114	**887**
2000	555	298	116	**969**
2001	634	330	118	**1,082**
2002	724	365	120	**1,209**
2003	829	400	123	**1,352**
2004	966	431	125	**1,522**
2005	980	450	127	**1,557**
TOTAL	**8,293**	**4,403**	**2,387**	**15,083**

Sources: Industry data and estimates
Notes: Includes side dishes and meal centres. The total includes available data for certain countries only and is therefore an underestimate of the true total.

Table 34 (Appendix 1): French Chilled Ready Meal Market Segmentation (2004)

Segment	Weight (tonnes)	Number of packs (million)
Pasta-based	26,966	80.9
Couscous	2,285	6.9
Paella	1,542	4.6
Other exotic	3,018	9.1
Regional	5,096	15.3
Meat-based	6,616	19.8
Fish-based	7,007	21.0
Poultry/rabbit-based	6,052	18.2
Game-based	18	0.1
Gratins and cooked vegetables	2,466	7.4
Total chilled ready meals	**61,066**	**175.8**

Source: SYNAFAP, personal communication. Note: Complete meals only

Table 35 (Appendix 1): Sales of Chilled Ready Meals in Eight Major EU Markets in 2004

Country	**Retail value (£Million)**	**Weight (k tonnes)**	**Number of packs (million)**
Belgium	23	4.6	14
Finland	200	41.4	124
France	750	150.0	450
Italy	120	24.0	75
Germany	304	60.8	183
Hungary	15	3.0	9
Spain	82	16.4	50
UK	1513	321.0	966
TOTAL	**3,007**	**621.2**	**1,871**

Source: Industry data.
Assumes three packs per kg

Table 36 (Appendix 1): German Chilled Food Market in 2002 and 2003

Chilled Food	Retail value (£Million)	Weight (k tonnes)	Number of packs (million)	Retail value (£Million)	Weight (k tonnes)	Number of packs (million)	% change	**Total number of packs (million)**
	2002			**2003**				
Complete meals	14.5	2.0	6.0	26.9	3.7	11.0	85	**17.0**
Stews	1.7	0.3	1.8	1.5	0.2	0.6	-14	**2.4**
Meat based meal centres	64.8	9.3	31.0	73.2	10.0	33.0	13	**64.0**
Fish based meal centres	1.7	0.3	0.9	0.7	0.1	0.3	-59	**1.2**
Pizza	13.6	2.0	5.0	14.9	2.0	5.0	10	**10.0**
Pasta	117.6	16.8	67.2	123.8	17.7	70.8	5	**138.0**
Fresh leaf salad	34.2	5.0	50.0	34.6	5.0	50.0	1	**100.0**
Soup	15.0	2.0	6.0	18.0	2.5	7.5	20	**13.5**
Dough	43.1	6.0	15.0	51.4	7.0	17.0	19	**32.0**
TOTAL	**306.3**	**43.7**	**182.9**	**345.0**	**48.2**	**195.2**	**13**	**378.1**

Source: Derived from CMA (Bundesverband der Feinkostindustrie, personal communication)
Assumes £7K/tonne and pack size is 250g

Table 37 (Appendix 1): Sales of Chilled Dough in France

Year	Weight (tonnes)	Number of packs (million)
1990	10,230	22
1991	12,523	26
1992	14,000	32
1993	22,000	45
1994	27,000	58
1995	30,302	70
1996	35,022	80
1997	36,400	95
1998	37,361	98
1999	40,000	105
2000	45,000	118
2001	50,000	131
2002	57,000	149
2003	64,499	169
2004	60,694	159
Total	**542,031**	**1,357**

Chapter three - The position in the UK, other European countries and internationally with respect to guidance on control of *C. botulinum* in chilled VP/MAP foods

3.1 Inventory of Guidance in Relation to Chilled Foods

The guidance documents inventory produced by the European Union funded Harmony Project (Martens, 1997, 1999) in relation to codes of good hygienic practice for minimally processed foods was used as the base data set for this chapter, with those documents referring to VP/MAP foods being selected. Documents issued post-Harmony were also reviewed for relevance to VP/MAP foods.

Table 3.1 summarises the scope of 45 existing GMP codes, guidelines, safety reports etc relating to VP/MAP/ROP (reduced oxygen packaging) chilled foods. The last column indicates the sectoral focus of each document:
M: manufacturers of minimally processed foods
C: catering sector
R: retail/distribution of chilled foods
D: domestic

The key documents are reviewed in the following sections.

Table 3.1 An outline summary the scope of 45 existing GMP codes, guidelines, safety reports etc relating to VP/MAP/ROP (reduced oxygen packaged) chilled foods

Organisation/Country	Year	Title of Document	Scope	Reference to VP/MAP/ ROP?	Sector
ACMSF 1992	1992	Report on Vacuum Packaging and Associated processes	Production, handling and storage of VP and other low oxygen chilled foods	Yes	M, C, R
ACMSF 1995	1995	Annual Report 1995, Annex III	Production, handling and storage of VP and other low oxygen chilled foods	Yes	M, C, R
AFIC, ASI, RWTAA (Australia)	1999	Australian Cold Chain Food Safety Programs	Handling, storage and transport of frozen foods, ice cream and chilled foods for retail sale and in food service outlets	Yes	M, C, R
Agriculture Canada	1990	Canadian Code of recommended practices for pasteurized/ MAP/ refrigerated food	Pasteurized products (before or after packaging) under vacuum or modified atmosphere	Yes	M
AIFST/Australian Cook Chill Council	2000	Guidelines for Chilled Food Production Systems including Food Safety Programs	Cook-chill systems – bulk foods, multi-portion packs, individual packs – catering and retail	Yes	M, C
AMI/NMA/SMA (USA)	2003	Best Practices for Handling Vacuum-Packed Sub-Primal Beef Cuts.	Safe handling of the primary and potential secondary end products from VP sub-primal beef cuts and clarification of testing of these products for pathogens.	Yes	M
ANZFA (applicable in Australia only)	2001	Standard 3.2.2 (Food Safety Practices and General Requirements)	Receipt, storage, processing, display, packaging, distribution, disposal and recall of food.	No	M, C, R
AFGC, AFIC, ASI, RWTAA (Australia)	1999	Australian Cold Chain Guidelines	Handling, storage and transport of frozen foods, ice cream and chilled foods for retail sale and in food service outlets	No	M, C, R
Australian Quarantine and Inspection Service (AQIS, Australia)	1992	Code of practice for heat-treated refrigerated foods packaged for extended shelf life.	Cook-chill products with extended shelf life (>5 days). Excluded: frozen meals, cured meat products, dairy products, fruit juices/purees	Yes	M, R
British Retail Consortium (UK)	2005	BRC Global Standard Food (Issue 4)	All retailer own label food products	No	M, R
Campden and Chorleywood Food Research Association (UK)	1996	Code of practice for the manufacture of vacuum and modified atmosphere packaged chilled foods with particular regard to the risks of botulism. Guideline No. 11	VP or MAP products, but notes that VP or MAP are not the only circumstances which can give rise to *C. botulinum* risks	Yes	M

Campden and Chorleywood Food Research Association (UK)	1992a	Guidelines for the good manufacturing and handling of MAP food products. Technical Manual No. 34	MAP foods, VP and air packed fresh fruit and vegetables. Excluded: other VP foods, such as cured, smoked and cook-chill products	Yes	M, R
Campden and Chorleywood Food Research Association (UK)	1992b	The microbiological safety of sous vide processing. Technical manual No. 39	Sous vide products	Yes	M
Canadian Food Inspection Agency	1997	Decisions: Best Before Date/Durable Life	MAP foods	Yes	M, R
CFSAN (USA)	2005	Food Code 2005	Establishes definitions, sets standards for management and personnel, food operations, and equipment and facilities; and provides for food establishment plan review, permit issuance, inspection, employee restriction, and permit suspension.	Yes	M
Chilled Food Association (CFA - UK)	2006	Best Practice Guidelines for the Production of Chilled Foods. 4th edition	Pre-packaged chilled prepared foods for retail sale	Yes	M
Chilled Food Association (CFA - UK)	1997	Guidelines for Good Hygienic Practice in the Manufacture of Chilled Foods. 3rd edition	Pre-packaged chilled prepared foods for retail sale	Yes	M
Codex Alimentarius Commission	1999	Code of hygienic practice for refrigerated packaged foods with extended shelf life	chilled foods with shelf life >5 days products with pH >4.6 and a_w >0.85	No	M
Codex Alimentarius Commission	2005 (draft)	Draft Standard for Smoked Fish	Internationally traded cold smoked fish	Yes	M
Department of Health (UK)	1989	Chilled and Frozen Guidelines of Cook-Chill and Cook-Freeze Catering Systems. DoH Guidelines.	Pre-cooked chilled foods for institutional catering	No	C
European Chilled Food Federation (ECFF)	1996	Guidelines for the hygienic manufacture of chilled foods	Chilled retailed foods with pH > 4.5 or a_w > 0.85	Yes	M
European Chilled Food Federation (ECFF)	1996 (pub 1999)	Sous vide: conclusions of an ECFF Botulinum working party	Chilled foods where there is no competition from other micro-organisms and/or where competitors are destroyed by a mild heat process	Yes	M

Elintarvikevirasto (EVI) (Finland)	2000	Suositus tyhjiöpakattujen kylmäsavustettujen ja graavisuolattujen kalavalmisteiden enimmäissäilytysajaksi	VP cold smoked and gravad fish preparations maximum storage times and temperatures	Yes	M, C, R
FDA	1993	Unrefrigerated garlic-, spice-in-oil mixes potentially hazardous.	Domestic and commercially produced garlic/spice-in-oil mixes	Yes	M, C, R, D
Food Institute Canada	No date	Canadian Code of Recommended Handling Practices for Chilled Food	Processed foods which are stored and distributed at temperatures of between -1°C and +4°C, and are sold away from the point of manufacture	Yes	M
FSA	No date	Is it safe to make my own flavoured oils at home using herbs?	Domestically produced flavoured oils with herbs, spice, vegetables	Yes	D
FSAI (Ireland)	2004	Cook-chill systems in the Food Service Sector	Cook-chill catering	No	C
Food Standards Agency Scotland	2005	Food Safety Guide for Farmers Markets in Scotland	Handling and storage of food sold at farmers markets	No	M, R
Health Canada	2002	Guidelines for the Production, Distribution, Retailing and Use of Refrigerated Pre-packaged Foods with Extended Shelf Life	Low acid (pH 4.6), high moisture (a_w>0.85) chilled foods	Yes	M, C, R
Health Canada	No date	Garlic in oil	Domestic/non-industrial production of garlic in oil	Yes	M, C, R, D
International Fresh-Cut Produce Association (IFPA - USA)	2001	Food Safety Guidelines for the Fresh-Cut Produce Industry	Sanitary procedures for the fresh-cut industry	Yes	M
International Fresh-Cut Produce Association (IFPA - USA)	2003	Packaging Design for Fresh-cut Produce	Selection of packaging approaches for fresh-cut produce	Yes	M
Joint Hospitality Industry Congress (UK)	1997	Industry Guide to Good Hygienic Practice: Catering Guide	All catered foods	No	C
MOFFA (UK)	2006	Draft Industry Guide to Good Hygiene Practice: Mail Order Foods	Mail order foods	Yes	M, R

NFPA (USA)	1989	Guidelines for the development, production, distribution and handling of refrigerated foods	Products that must be refrigerated to retard spoilage and to help prevent growth and toxin production by pathogenic microorganisms.	Yes	M, C, R
PTK/ETL/SKKL (Finland)	2003a	Helposti Pilaantuvien Pakattujen Kalojen ja Kavalmisteiden Säilyvyysmerkinnät ja Säilyvyyden Varmistaminen	Highly perishable packed fish and fish preparations' storage instructions and the determination of shelf life	Yes	M, R
PTK/ETL/SKKL (Finland)	2003b	Helposti Pilaantuvien Pakattujen Lihavalmisteiden ja Valmisruokien Säilyvyysmerkinnät ja Säilyvyyden Varmistaminen	Highly perishable packed meat products' and ready to eat foods' storage instructions and the determination of shelf life	No	M, R
PTK/ETL/SKKL (Finland)	2003c	Tuoreen Lihan ja Raakalihavalmisteiden Säilyvyysmerkinnät ja Säilyvyyden Varmistaminen	Fresh meat and raw meat preparations' storage instructions and the determination of shelf life	No	M, R
Sous Vide Advisory Committee (UK)	1991	Code of practice for sous vide catering systems	Catered sous vide products with a shelf life ≤ 8 days	Yes	C
Syndicat National des Fabricants des Plats Préparés (SYNAFAP - France)	1995	Aide à la maîtrise de l'hygiène alimentaire	Implementation of GHP and HACCP in the production of refrigerated ready-to-eat meals	Yes	M
SYNAFAP (France)	Draft, 2006		GHP and HACCP in the production of refrigerated ready-to-eat meals	Yes	M
TNO The Netherlands	1994	Code for production, distribution and retail of chilled pasteurized meals with extended shelf life	Cook-chill products with extended shelf life (11 to 42 days) Excluded: products with pH < 4.6 or a_w < 0.96 and VP sliced meats	Yes	M, R
Transport en Logistiek Nederland	1996	Transport, distribution and retail of food products.	All food products	No	R
University of California (UC Davis) (USA)	2001	Optimal Controlled Atmospheres for Horticultural Perishables	Choice of atmospheres for various produce (prepared and whole)	Yes	M
UNIPI (Italy)	1999	Guidelines for the application of general principles of food hygiene and the HACCP system in the pasta production industry	Fresh and dry pasta production	Yes	M
US Chilled Foods Association (USA)	1990	Technical Handbook for the Chilled Foods Industry	HACCP descriptions and flow diagrams common across chilled food product types.	Yes	M

3.2 Guidance at the European Level

3.2.1 EU Food Regulation

From 1 January 2006, new food hygiene legislation has applied throughout the EU. In July 2000, the European Commission published a package of five measures to update and consolidate the 17 existing (vertical) hygiene directives. The texts were adopted on 29 April 2004 and published in the Official Journal (OJ) of the European Union on 30 April 2004. The EU food hygiene regulations package comprises:

- Regulation 852/2004 on the hygiene of foodstuffs
- Regulation 853/2004 laying down specific hygiene rules for food of animal origin
- Regulation 854/2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption
- Directive 2004/41 repealing the previous EU legislation or, in some cases, amending still existing legislation
- Directive 2002/99 laying down animal health rules on products of animal origin for human consumption.

The regulations have been amended since by the EU implementing measures and in the case of 854/2004 by Regulation (EC) 882/2004, the Official Feed and Food Controls Regulation.

On 22 December 2005 the Commission published the remaining implementing and transitional measures that support the application of the EU hygiene legislation in the EU Official Journal. These regulations are:

- Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs
- Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under 853/2004 and for the organisation of official controls under 854/2004 and 882/2004, derogating from 852/2004 and amending 853/2004 and 854/2004
- Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for trichinella in meat
- Regulation (EC) No 2076/2005 of 5 December 2005 laying down transitional arrangements for the implementation of 853/2004, 854/2004 and 882/2004 and amending 853/2004 and 854/2004

These now join Regulation (EC) No 1688/2005 of 14 October 2005 implementing 853/2004 as regards special guarantees concerning salmonella for consignments to Finland and Sweden of certain meat and eggs.

The new hygiene regulations build on the general principles of food law established by Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

The General Food Law is supplemented by targeted legislation on a raft of food safety issues, such as use of pesticides, food supplements, colourings, antibiotics and hormones in food production.

In summary, the new EU hygiene legislation:

- Sets out the duty of food business operators to produce food safely.
- Requires food business operators (except primary producers) to implement a permanent procedure, or procedures, based on HACCP principles.

- Is structured so that it can be applied flexibly and proportionately according to the size and nature of the business.
- Requires registration or approval for certain food establishments.
- Allows for the development of guides to good practice for hygiene and for the application of HACCP principles by food business operators.
- Allows a special provision to ensure flexibility for food produced in remote areas and for traditional production and methods.

However, beyond there being a general product safety requirement there is no specific EU legislation relating to VP or MAP chilled prepared foods.

In practice, controls are stipulated in national rules or guidance (e.g. French legislation, CCFRA Industry Code, Chilled Food Association guidelines) in certain Member States, or, on a broader basis, through industry guidance (e.g. European Chilled Food Federation), compliance with which is voluntary.

3.2.2 European Industry Guidance

The European Chilled Food Federation in 1996 issued the first edition of its Guidelines for the Hygienic Manufacture of Chilled Foods (ECFF, 1996). With respect to the control of *C. botulinum* this built on the outputs of an international working party of researchers/academics and industry experts convened by ECFF, which published a report particularly of the safety of chilled products where there is no competition from other microorganisms and/or where competitors are destroyed by a mild heat process. The report was published as a review in Food Control (Gould, 1999).

The ECFF Botulinum Working Party concluded that controls for long shelf life hermetically sealed packs should be either:

- 6D process (non-proteolytic *C. botulinum* spores): 90°C/10 min and <10°C, or
- <6D process (non-proteolytic *C. botulinum* spores) and <3°C, or
- Demonstrably effective hurdles versus non-proteolytic *C. botulinum* spores

The 1996 ECFF Guidelines included the following example “if a chilled product is to be packed in a reduced oxygen atmosphere and has a shelf life of >10 days, one or more of the following hurdles should also be used to control non-proteolytic (psychrotrophic) *C. botulinum*:

- Heat to a temperature/time combination equivalent to 90°C for 10 min (or equivalent); or
- Adjust a_W to < 0.97; or
- Increase acidity to < pH 5.0; or
- Use combinations of a_W, pH, atmosphere, temperature etc that demonstrably will inhibit the growth of non-proteolytic (psychrotrophic) *C. botulinum* within the shelf life and storage conditions”

Work is currently ongoing on the second edition of the ECFF guidance, with strong pressure being brought by some members for an approach reflecting actual loadings of non-proteolytic *C. botulinum* spores on raw materials rather than a blanket 6-log reduction approach.

See section 3.3.3 for the approach being sought by SYNAFAP, the French delegation to ECFF.

3.3 Guidance in EU Member States

EU Member States can introduce specific hygiene provisions provided they are not less stringent than the EU hygiene requirements and do not result in barriers of trade. In many Member States, more specific microbiological and temperature controls are laid down.

The publication of EC Regulation 2073/2005 on Microbiological Criteria for Foodstuffs in January 2006 harmonised many microbiological criteria applicable to foodstuffs, in particular regarding Salmonella and *Listeria monocytogenes*, the latter in relation to ready to eat products.

There remain many national criteria in EU Member States which have not been harmonised. However, none of these relate to non-proteolytic *C. botulinum* since it is not valid to set a criterion (and therefore a sampling plan) in relation to organisms which rarely occur.

There is no single EU legislated temperature for multi-component products. Differences in national temperature rules constitute a significant barrier to trade since they can affect the basis on which shelf life is determined.

Some examples of variations in national temperature rules are given in Chapter 4 where comment is also made regarding the conclusions of the 1996 SCOOP project (EC Scientific Cooperation) on temperature control.

Further differences in temperature requirements are evident in the different national GMP codes discussed below.

In practice, controls are stipulated in national rules or guidance in certain Member States (e.g. French legislation, CCFRA Code, Chilled Food Association Guidelines), or, on a broader basis, through industry guidance (e.g. European Chilled Food Federation), compliance with which is voluntary.

3.3.1 The position in the UK

National legislation in the form of Statutory Instruments (SIs) in England, and equivalent legislation in Scotland, Wales and Northern Ireland, is required to enforce EU regulations. In relation to EU hygiene legislation these cover:

- offences, penalties and powers of entry
- revocation of existing implementing legislation
- enacting the national measures required or provided for in the EU regulations
- any consequential amendments (where the revocation of existing legislation requires changed references in other pieces of legislation)

The Food Hygiene (England) Regulations 2006 (SI 2006/14) came into force on 11 January 2006 and also applied the provisions of the EU Microbiological Criteria Regulation 2073/2005.

The Official Feed and Food Controls (England) Regulations 2006 (SI 2006/15) also applied from 11 January 2006. These regulations apply the EU Official Feed and Food Controls Regulation (OFFC) in England.

There are no legislated UK requirements specific to VP/MAP chilled foods. However, ACMSF reports (1992 and 1995), and various industry guidance documents exist.

The voluntary ‘10 day rule’ (CCFRA, 1996) is generally applied in the UK for foods stored at chill (chill temperature is specified as a maximum of 8°C in England, Wales and Northern Ireland. In Scotland the requirement is to keep chilled food in “a refrigerator, or refrigerating chamber, or a cool ventilated place” (Food Law 2006)).

3.3.1.1 ACMSF Guidance

The 1992 ACMSF recommendations on the safe production of vacuum and MAP chilled foods with respect to non-proteolytic *C. botulinum* was published formally (ACMSF, 1992). It

recommended a maximum shelf life of 10 days at 10°C maximum unless appropriate controls (formulation and/or processing) were in place. The recommendations were taken up widely by professional food manufacturers and retailers in the UK and were taken forward into various other guidance including internationally.

Later, in response to questions raised by the team drawing up an industry code of practice (at CCFRA), the ACMSF recommendations were revised (ACMSF, 1995). These revisions were made on the basis of a review of 31 references from the literature on the production of toxin by non-proteolytic *C. botulinum* within 10 days at ≤10°C. Most of these were challenge tests carried out in food materials, not foods as purchased/eaten in the UK. A maximum shelf life of 5 days at 10°C maximum or 10 days at 5°C maximum was recommended unless appropriate controls (formulation and/or processing) were in place. The ACMSF 1995 approach was not taken up by industry or included in other guidance.

3.3.1.2 Code of Practice for the Manufacture of Vacuum and MAP Chilled Foods (CCFRA, 1996)

This was MAFF-funded work through a working group of manufacturers, retailers and MAFF and DH representatives. It aimed to crystallise various guidance into practical advice. One of the recommendations included was a maximum shelf life of 10 days at 8°C maximum unless appropriate controls (formulation and/or processing) were in place.

Based on previous evidence that growth and toxin production did not occur at <3°C, the guidance recognised that any period of storage at <3°C (e.g. during the manufacture stages before the product is distributed for retail display or other use) does not contribute to the designated shelf-life.

3.3.1.3 Chilled Food Association guidance

The Chilled Food Association in its 2006 and 1997 guidance for prepared pre-packed foods sold at retail maintains the 10 day rule, based on storage at 8°C maximum (CFA, 1997; CFA, 2006). Beyond this shelf life demonstrably effective controls must be in place with respect to non-proteolytic *C. botulinum*. This is applicable to all chilled products, irrespective of whether or not they are VP, MAP or packed in air.

3.3.1.4 Mail Order Foods

The Mail Order Fine Foods Association (MOFFA) in February 2006 issued a draft Guide to GHP for Mail Order Foods for comment (MOFFA, 2006). The document is a Guide under EU hygiene legislation (852/2004) and makes the following points in relation to VP/MAP products:

- Technically it may be necessary to have a salt level of 3.5% (aqueous) throughout a VP food to control *C. botulinum*.
- For vacuum and MAP foods where chill temperatures (warmer than 3°C but cooler than 8°C) alone are used to control *C. botulinum*, a maximum total shelf life of 10 days should be assigned.
- For specific high risk products, e.g. VP foods, special precautions may be required to ensure a satisfactory product. Advice should be sought from your supplier, your local Environmental Health Service, Enforcement Officer or other expert. For vacuum or MAP foods the Industry Code (CCFRA, 1996) can be consulted.
- Whatever aspect of the product you are relying on, to ensure the product is safe to eat when it arrives with the consumer (e.g. temperature, and/or salt content and/or acidity and/or water activity), check it is in place, at least twice a year.

3.3.1.5 Sous Vide Advisory Committee

SVAC (1991) issued a code of practice for sous vide catering systems, applicable to products with a shelf life of no more than 8 days. Heat processes are specified (26 min at 80°C or 11 min at 85°C or 4.5 min at 90°C or 2 min at 95°C) in relation to non-proteolytic *C. botulinum* type E, followed by storage at 0-3°C.

3.3.1.6 FSA Consumer Guidance on Homemade Flavoured Oils

This guidance, which is not easy to locate within the FSA website (being found when searching for 'flavoured oil' but not 'herbs in oil') acknowledges that "even though recipes for flavoured oils can be found in cookery books, magazines and websites, these might not have considered the risk of botulism". The guidance states that "the safest option is to make a small quantity and use it on the day you have made it. If you have some left over, put it in the fridge straight away and use it within a week."

3.3.2 The position in Finland

The Finnish Food Authority (EVI) in 1991 recommended the storage of VP fish preparations at +3°C maximum and for no more than 3 weeks. This recommendation was revised in 2000 when EVI recommended the storage of VP fish preparations (cold smoked and gravad) at as low chill temperatures as possible, and at the most at +3°C for 10-14 days (EVI, 2000). This was based on mathematical modelling of *Listeria monocytogenes* growth potential. However, if documentary evidence is available that the temperature is no more than +3°C throughout the whole of the chill chain then the shelf life can be up to 3 weeks.

3.3.3 The position in France

The Arrêté of 26 June 1974 and related memoranda of 1988 and 1992 apply to the production of chilled prepared meals and their allowed shelf life. However their scope now only covers meals made from products from animal origin other than fish and meat products (i.e. milk, eggs).

The 1974 "Arrêté" limited shelf life of all pre-cooked chilled products to 6 days. The "Note de Service du 31 Mai 1988" (modified in 1992) established changes in manufacturing protocols to extend shelf life up to 42 days, according to the type of products (sous vide or not), the pasteurisation value (Pv) and the attained core temperature. The 1988 legislation enabled ready meals reaching a centre temperature of 65°C and a process equivalent of 70°C for 100 min (comparable to 90°C for 1 min, or 0.6 log inactivation of spores of non-proteolytic *C. botulinum*) to have a shelf life of up to 21 days, although the precise shelf life was to be determined by the manufacturer. Products reaching a centre temperature of 70°C and receiving equivalent lethality of 1,000 min (i.e. equivalent to 90°C for 10 min) could have a shelf life of 42 days.

Pasteurisation values are based on the heat resistance of the opportunistic pathogen *Enterococcus* (*Streptococcus) faecalis* with D_{70} = 2.95 min and z = 10°C. The shelf life that can be safely achieved by minimally processed foods is therefore linked to the pasteurisation process given.

For ready-to-eat meals made from meat and fish products, the shelf life is set by the manufacturer using a protocol developed by industry with AFNOR (see Chapter 4).

French industry guidance is currently being finalised by SYNAFAP and proposed for inclusion in ECFF guidance, it provides a series of approaches:

The manufacturer shall implement measures such that the product remains safe until its usage (depackaging or pack opening), notably:

- The type of packaging used (taking account of the risk of cross contamination, MAP, etc);
- The labelling of products and the description of usage, storage, etc;

- Product shelf life: indication of a use by date.

Factors influencing shelf life are, notably:

- the type of microorganisms and the level of contamination at the end of production, which are related to:
  - the control of initial contamination of ingredients and growth during production (i.e. implementing GMP/GHP);
  - the application of a procedure aiming to reduce potential product contamination, e.g. thermal treatment, acidification;
- the potential for growth of microorganisms during distribution, which will depend on:
  - the physicochemical composition of the product;
  - the use of inhibitory factors or minimising the multiplication of microorganisms ('hurdles'), e.g. additives, MAP;
  - the storage temperature;
- the possibility of microbial destruction before product use (e.g. requirement to cook before use).

These elements are taken into account in the hazard analysis. For example, if a product is cooked in the final packaging, with a core temperature of 70°C for 10 minutes, and GMP/GHP are applied, the dangers in relation to vegetative pathogens are controlled, whatever the shelf life. Factors to take into account will therefore be sporeforming pathogens (and possibly sporeforming spoilage microorganisms). The shelf life will therefore take account of the initial potential level of contamination by sporeformers, the growth potential from spores and the possibility of toxigenesis. In relation to *C. botulinum*, two scientific studies are important:

- A study of the contamination of ingredients/raw materials used in France indicated that there was <10 viable *C. botulinum* organisms/kg. A number of samples were found to be contaminated with *C. botulinum* strains forming type A and/or B toxin, but not with *C. botulinum* strains forming type E toxin (Carlin *et al*., 2004). One limitation of this study, however, was that it did include positive controls to confirm the limit of the detection (i.e. it was not confirmed that low numbers of *C. botulinum* could be detected in all foods tested).
- A study was carried out with cod inoculated with two strains of non-proteolytic *C. botulinum* (at 10^4-10^6 spores per 110g pack, a concentration approximately 20-100 times higher than natural contamination levels reported in the literature). Various heat treatments were applied in-pack, and then different storage regimes employed. The study was carried out at l'Institut Pasteur de Lille (1996) and is also discussed in Chapter 6. Briefly, this study showed that:
  - A core temperature of 90°C for 10 minutes or equivalent (e.g. 83°C for 100 minutes), destroyed the inoculated spores, while a core temperature of 75°C for 85 minutes, reduced the spore population by a factor of 10 (for the type E strain) or by 5 (for the type B strain).
  - Combinations of heat treatment and storage regime were identified that prevented toxin formation within 30-35 days (Table 3.2). These results are consistent with those obtained with sterile minced beef by Fernandez and Peck (1997, 1999). These and other studies with sterile minced beef provide details of combination of heat treatments less than 90°C for 10 min, that can be combined with pH, NaCl concentration, incubation temperature and shelf-life to prevent toxin formation from 10^6 spores of non-proteolytic *C. botulinum* (Peck and Stringer, 2005). For example:
    - 90°C/ 10 min combined with storage at 8°C prevented toxin formation in 50 days
    - 80°C/ 98 min combined with storage at 8°C prevented toxin formation in 40 days
    - 75°C/284 min combined with storage at 8°C prevented toxin formation in 30 days
    - 80°C/ 11 min combined with storage at 8°C prevented toxin formation in 20 days

Table 3.2 Effect of specified heat treatments and storage temperature regimes on time to toxin formation by non-proteolytic *C. botulinum* in cod (data from l'Institut Pasteur de Lille, 1996)

Storage temperature	Time (days) to toxin formation for specified heat treatment delivered			
	75°C 85-90 min	80°C 93 min	83°C 100 min	90°C 10 min
4°C 30d	30/NP*	nt**	nt	nt
4°C 10d, then 8°C 25 d	30/34	35/NP	35/NP	35/NP
4°C 10d, then 22°C 4h, then 8°C 25 d	21/30	30/35	35/NP	35/NP
8°C 20d	20/NP	30/35	35/NP	35/NP

Note: 10^5-10^6 spores of non-proteolytic *C. botulinum* type B added per 110g pack prior to heat treatment

* First number = last day negative for toxin, second number = first day positive for toxin

NP = Not positive, nt = not tested

The SYNAFAP approach therefore aims to take account of natural levels of contamination and to tailor the heat process accordingly, not necessarily using a 6-log reduction process (i.e. equivalent to 90°C for 10 minutes). Account is also taken of the temperatures anticipated in the controlled chill chain. Sous vide product shelf lives up to 30 days are mooted by the guidance and have existed on the French market for two decades.

3.3.4 The position in Italy

UNIPI (1999) published guidelines in relation to pasta production, applicable to fresh and dry pasta. Process parameters are for the manufacturer to determine on the basis of HACCP, but lethal rate tables are included in relation to *Listeria monocytogenes* and non-proteolytic *C. botulinum*. Storage temperature and shelf life are not specified, this again being for the manufacturer to determine on the basis of HACCP.

3.3.5 The position in the Netherlands

TNO (1994) issued a code developed with industry in relation to cook-chill products (excluding VP sliced meats) with shelf life 11-42 days. The code requires a total 6D heat process of 90°C/10 mins (equivalent) including if the product is duo pasteurised (cooked, filled, sealed, cooked). Final product storage temperatures of 0-3°C are referred to for post-production storage on-site and a maximum of 6 weeks post-production is allowed, including a maximum of 3 weeks to consumption in the commercial and domestic chill chain (0-5°C) and 1 week maximum consumer storage (0-5°C).

3.4 Guidance outside the EU

3.4.1 The position in Australia

AQIS (1992) set a 10 day limit for MAP/VP foods held at $\leq$ 5°C (unless frozen), or >5 days if storage is at $\leq$ 3°C. For storage at >5°C to 10°C a 6D process for non-proteolytic *C. botulinum* is required: Extended shelf life is possible by storage at $\leq$ 3°C. If the product is at >10°C, it should be discarded. The Guidance is no longer in print however.

AFIC/ASI/RWTAA (1999) reiterates legal temperature requirements (0-4°C but never more than 5°C), including for MAP products.

AIFST/AFIC/ACCC (2000) guidelines are applicable to a variety of chilled food production systems including cook-chill, sous vide and MAP. The emphasis is on low temperature (0-3°C) storage and the selection of heat process "according to the recipe". Temperatures of 70°C/2 min or 90°C/11 min equivalent are referred to. The guidance does not give specific shelf life limitations but states 'long shelf life with correct practice can be achieved. It has been found that some products have a shelf life of up to 45 days.' The guidelines appear to be designed to apply to the catering sector rather than retail and are not believed to be complied with in the retail sector.

3.4.2 The position in Canada

3.4.2.1 MAP Products

The Canadian Code of Recommended Manufacturing Practice for Pasteurized/Modified Atmosphere Packed/Refrigerated Food (Agriculture Canada, 1990) states that:

- Care should be taken to prevent the product temperature from rising above 10°C during cold filling
- Product should be maintained at temperatures of less than 4°C (or above 65°C).
- Refrigerated products to be used as ingredients or prepared foods should be held at temperatures below 4°C at all times
- The processed final product must be kept refrigerated (-1 to +4°C) at all times
- Whenever chilled food is received with the product temperature of +7°C or higher the manufacturer shall be notified immediately and 'special handling instructions requested'.
- Chilled food storage facilities/retail display cases must be capable of maintaining product temperature between -1 and +4°C.

The emphasis is therefore on low temperature storage.

3.4.2.2 Chilled Foods with Extended Shelf life

Health Canada's 1992 Guidelines for the Production, Distribution, Retailing and Use of Refrigerated Pre-packaged Foods with Extended Shelf life note that 'under Division 27 of the Food and Drug Regulations, refrigeration is defined as 'exposure to a temperature of 4°C or less'. However, Provincial regulatory provisions for refrigeration range from no stipulated transportation temperature requirements (5 provinces) to <5°C (2 provinces), and regarding storage from voluntary 4°C (1 province) to up to 5°C in another.

With respect to shelf life, if this exceeds 10 days, 'the processor should on request, make available appropriate data to the agency bearing responsibility for food safety, showing that the food in question can safely be marketed for the intended shelf life. Such information should include results of microbiological challenge tests involving food poisoning or appropriate non-pathogenic organisms placed in the food and incubated under conditions of temperature abuse. Shelf life tests should also be performed to determine when spoilage occurs relative to the growth of potential pathogens.'

It is notable that the 10 day limit 'is based on considerations related to the time required for *Listeria monocytogenes* to reach levels of concern in a food readily supporting its growth under conditions of appropriate refrigeration, assuming an initial level of 1 cell/g'. i.e. *C. botulinum* is not highlighted as an organism of particular concern in relation to shelf life, but rather with respect to packaging technique.

The document states that Federal/Government agencies' microbiological surveillance programs 'should be instituted to determine the presence of foodborne pathogens in those products for which the shelf life has not been suitably validated by the processor. Priority for monitoring should be given to new extended shelf life foods (e.g. processed by sous vide technology) rather than products such as cured meats which generally have a long established record of safety.'

3.4.2.3 Handling Practices for Chilled Food

The Food Institute of Canada in its undated Canadian Code of Recommended Handling Practices for Chilled Food reiterates -1°C to +4°C as the storage temperature for chilled foods but also states that:

- 'Processed products intended for chilled distribution and sale should be designed with additional hurdles to inhibit food spoilage and poisoning, e.g. pH less than 4.5, reduced water activity, vacuum or modified atmosphere packaging.'

VP and MAP are therefore considered as hurdles to microbiological deterioration.

- Whenever chilled food is received with the products temperature of +7°C or warmer, the warehouse/receiver shall immediately notify the manufacturer and request instruction for special handling. These instructions may consist of any available method for effectively lowering the temperatures such as low temperature rooms with air circulation and proper use of dunnage or separators in stacking.'

The emphasis is therefore on low temperature storage.

3.4.2.4 Best Before/Durable Life

In its 12/1/91 Decision on the use of best before dates/durable life, the Canadian Food Inspection Agency provides the following information indicating no particular shelf life limitation for MAP meat:

"Question: When meat is pre-packed in individual portions at a manufacturing plant and shipped in retail stores in outer containers that have been flushed with a modified atmosphere (i.e. CO_2) designed to extend shelf-life, is the meat manufacturing plant required to label the individual portions of meat with a 'best before' date (durable life date)?

Answer: No, although section B.01.007 of the Food and Drug Regulations requires date marking on food products that have a durable life of less than 90 days, administratively, these products are not required to be so labelled by the manufacturer. The durable life of these products is largely dependent upon when the outer shipping container is opened and the pre-packaged product is exposed to air. Consequently, a best before date established by the manufacturer could be potentially misleading to the consumer. These products are date labelled by the retailer."

3.4.2.5 Garlic in Oil

Following an outbreak in Vancouver in 1985 in relation to a temperature abused garlic in oil product, Health Canada issued guidance targeting domestic/non-industrial production on the safe preparation of garlic in oil. This states "when you make it at home and use it right away it is a safe product. It's also safe if you keep it refrigerated on a continuous basis, and use it within a week. Never store garlic in oil at room temperature. Throw away any that has been in the refrigerator for more than a week."

3.4.3 The position in the USA

The 2005 US FDA CFSAN Food Code (CFSAN, 2005) stipulates the procedures for reduced oxygen packaged (ROP) products, and includes *Listeria monocytogenes* as a pathogen of concern that needs to be controlled in addition to *C. botulinum*.

The Code requires that bagged products must be cooled to 34°F (1°C) within 48 hours and held for no more than 30 days, unless a variance is obtained from the authorities. This appears to be based on *Listeria monocytogenes* growth potential rather than *C. botulinum*.

If foods are packaged using cook-chill (defined as being cooked then hot-filled under reduced oxygen) or sous vide, other food safety measures must be taken including establishment of a HACCP plan, records retention for 6 months, and no sale of the ROP bagged product to another business or consumer that may not have adequate temperature control. However, variances are again available on application to the authorities.

The 2005 Food Code also allows reduced oxygen packaging for hard cheeses, semi-soft cheeses, and pasteurised processed cheeses, but limits shelf life to 30 days. ROP of unfrozen fish is not permitted.

3.4.3.1 Reduced Oxygen Packaging

The 2005 Food Code requires that except for a food establishment that obtains a variance or is otherwise exempt, an establishment that packages potentially hazardous food using a reduced oxygen packaging method shall have a HACCP plan and addressing the need to:

- ensure that there are at least two barriers in place to control the growth and toxin formation of *C. botulinum* and the growth of *Listeria monocytogenes*. These are described as being chilled storage (at 5°C or less) and at least one of the following criteria:
    - an a_W of 0.91 or less, or
    - a pH of 4.6 or less, or
    - a cured or irradiated meat or poultry product produced at a food processing plant regulated by the USDA, and is received in an intact package, or
    - a food with a high level of competing organisms such as raw meat or raw poultry;
- instructions are required on-pack to maintain the food at 5°C or below, and discard the food if within 14 calendar days of its packaging it is not served for on-premises consumption, or consumed if served or sold for off-premises consumption;
- the refrigerated shelf life is limited to no more than 14 calendar days from packaging to consumption, except the time the product is maintained frozen, or the original manufacturer's "sell by" or "use by" date, whichever occurs first;

However, a food establishment can obtain a variance from the regulatory authority before packaging food using a reduced oxygen packaging method where a barrier to *C. botulinum* does not exist in addition to refrigeration.

3.4.3.2 Cheese

The 2005 Food Code allows a food establishment may package cheese using a reduced oxygen packaging method without obtaining a variance if

- the cheese is commercially manufactured within a HACCP plan, and
- has no other ingredients added, and
- meets the Standards of Identity for hard cheeses, pasteurized processed cheese or semisoft cheeses, and
- it labels the package with a "use by" date that does not exceed 30 days or the original manufacturer's "sell by" or "use by" date, whichever occurs first; and
- discards the reduced oxygen packaged cheese if it is not sold for off-premises consumption or consumed within 30 calendar days of its packaging.

3.4.3.3 Fish

The 2005 Food Code states that except for fish that is frozen before, during, and after packaging, a food establishment may not package fish using a reduced oxygen packaging method.

3.4.3.4 Cook-Chill (US definition) or Sous Vide

The 2005 Food Code states that a food establishment may package food using a cook-chill or sous vide process without obtaining a variance if it implements a HACCP plan and the food is:

- Prepared and consumed on the premises, or prepared and consumed off the premises but within the same business entity with no distribution or sale of the bagged product to another business entity or the consumer,
- Cooked to heat all parts of the food to a specified temperature/time
- Protected from contamination after cooking
- Placed in a package or bag with an oxygen barrier before cooking, or placed in a package or bag immediately after cooking and before reaching a temperature below 57°C
- Cooled to 5°C in the package or bag and then cooled to 1°C or less within 48 hours of reaching 5°C, and:
    - Held at 1°C and consumed or discarded within 30 days after the date of preparation, or
    - If removed from a storage unit that maintains food at 1°C, held at 5°C or less for no more than 72 hours before consumption.

The emphasis is on low storage temperature and the control of *Listeria monocytogenes*.

3.4.3.5 Cooking

Heat processes stipulated by FDA are significantly milder than those used by UK industry. The 2005 Food Code requires, with exceptions, raw animal foods such as eggs, fish, meat, poultry, and foods containing these raw animal ingredients, to be cooked throughout to a temperature and one of the time/temperature combinations for ratites and injected meats if they are comminuted (Table 3.3).

Table 3.3 Heat treatments included in the 2005 Food Code for comminuted ratite and injected meats

Minimum	
Temperature (°C)	**Time**
63	3 minutes
66	1 minute
68	15 seconds
70	<1 second (instantaneous)

A heat treatment of 74°C or above for 15 seconds is required for the cooking of poultry, baluts, wild game animals, stuffed fish, stuffed meat, stuffed pasta, stuffed poultry, stuffed ratites, or stuffing containing fish, meat, poultry, or ratites.

In the case of whole meat roasts including beef, corned beef, lamb, pork, and cured pork roasts such as ham, it is necessary to heat all parts of the food to one of the time-temperature combinations shown in Table 3.4

Table 3.4 Heat treatments included in the 2005 Food Code for whole meat roasts

Temperature (°C)	Time in minutes[1]	Temperature (°C)	Time in seconds[1]
54.4	112	63.9	134
55.0	89	65.0	85
56.1	56	66.1	54
57.2	36	67.2	34
57.8	28	68.3	22
58.9	18	69.4	14
60.0	12	70.0	0[2]
61.1	8		

[1] holding time may include post-oven heat rise
[2] to reach 70.0°C

3.4.3.6 Cooling

The 2005 Food Code requires cooked potentially hazardous food to be cooled:

- Within 2 hours from 57°C to 21°C; and
- Within a total of 6 hours from 57°C to 5°C or less, or to 7°C or less.

3.4.3.7 Date Marking

Except for infant formula and some baby food, product dating is not required by Federal regulations. There is no uniform or universally accepted system used for food dating in the United States. Although dating of some foods is required by more than 20 states, there are areas of the country where much of the food supply has some type of open date and other areas where almost no food is dated (USDA, 2001).

Based on the results of FDA's 2001 *Listeria* risk assessment and the recommendations from the 2004 Conference for Food Protection, FDA re-evaluated date marking provisions and focused its recommendations for date marking in the 2005 Food Code on high-risk foods with respect to *Listeria* contamination.

It is notable that the 2005 Food Code exempts 'deli salads' (e.g. ham, chicken, egg, seafood, pasta, potato, and macaroni) prepared and packaged in a food processing plant since 'Scientific data support the exemption of these products because deli salads prepared and packaged by a food processing plant contain sufficient acidity and preservatives to prevent the growth of *Listeria monocytogenes*.'

Whether this statement truly applies to all deli salads manufactured commercially in the USA is not known however.

3.4.3.8 Herbs/Vegetables in Oil

Guidance was developed by the FDA in 1993 targeting domestic and commercial industrial production. It:

- requires such mixes, especially those prepared fresh at home, to be kept refrigerated and states that people should dispose immediately of any products suspected to be spoiled or to have been stored unrefrigerated
- requires manufacturers to stop making garlic-in-oil mixes that rely solely upon refrigeration for safety. Commercial mixes are required to contain specific levels of microbial inhibitors, usually acidifying agents such a phosphoric or citric acid.

- Recommends that consumers do not prepare any homemade spice-in-oil, -margarine or –butter recipes for 'extended storage' because the protective additives used in commercial mixes are not generally available for homemade products

3.4.3.9 Fresh-Cut Produce

IFPA (2001) in its food safety guidelines for the fresh-cut produce industry states that:

- *"C. botulinum* associated with fresh produce will not grow or produce toxin at temperatures below about 10°C, in acid conditions (pH<4.6) or in the presence of a normal concentration of oxygen in the atmosphere". [It is noted that while the comment on oxygen may apply to some produce (although removal of oxygen through product respiration might be an issue), the presence of oxygen is unlikely to restrict toxin formation by non-proteolytic *C. botulinum* in many chilled foods].
- "Growth of spoilage microorganisms on fresh produce normally causes organoleptic deterioration before *C. botulinum* can produce toxin, and rapid growth of [non-pathogenic and facultative] microorganisms should contribute to spoilage before toxin production by proteolytic *C. botulinum* if temperature abuse (>10°C) occurs."

The guidelines appear to focus on the hazard presented by proteolytic *C. botulinum*.

Reference is made to the maximum recommended product temperature being 4.4°C (40°F).

The University of California (2001) issued a compendium of controlled (and modified) atmospheres for horticultural perishables, in which gas mixes and storage temperatures are recommended. For example, for cut or shredded lettuce 0-5°C is given as the recommended temperature and 0°C as optimum. No shelf lives are specified since 'in general, overt gross spoilage of fresh-cut produce occurs well before [*C. botulinum*] toxin is produced on shredded cabbage, shredded lettuce, broccoli florets, sliced carrots and rutabaga. Not only does the endemic microflora on fresh-cut produce play an important role in signalling end of shelf life but it is believed to suppress toxin production by *C. botulinum* (Larson and Johnson, 1999). However, some products such as butternut squash and onions have been demonstrated under temperature abuse conditions to have the potential of being acceptable after detection of botulinal toxin (Austin *et al.*, 1998).'

IFPA (2003) set out packaging approaches for fresh-cut produce, including MAP gas mixes and recommended temperatures (0-5°C). However, no shelf life limitation is given, but reference is made to produce spoiling prior to growth of *C. botulinum.*

Emphasis is on low temperature storage rather than shelf life limitation.

3.4.3.10 VP Sub-Primal Beef Cuts

Guidance regarding the vacuum packing of sub-primal beef cuts that are produced and sold as whole muscle cuts has been developed by the American Meat Institute, National Meat Association and Southwest Meat Association (AMI/NMA/SMA, 2003). However, it makes no mention of specific *C. botulinum* controls, noting that the temperature "at which foodborne pathogens do not grow is 7°C for *Salmonellae* and 7-8°C for pathogenic *E. coli"* and stating that "temperature control throughout the disassembly, storage distribution and further processing at all stages is extremely important. Under no circumstances should boxed sub-primal cuts be left in non-refrigerated cutting rooms, kitchens or unrefrigerated shipping or receiving docks for more than one hour".

3.4.3.11 Refrigerated Foods

NFPA (1989) states that "the establishment of an adequate thermal process to destroy vegetative cell pathogens should consider the number of organisms that are likely to be present in the unprocessed products and the greatest potential heat resistance of the target organism. In most cases, a process designed to destroy 2 log cycles above the maximum level likely to be found will be sufficient. When a product will tolerate a more severe heat process, the product should be processed to destroy the spores of non-proteolytic (psychrotrophic) *C. botulinum*. When a product will not tolerate a process severe enough to destroy these bacterial spores, the risk presented by non-proteolytic (psychrotrophic) and other Groups of *C. botulinum* must be considered and controlled to prevent outgrowth and toxin production".

Specific thermal processes are not given, and 4.4°C is referred to as the recommended storage temperature. No shelf life guidance is given.

3.5 International Trade - Codex Alimentarius Commission

3.5.1 Refrigerated Packaged Foods with Extended Shelf Life

CODEX standards and Codes relate to internationally-traded goods. The Codex Committee on Food Hygiene in 1999 produced a 'Code of Hygienic Practice for Refrigerated Packaged Foods with Extended Shelf Life' (CAC/RCP 46). Extended shelf life is defined in this case as being more than 5 days since the Codex mass catering code is applicable to products with shorter shelf lives. The code gives recommendations for processing, packaging, storage and distribution of refrigerated packaged foods, based on HACCP principles. This code must be used in addition to the Codex General Principles of Food Hygiene (Alinorm 97/13A).

The Code covers low-acid foodstuffs (whatever their packaging format) that are at risk from microbiological pathogens because of their extended shelf life. The Code excludes those food products for which there is already a specific Codex Alimentarius Code of Practice (see Codex website for examples (http://search.fao.org/opensearch, accessed 16/03/06)). The Code does not stipulate particular thermal treatments, shelf lives or storage regimes, but states that:

> "It is the responsibility of the manufacturer to ensure that the product is safe throughout its shelf-life, taking into consideration the potential for temperature abuse. This may warrant the use of hurdles to microbial growth in addition to refrigeration. When using the hurdle concept for product development, even where refrigeration is the sole hurdle, the effect of the hurdle(s) on product safety and shelf life should be considered thoroughly. Predictive microbiological models may be used to estimate both the effectiveness of preservation conditions and the effects of modifying product composition and varying handling/storage conditions on safety. Unless scientific evidence previously exists, challenge studies should be conducted to confirm the effectiveness of the chosen hurdle(s) against the pathogen(s) of concern."

Product shelf life is stated to depend on a number of factors, such as:

- product formulation (e.g. decreased pH, decreased a_w, other hurdles)
- scheduled heat or other preservation treatments
- cooling methods applied to product
- type of packaging (e.g. hermetically sealed or not, MAP)
- storage temperature
- other hurdles

3.5.2 Fish and Fishery Products

A Code of Practice for Fish and Fishery Products is currently being developed by CODEX, the latest publicly available draft of which is included in the report of the 2005 meeting of the CODEX Committee for Fish and Fishery Products (ALINORM 05/28/18 Appendix IX).

- The draft states in relation to VP and MAP products that: "The extent to which the shelf-life of the product can be extended by vacuum or MAP will depend on the species, fat content, initial bacterial load, gas mixture, type of packaging material and, especially important, the temperature of storage. Refer to Appendix 1 for process control issues in MAP".

- MAP should be strictly controlled by:
  - monitoring the gas to product ratio;
  - types and ratio of gas mixtures used;
  - type of film used;
  - type and integrity of the seal;
  - temperature control of product during storage;
- occurrence of adequate vacuum and package;
- fish flesh should be clear of the seam area;
- packaging material should be inspected prior to use to ensure that it is not damaged or contaminated;
- packaging integrity of the finished product should be inspected at regular intervals by an appropriately trained personnel to verify the effectiveness of the seal and the proper operation of the packaging machine;
- following sealing, MAP or vacuumed products should be transferred carefully and without undue delay to chilled storage;
- Ensure that adequate vacuum is attained, and the package seals are intact.

As mentioned above, Appendix 1 of this document relates to MAP, and is entitled "Good process controls are essential when packing fillets and similar products in a modified atmosphere" and follows below.

- Modified atmosphere packing (MAP), in which the composition of the atmosphere surrounding the fillet is different from the normal composition of air, can be an effective technique for delaying microbial spoilage and oxidative rancidity in fish.

- For white fish gas mixtures containing 35-45% CO_2, 25-35% O_2 and 25-35% N_2 are recommended. Gas mixtures containing up to 60% CO_2 in combination solely with N_2 are recommended for oily fish. The inclusion of CO_2 is necessary for inhibiting common aerobic spoilage bacteria such as *Pseudomonas* species and *Acinetobacter/Moraxella* species. However, for retail packs of fillets or similar products, too high a proportion of CO_2 in the gas mixture can induce pack collapse, excessive drip and may cause bleaching.

- Other gases, N_2 and O_2, are included as diluents to prevent these effects. O_2 is preferentially excluded from oily fish in MA packs so as to inhibit oxidative rancidity. A gas/product ratio of 3:1 is commonly recommended. Any reductions in this ratio can result in an impaired shelf-life extension.

- The extent to which the shelf-life of the product can be extended by MAP will depend on the species, fat content, initial bacterial load, gas mixture, type of packaging material and, especially important, the temperature of storage. Determination of the shelf life of a particular product should be by a suitably qualified person such as a food technologist or microbiologist. Since fish can be contaminated with non-proteolytic *C. botulinum* type E great care has to be exercised when determining the shelf life. Although it is generally accepted that non-proteolytic *C. botulinum* does not grow at temperatures below +3°C, other factors, e.g. salt content or pH etc., can also have an inhibitory effect. Thus when determining the

shelf life of MAP fresh fish it is advisable to do challenge tests on the product which accurately reflect the product conditions and storage and distribution environment. It is very important to note that the inclusion of O_2 does not preclude the growth of non-proteolytic *C. botulinum* type E and temperature control throughout the shelf-life of the product is very important. In many circumstances it is considered undesirable to use ice to cool these packs and therefore mechanical refrigeration methods are preferred.

- Seal integrity of MA packs is a critical control point since it determines whether a MA pack is susceptible to external microbial contamination and air dilution of the gas mixture. Essential checks on heat sealing should include proper alignment of the sealing heads or jaws, dwell time, temperature, pressure and machine speed.

- Great care should be taken to ensure that the seal area is not contaminated with product, product drip or moisture since seal integrity may be reduced. In addition, the quality of the film used is important, particularly with regard to gas permeability, and only film with a clearly defined specification from reputable manufacturers should be used.

- Maintenance of the correct gas mixture injected into MA packs is essential to ensure product quality, appearance and shelf life extension. For these reasons routine gas analysis of MA packs should be included as part of the process control. Analysis of the gases within MA packs can indicate faults with seal integrity, MA materials, MAP machinery or gas mixing prior to flushing. The use of continuous gas analysers is recommended. Immediate gas analysis following packing is necessary as CO_2 absorption takes place rapidly.

3.5.3 Smoked Fish

A Draft Standard for Smoked Fish is being developed within the CODEX Fish and Fishery Products Committee, although it is at an early stage at the time of writing (Step 3).

Para 5.6 of the current draft (ALINORM 05/28/18, Appendix V) states in relation to *C. botulinum* that: “Toxins of *C. botulinum* are not allowed in smoked fish products. The formation of *C. botulinum* toxin can be controlled through an application of science-based options involving packaging type, storage temperature, and the use of salt in the water phase. The table shown in Annex 1 addresses these control options”, and is reproduced as Table 3.5. Annex 1 on ”Control and prevention of *C. botulinum* toxin formation” states that:

- Countries where the products are to be consumed can be expected to make their science-based risk management choices within this framework, i.e. select some option and exclude others, based on conditions within the country (e.g. nature and enforcement of refrigeration and shelf life controls, transportation times and conditions, variability in amount of salt in the water phase that could occur despite best efforts to achieve a required percentage), and the level of protection that the country chooses for itself for this particular risk.

Table 3.5 Control and prevention of *C. botulinum* toxin formation in smoked fish (Draft CODEX)

Storage temperature	Packaging	Water phase salt *	Comments
0°C to 3°C	Any	No minimum water phase salt is needed.	Temperature monitoring required on each package
>3°C to 5°C	Aerobically Packaged	No minimum water phase salt is needed. Nonetheless, where there is a reasonable possibility of severe time/temperature abuse, the country where the product is being consumed might choose a water phase salt barrier of at least 3.0% to 3.5% as a precautionary measure.	Storage temperature is for the control of pathogens generally and for quality. In air-packaged products, aerobic spoilage organisms provide sensory signs of spoilage before the formation of toxin by *C. botulinum.* However, even in air packaging it is possible for anaerobic micro-environments to exist and toxin may form if the product is subject to severe time/temperature abuse. For that reason, the country where the product is consumed may still require water phase salt as a barrier to growth of non-proteolytic *C. botulinum* if there are concerns about the ability of transporters, retailers or consumers to maintain time/temperature control.
Frozen ≤ -18°C	Reduced Oxygen (inc. VP and MAP**)	No minimum water phase salt is needed for safety.	*C. botulinum* toxin cannot form when product is frozen. Because toxin production can occur after thawing, labelling information about the need to keep frozen, to thaw under refrigeration, and to use the product immediately after thawing is important.
>3°C to 5°C	Reduced Oxygen (inc. VP and MAP)	Water phase salt at minimum level of between 3.0- 3.5% may be selected by the country where the product is to be consumed.	Water phase salt at a minimum level of between 3.0-3.5% (water phase salt) in combination with chilling will significantly delay (or prevent) toxin formation.
>5°C to 10°C	Reduced Oxygen	5% Water Phase Salt	Non-proteolytic *C. botulinum* is controlled under these conditions.

* As an alternative to water phase salt, time/temperature controls alone may be used. *C botulinum* cannot grow and produce toxin at or below 3°C. Other time/temperature combinations exist that similarly control the formation of toxin (Skinner and Larkin, 1998) Where enforcement of shelf life as well as consumer acceptance of shelf life are norms, the country may select a system that relies on the combination of existing storage temperature conditions (i.e. during transport, retail storage, and consumer storage) and shelf life limitations. However, in countries where consumer acceptance and regulatory enforcement of shelf life are not norms, continuous monitoring, such as that provided by time/temperature integrators on consumer packages, may be selected as a control by the country where the product will be consumed. The necessity for time/temperature integrators exists because, unlike freezing, temperature control through refrigeration is not a visual condition and cannot be determined without an additional monitoring control.

** As new technologies are developed, e.g. modified atmosphere with high oxygen, new controls may be defined.

3.6 Summary of Key Stipulated Process Parameters for VP/MAP/ROP Chilled Foods in Different Documents

Table 3.6 Summary of key stipulated process parameters for chilled foods in different documents

The process parameters summarised below must only be applied within the context of the original document.

Reference	Pasteurisation	Other control parameters	Post process chilling	Pre-distribution storage temperature	Distribution/ retail storage	Shelf life	Basis
ACMSF 1992	If shelf life >10 days: 90°C/10 mins	If >10d under chill use at least one hurdle if not heated 90°C/10 mins equiv. i.e. pH<5, $a_w \leq 0.97$ throughout, NaCl 3.5% (aq) throughout or combination of heat and hurdles shown to prevent growth and toxin formation by non-proteolytic *C. botulinum*	Not specified	10d max at 10°C unless non-proteolytic *C. botulinum* control in place in addition to chill			Predictive models, industry practice, product history, chill chain performance
ACMSF 1995	90°C/10 mins unless stored at either 5-10°C for ≤5 days or 10 days maximum at <5°C	As ACMSF 1992	Not specified	Where chilled storage is the sole controlling factor, chilled foods stored at 5-10°C should have an assigned shelf-life of ≤5 days. Where a shelf-life of up to 10 days is required, the Group recommended that the storage temperature should be at ≤5°C.			Growth in broth and fish/turkey homogenate challenge test data
AFIC, ASI, RWTAA 1999	Not applicable	Not specified	Not applicable	0°-4°C, never warmer than 5°C		Not specified	Australian law
Ag Canada, 1990	Determined by manufacturer. Reference is made to French legislation	Product ≤10°C before cold filling	to ≤ 4°C in 120 min	-1° to 4°C		Not Determined by manufacturer.	French legislation?

Reference	Pasteurisation	Other control parameters	Post process chilling	Pre-distribution storage temperature	Distribution/ retail storage	Shelf life	Basis
AIFST/ ACCC 2000	Cook-chill cook tank: VP products placed in water tank or steam and slow cooked until the desired temperature is reached - either 70°C/2 mins or 90°C/11 mins equiv or 'according to recipe'	Determined by manufacturer (GMP)	Begin chilling within 30 mins of cooking. Chill to 3°C within 90 mins	-1° to 3°C		'Long shelf life with correct practice can be achieved.' 'It has been found that some products have a shelf life of up to 45 days.' Micro testing and quality evaluation must be carried out when setting up the process and on a regular basis.	Not stated/ HACCP
	Cook-chill sous vide: either 70°C/2 mins or 90°C/11 mins equiv or 'according to recipe'	Determined by manufacturer (GMP)		-1° to 3°C		'It has been found that some products have a shelf life of up to 45 days.' 'Micro testing and quality evaluation must be carried out when setting up the process and on a regular basis, at least annually.'	Not stated/ HACCP
	Cook-chill VP & MAP/gas flushing: 70°C/2 mins at core. Extended shelf life: time-temperature control to eliminate *C. botulinum*	Determined by manufacturer (GMP)	Not specified	MAP meat <3°C		Note shelf life tests need to recommence after repackaging as previous tests cannot be relied upon	Not stated
AMI/NMA/SMA	Not applicable	Chill, avoid cross-contamination	Not applicable	7°C implied		Not stated	Lower growth temperature of *Salmonella*
AQIS 1992	6D reduction of target organism: Lm D70=0.34 min, z=7.5°C non-prot *C. bot* , D80=21.6 min, z=9°C	Determined by manufacturer (GMP)	Chilling to start within 30 min. To reach storage temperature in 4h.	Extended shelf life: ≤3°C or if 6D process for non-prot. *C. bot*: ≤ 5°C. If >5°C, shelf life max 5 days. If product >10°C, discard product		>5 days if ≤ 3°C or if ≤ 5°C eat within 10 days of purchase unless frozen	Not stated
CCFRA 1996	Short shelf life: 70°C/2 min equiv Long shelf life: 90°C/10 min equiv or <90°C/10 mins equiv + at least one hurdle Note: shelf life is not impacted by storage at ≤3°C since there will be no growth of *C. botulinum*.	>10d under chill use at least one hurdle if not heated 90°C/10 mins equiv. i.e. pH<5, a_w ≤0.97 throughout, NaCl 3.5% (aq) throughout or combination of heat and hurdles shown to prevent growth and toxin formation by *C. botulinum*	Chilling to start within 30 min. of cooking As quick as possible Portion size pack: to 5°C in 90 min. Larger quantity: from 50°C to 10°C in 4 hours	If ≤3°C then no reduction of total shelf life. If ≥3°C and ≤8°C then shelf life 'clock' to be started	≤8°C	short shelf life: ≤10 days long shelf life: >10 days	ACMSF 1992 + England, Wales & NI legal temperatures

Reference	Pasteurisation	Other control parameters	Post process chilling	Pre-distribution storage temperature	Distribution/ retail storage	Shelf life	Basis
CCFRA 1992a	Not specified - refers to CFA (1989) guidelines	Hygiene Gas mixtures Chill	Not specified	-1°C to +5°C, depending on product		Various, depending on product	Various industry and other guidance
CCFRA 1992b	90°C/10 mins (shelf life >10d) 70°C/100 mins (shelf life 1-10d)	Not specified	Chilling to start within 30 min. of end of cooking and reach 0-3°C in 90 min.	0-3°C	0-3°C if product > 10°C, discard product	1-10 days: short 10-42 days: long	Various published literature (90°C/10 mins), then current French legislation (70°C/100 mins)
CFA 2006	Shelf life 10 days max: equiv to 70°C/2 mins Shelf life >10 days: equiv to 90°C/10 mins or other measures demonstrably controlling non-prot *C. botulinum*	If >10d (VP/MAP/air) under chill use at least one hurdle if not heated 90°C/10 mins equiv. i.e. pH<5, a_w <0.97, or use demonstrably effective combination of hurdles. GMP.	Chilling as soon as it is practicable after heating, assembly or air drying has been completed. The cooling rate must be such that significant growth of surviving microorganisms is prevented.	Determined by manufacturer but ≤8°C	≤8°C	1-10 days: short – limit *Listeria monocytogenes* survival/growth potential >10 days: long – control measures to be determined by manufacturer to assure safety	ECFF Botulinum WP, CCFRA 1996
CFA 1997	Shelf life 10 days max: equiv to 70°C/2 mins Shelf life >10 days: equiv to 90°C/10 mins or other measures demonstrably controlling non-prot *C. botulinum*	Demonstrably effective combination of hurdles. GMP	As quick as possible through temperature range 63°C to 8°C	≤5°C	≤8°C preferable: ≤5°C	10 days maximum for unless non-proteolytic *C. botulinum* controls in place	CCFRA, 1996
CFIA, 1997	Not applicable	Not specified	Not specified	Not specified		Not specified	Not stated
CFSAN	Various mild processes, e.g. Raw animal foods: • 63°C/3 mins or • 66°C/1 min or • 68°C/15 secs or • 70°C/<1 sec Poultry/stuffed products: 74°C/15 secs	Chill + aw≤0.91 or pH≤4.6 or is cured meat or has a high level of competing organisms e.g. raw meat/fish	Cooked potentially hazardous food is required to be cooled within 2h from 57°C to 21°C; and within a total of 6h from 57°C to 5°C or less, or to ≤7°C.	5°C max		14 days: ROP products 30 days: ROP cheese	Various FDA documents. Control of *Lm* and *C. botulinum* (including proteolytic)
	• Whole meat roasts: range from 54.4°C/112 mins to 70°C/0 secs (instantaneous)		Cook-chill and sous vide: cooled to 5°C in the package or bag then cooled to ≤1°C within 48h of reaching 5°C	Cook-chill and sous vide foods: 1°C max		30 days: cook-chill and sous vide foods	

Reference	Pasteurisation	Other control parameters	Post process chilling	Pre-distribution storage temperature	Distribution/ retail storage	Shelf life	Basis
CODEX (draft)	Not applicable	NaCl 3-3.5% (aq) at >3°C to 5°C, or NaCl 5% (aq) at >5 to 10°C	Not applicable	>3 NaCl to 5°C or >5 NaCl to 10°C (select NaCl accordingly)		Not specified. Determined by manufacturer	Skinner/ Larkin
ECFF 1996	Up to 10d shelf life under chill: 70°C/2 mins equiv >10d shelf life under chill: 90°C/10 mins equiv or use combination of hurdles	Alternatively use combination of demonstrably effective hurdles	'The cooling rate must be such that significant growth of surviving microorganisms is prevented, e.g. to 10°C within 2h'	≤3°C predist. storage (sous vide products)	≤4°C (sous vide products)	Determined by manufacturer on basis of demonstrable safety	ECFF Botulinum WP/ACMSF 1992
ECFF Botulinum WP 1999	Long shelf life (>5 days): 90°C/10 mins equiv, or <90°C/10 mins equiv and 3°C, 'other preservative factors are present and operating'	Alternatively use combination of demonstrably effective hurdles	Not specified	Up to 5d shelf life without non-prot *C. botulinum* control or storage <3°C or combination of demonstrably effective hurdles		Up to 5d or storage <3°C, or 6-log reduction process for non-prot *C. botulinum,* or intrinsic factors (hurdles) inc. lower heat process in-pack	Independent scientific review
EVI 2000	Not applicable	Not specified	Not specified	+3°C maximum		3 weeks at +3°C max	Predictive modelling (*Lm* growth)
FDA, 1993	Not applicable	Domestic: refrigerate Commercial: Add microbial inhibitors (e.g. citric or phosphoric acid)	Not specified	Refrigerate		Not specified	Not specified
Food Institute Canada	Determined by manufacturer	Products should be designed with additional hurdles to inhibit food spoilage and poisoning, e.g. pH 4.5, reduced a_w, VP or MAP	Determined by manufacturer	-1°C to +4°C. If ≥7°C on receipt instruction for special handling shall be requested from the manufacturer. These instructions may consist of lowering the food temperature.		Determined by manufacturer	Unknown
FSA	Not applicable	Chilled	Refrigerate	Refrigerate		1 week maximum	Not specified

Reference	Pasteurisation	Other control parameters	Post process chilling	Pre-distribution storage temperature	Distribution/ retail storage	Shelf life	Basis
Health Canada	Determined by manufacturer	Determined by manufacturer	Not specified	+4°C maximum		Manufacturer to make available appropriate data showing safety for intended shelf life if >10 days	10 day cut off based on *Listeria monocytogenes* growth potential
Health Canada	Not applicable	Chilled	Refrigerate	Refrigerate		1 week maximum	Not specified
IFPA	Not applicable	Chilled	Not specified	+4.4°C maximum		Not specified	Various research
IFPA	Not applicable	Chilled	Not specified	0-5°C		Not specified	Various research
MOFFA	Not applicable	'3.5% NaCl (aq) may be necessary throughout a VP food'	Not applicable	8°C max 'may be required by law' but note general exemption for mail order foods		Limit shelf life for VP/MAP foods where temperature is the only control and is >3°C to no more than 8°C	CCFRA 1996
NFPA, US 1989	5D reduction of *E. coli* O157:H7 in ground beef 7D reduction of *Salmonella* in poultry	Determined by manufacturer (GMP)	Not specified for MAP/VP	< 4.4°C	< 5°C	Scientific evidence needed to support labelled shelf-life	Not specified
PTK/ ETL/ SKKL	Not applicable	Not specified	Not specified	0-3°C	0-3°C (max 6°C)	Not specified, but limited by document 16	Predictive modelling of *Lm* growth potential
SVAC 1991	Targeting non-prot. *C. bot.* type E, D_{80}=4.3 min, z=13.2°C: 80°C/26 min, or 85°C/11 min, or 90°C/4.5 min, or at 95°C/2 min.	Not specified	Chilling to start within 30 min. of cooking. Reach 0-3°C in 90 min.	0-3°C		Max. 8 days	Not stated
SYNAFAP 1995	Determined by the manufacturer (after hazard analysis)	Determined by manufacturer (GMP)	Minimum time between 60°C and 10°C: gen. less than 2h	<4°C		Determined by manufacturer using shelf life validation protocol	HACCP
SYNAFAP (Draft, 2006)	Determined by the manufacturer: for sous vide it is acceptable to take account of the natural level of contamination and adjust the process (log reduction) accordingly	Determined by manufacturer (GMP)		<4°C		For sous vide, a shelf life of 21 days, even 30 days, is acceptable, taking account of the natural level of contamination, respecting GHP and under controlled chill chain conditions.	Carlin *et al* spore loading study, Cemagref/ANIA chill chain study, Institut Pasteur Lille challenge test study

Reference	Pasteurisation	Other control parameters	Post process chilling	Pre-distribution storage temperature	Distribution/ retail storage	Shelf life	Basis
TNO 1994	90°C/10 min total (including duo pasteurisation option) Reference org. non-prot. *C. bot.* type B. D_{90}=10 min. z=10°C if T>90°C or z=7°C if T<90°C	Determined by manufacturer (GMP)	to 10°C in 2h to ≤ 3°C in 12 hours	0-3°C	0-5°C	Max. 42 days including max. 3 weeks outside manufacturers' facility max. 1 week in domestic fridge	CODEX HACCP, ACMSF 1992
UC 2001	Not applicable	MAP gas mixtures	Not specified	Dependent on product, e.g. 0-5°C for MAP shredded lettuce		Determined by manufacturer	Not specified
UNIPI	70°C/2 mins or 90°C/10 mins Choice determined by manufacturer	Determined by manufacturer (GMP)	Determined by manufacturer	Not specified		Determined by manufacturer	HACCP
US CFA	Determined by the manufacturer	Determined by manufacturer (GMP)	Determined by manufacturer	1.7°C (35°F)	Not specified	Determined by manufacturer	HACCP

3.7 Conclusions

Overall approach

- There are no legal requirements in the UK in relation to VP/MAP chilled foods, except that they should be stored at a temperature of 8°C or less (as defined in law in England, Wales and Northern Ireland. In Scotland the requirement is to keep chilled food in "a refrigerator, or refrigerating chamber, or a cool ventilated place") and produced using HACCP principles, as required under European hygiene law. Recommendations have been made by the ACMSF (1992 and 1995), and there is guidance in an industry code and other industry documents.
- The 1992 ACMSF recommendations on the safe production of VP/MAP chilled foods with respect to non-proteolytic *C. botulinum* were published formally (ACMSF, 1992). The report included the recommendation of a maximum shelf life of 10 days at 10°C maximum unless appropriate controls (formulation and/or processing) were in place. The recommendations were taken up widely by professional food manufacturers and retailers in the UK and were taken forward into various other guidance including internationally.
- In 1995, one of the key ACMSF recommendations was revised (ACMSF, 1995). In particular the recommendation of a maximum shelf life of 10 days at 10°C maximum unless appropriate controls were in place, was replaced with the recommendation of a maximum shelf life of 5 days at 10°C maximum or 10 days at 5°C maximum unless appropriate controls were in place. This change was made on the basis of a review of 31 references from the literature on toxin production by non-proteolytic *C. botulinum* within 10 days at $\leq$10°C (mostly challenge tests carried out in food materials). The ACMSF 1995 approach was not taken up by industry or included in other guidance.
- An Industry Code of Practice for the manufacture of VP/MAP chilled foods was written by a working group of manufacturers, retailers and MAFF and DH representatives (CCFRA, 1996). It aimed to crystallise various guidance into practical advice. One of the recommendations included was a maximum shelf life of 10 days at 8°C maximum unless appropriate controls (formulation and/or processing) were in place. The code recommendations were taken up widely by professional food manufacturers and retailers in the UK and were taken forward into various other guidance.
- Both in the UK and internationally, a variety of approaches are described, and most allow for flexibility of control measures on the basis of safety being demonstrable by the manufacturer on the basis of HACCP.

Thermal Processes

- Many documents refer to 6-log non-proteolytic *C. botulinum* processes for long shelf life products (90°C/10 min or equivalent), which are based on the ACMSF 1992 approach. However, different z values are referred to, resulting in a range of processes that are intended to be equivalent, but which are not in reality. The appropriate choice of z value is a matter that needs to be addressed by research.
- The use of heat treatments less than 90°C/10 min (or equivalents) can be effectively combined with storage temperature and shelf-life to prevent toxin formation from 10^6 spores of non-proteolytic *C. botulinum* (i.e. provide a 6-log non-proteolytic *C. botulinum* process). For example, heating at 80°C for 11 min and for 98 min, prevented toxin formation at 8°C in 20 days and 40 days, respectively.
- In France, legislation has permitted the use of heat treatments that deliver less than a 6-log non-proteolytic *C. botulinum* process. For example, the 1988 legislation enabled ready meals receiving a process equivalent of 70°C for 100 min (core temperature of 65°C; and equivalent to only 90°C for 1 min) to have a shelf life of up to 21 days, although the precise shelf life was to be determined by the manufacturer.
- The French retail sous vide industry includes in its current approach the use of less than a 6-log non-proteolytic *C. botulinum* process. Heat treatments less than 90°C/10 min (or

equivalents), such as 80°C for 93 min, combined with chilled storage regimes have been shown to provide for shelf lives of up to 30 days.
- All approaches either leave the selection of thermal processes to the manufacturer to determine using HACCP, or provide example thermal equivalents, with flexibility for equivalents or otherwise demonstrably effective processes to be used.

Post-Process Chilling
- In all cases rapid chilling of cooked foods is recognised as being a key CCP. However, a range of time/temperature combinations are specified. CFA and ECFF guidance require chilling such that significant growth of surviving microorganisms is prevented.

Hurdles/Intrinsic Factors
- Examples of single hurdles targeted at control of non-proteolytic *C. botulinum* are included in many documents, such as those produced by the ACMSF (1992, 1995), and the Industry Code of Practice (CCFRA, 1996). Examples of the hurdles include, pH<5 throughout, $a_w \leq 0.97$ throughout, and NaCl 3.5% (aq) throughout.
- Some documents appear to be targeted at control of other pathogens such as *Listeria monocytogenes* and proteolytic *C. botulinum* (e.g. CFSAN, 2005), rather than non-proteolytic *C. botulinum*.
- The importance of HACCP is stressed, and allow for the manufacturer to select appropriate hurdles (e.g. CFA, ECFF and SYNAFAP guidelines).

Chilled Temperatures
- Storage at temperatures up to 3°C are generally recognised as being a means of preventing the growth and toxin formation by non-proteolytic *C. botulinum*.
- In all approaches the main emphasis is on low temperature storage (not necessarily with specific limitation of shelf life). However, the specified temperatures vary. For example the Canadian and French approaches refer to 4°C as the national legal maximum, while in England, Wales and Northern Ireland this is 8°C. In Scotland the requirement is to keep chilled food in “a refrigerator, or refrigerating chamber, or a cool ventilated place”.
- Cross-reference needs to be made to the actual performance of the chill chain, including in the home.

Shelf life
- A wide range of approaches exist, but the emphasis is on demonstrable safety and HACCP.
- In many cases the shelf-life relies on a controlling factor in addition to storage at chill temperature alone. For example, draft French industry guidance reflecting longstanding practice for sous vide foods allows for a shelf life of 21 days and potentially up to 30 days for products subjected to less than 6-log thermal reduction of non-proteolytic *C. botulinum*, provided that GHP is respected, and the product is distributed under controlled chill chain conditions.
- Other advice relies on storage at 3°C or below. For example, Finnish Government advice in relation to cold smoked and gravad fish is for shelf life to be a maximum of 3 weeks at 3°C, being based on the potential for *Listeria monocytogenes* growth rather than non-proteolytic *C. botulinum*.
- A general feature of many guidance documents is that a shelf life of 10 days is permitted under chilled storage conditions, and that if a shelf life of greater than 10 days is required then the manufacturer is required to make available appropriate data demonstrating safety. The importance of HACCP is stressed.

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Chapter Four - Storage and Handling of Chilled Food

Recent decades have seen a considerable increase in legislation setting maximum temperatures during the production, distribution and retailing of chilled food. Surveys on the temperature performance of commercial chill chains are relatively scarce however, and official sampling of the microbiological quality of commercially available foodstuffs often fail to report product temperatures at the time of sampling.

As soon as the food is purchased by the consumer it is outside any legislative requirements. Consumer handling of products may not be as intended or envisaged by the manufacturer. Again there are relatively few published studies on the performance of domestic refrigerators and on consumers' attitudes to chilled food and their handling procedures in the home.

4.1 Pre-distribution temperatures

No published data are available regarding pre-distribution storage temperatures, i.e. product storage by manufacturers prior to despatch to their customers.

However, UK manufacturers' on-site storage and despatch of chilled prepared foods is usually at ≤5°C, and often under "deep chill".

General French chilled food manufacturing industry practice is reported to involve storage at <4°C and can be for a relatively long term period (from days to approximately three weeks) (SYNAFAP, Personal communication).

Storage at such low temperatures effectively extends shelf life in relation to non-proteolytic *C. botulinum* since growth rates are so slow, or there is no growth at all if <3°C. This principle is reflected in the CCFRA (1996) industry code (see Chapter 3).

4.2 Legislated commercial temperatures

Nationally legislated temperature rules and commercial agreements set maximum temperatures for certain commercial chilled foodstuffs. A lack of an EU harmonised approach is believed to be largely due to the uneven development of markets internationally (see Tables 4.1 and 4.2, the following text, and SCOOP report (1996)).

Table 4.1: Retail temperature requirements in various European countries (ECFF, Personal communication)

Product Type	Required retail temperature (°C) in specified country						
	Belgium	Finland	France	Germany*	Italy	NL	UK***
Air or product temperature?		Air except protein	Air				Product
Fresh-cut produce	7 LN	8 LN	4 LN	7		7 LN	8 LN
Soups, Sauces	7 LN	6 NGL	4 LN			5	8 LN
Sandwiches	7 LN	6 NGL				7 LN	8 LN
Ready to cook dough	7 LN					7 LN	8 LN
Fresh pasta, plain & filled	7 LN	8 LN			4 LN	5	8 LN
Fresh gnocchi	7 LN	8 LN			NGL being developed	5	8 LN
Pizza	7 LN	6 NGL				7 LN	8 LN
Savoury pastries and quiches	7 LN	6 NGL				7 LN	8 LN
Dressed salads, dips	7 LN	6 NGL	4 LN	7		7 LN	8 LN
Sweet pastry desserts	7 LN	8 LN				5	8 LN
Other chilled ready meals	7 LN	6 NGL 8 LN	4 LN			5	8 LN
Raw red meat	7 LEU	4 (max 6) NGL	7 LEU	7 LEU	7 LEU	7 LEU	7 LEU
Raw poultry	4 LEU	4 (max 6) NGL	4 LEU	4 LEU	4 LEU	4 LEU	4 LEU
Raw game	7 LEU	4 (max 6) NGL	7 LEU	7 LEU	4 (small size); 7 (big size) LEU	7 LEU	4 LEU
Minced red meat	2 LEU	2 LEU	2 LEU	2 LEU	2 LEU	7	2 LEU
Minced poultry meat	4 LEU	4 LEU	4 LEU	4 LEU	4 LEU	4	4 LEU
Meat preparation	4 LEU	4 LEU	4 LEU	4 LEU	4 LEU	4	4 LEU
Chilled cooked meat products	7 LN	6 NGL	4 LN			5	8 LN
Chilled cured meat products	7 LN	7 LN				7	8LN
Chilled fermented meat products	7 LN	7 LN				7	8LN
Raw fish, cooked and chilled crustaceans and molluscs	melting ice LEU	0 to 3 NGL	melting ice LEU	2	0 to 4 LN	4	melting ice LEU
Cured fish		6 NGL			0 to 4 LN	7	
Smoked fish	4 LN	6 NGL**			0 to 4 LN	7	8 LN
Dairy Desserts	7 LN	8 LN	4 LN	10	4 LN	7	8 LN
Fermented dairy products, not heat treated	7 LN	8 LN		10	4 LN	7	
Non cured cheese	7 LN	8 LN		10		7	
Cured cheese		8 LN		10		7	

LEU = covered by EU legislation; LN = covered by national legislation; NGL = covered by national guidelines other than those adopted by legislation

* Germany: Legal temperature requirements only exist for milk, meat and fish. For other products temperatures are on the basis of convention. ** Finland, cold smoked VP fish and raw lightly salted fish (gravad) 0 to 3°C (national commercial agreement & recommendation of National Food Agency). ***UK, chill temperature is specified as 8°C in England, Wales and NI. In Scotland, chilled food is to be stored in a refrigerator, or refrigerating chamber, or a cool ventilated place.

Canada: Product temperature should be maintained between -1°C and 4°C. If product is found to be 7°C or warmer on receipt by a warehouse operator or in foodservice "instructions for special handling" should be requested from the manufacturer. These instructions may consist of any available method for effectively lowering temperatures such as low temperature rooms with air circulation and proper use of dunnage or separators in stacking. (Food Institute Canada, undated).

USA: Refrigerated, potentially hazardous food (i.e. requiring time/temperature control) shall be at a temperature of 5°C or below when received, unless a specific law applies (e.g. milk, molluscan shellfish). Cook-chill in reduced oxygen packages or sous vide must be cooled to 5°C in the package or bag and then cooled to 1°C or less within 48 hours of reaching 5°C, and either held at 1°C and consumed or discarded within 30 days after the date of preparation, or if removed from a storage unit that maintains a 1°C food temperature, held at 5°C or less for no more than 72 hours before consumption (CFSAN, 2005).

CODEX: For refrigerated foods, an important safety hurdle to control microbial growth is refrigeration (for example, +4°C). Any recommendation for specific temperatures should be considered guidelines only. The actual temperatures used will depend upon the requirements for the product, and processes used in terms of safety (CODEX, 1999).

Table 4.2: Maximum Retail Storage Temperatures for Chilled Ready Meals in some EU Countries (CCFRA, 2004)

Country	Maximum legal retail storage temperature (°C)
Belgium	7
Denmark	5
Finland	8*
France	≤4
Spain	0-3
Sweden	<8
UK	8**

* In Finland, commercial agreement between retailers and manufacturers to operate at 6°C.
**In UK, chill temperature is specified as 8°C in England, Wales and Northern Ireland. In Scotland, chilled food is to be stored in a refrigerator, or refrigerating chamber, or a cool ventilated place.

The EU SCOOP Report (1996) states that "it is not necessary to recommend a single storage temperature for all chilled foods, irrespective of the processing received and, conversely, a large number of product-specific temperatures would not be practical. In practice, both temperature and shelf life need to be considered together." The report made a number of recommendations for further work to be carried out, which are still valid:

1. To clearly define the shelf life concept(s) and to agree common definitions at a European Community level
2. The Commission should consider conducting a survey of food and air temperatures in retail display cabinets in Member States to indicate the practicability of setting broad or product-specific temperatures across the Community
3. To monitor temperature fluctuations in the chilled food chain from production through to the point where the consumer selects the product
4. Options for the accurate prediction of food temperature behaviour during transport, storage and retail display should be explored

5. Ways of linking temperature requirements in 'vertical' directives [Note: no longer in force] with any temperature requirements at the retail level should be explored
6. The role food labelling might have in ensuring that foods are held at appropriate temperatures and with a satisfactory shelf life should be explored
7. Data relating to the microbial safety of foodstuffs should be collected and presented in a systematic and well structured format to aid the dissemination of important information to relevant authorities
8. Information on specific factors such as pH and a_w need to be available, particularly for more complex foods, e.g. recipe dishes/fermented foods.

Internationally, the UN "Agreement on the International Carriage of Perishable Foodstuff and on the Special Equipment to be used for such Carriage" (ATP) applies. ATP is an Agreement between States, and there is no overall enforcing authority. ATP applies to transport operations performed on the territory of at least two of Contracting Parties (i.e. countries – see below). In practice, highway checks are carried out by Contracting Parties, and non-compliance may then result in legal action by national authorities against offenders in accordance with their domestic legislation. ATP itself does not prescribe any penalties.

A number of countries, including the UK, have adopted the ATP as the basis of their national legislation: Austria; Azerbaijan; Belarus; Belgium; Bosnia and Herzegovina; Bulgaria; Croatia; Czech Republic; Denmark; Estonia; Finland; France; Georgia; Germany; Greece; Hungary; Ireland; Italy; Kazakhstan; Lithuania; Luxembourg; Monaco, Morocco; Netherlands; Norway; Poland; Portugal; Romania; Russian Federation; Serbia and Montenegro; Slovakia; Slovenia, Spain; Sweden; The former Yugoslav Republic of Macedonia; UK; USA; and Uzbekistan.

However, the Agreement's product scope is limited as international trade in prepared chilled foods is also limited. Examples of agreed distribution temperature are shown in Table 4.3.

Table 4.3: International Distribution Temperature Agreement - Conditions for the Carriage of Certain Foodstuffs which are Neither Quick (deep)-frozen nor frozen (ATP, 2003)

Foodstuff	Maximum temperature (°C)	Notes
Red offal	3	In principle the duration of carriage should not exceed 48h
Butter	6	
Game	4	
Milk (raw or pasteurised, for immediate consumption)	4	In principle the duration of carriage should not exceed 48h
Industrial milk	6	In principle the duration of carriage should not exceed 48h
Dairy products (yoghurt, kefir, cream and fresh cheese)	4	In principle the duration of carriage should not exceed 48h 'Fresh cheese' means non-ripened (non-matured) cheese which is ready for consumption shortly after manufacturing and which has a limited shelf life
Fish, molluscs and crustaceans	Must always be carried in melting ice	In principle the duration of carriage should not exceed 48h
Meat products	6	Except for products stabilised by slating, smoking, drying or sterilisation
Meat (other than red offal)	7	
Poultry and rabbits	4	

4.3 Commercial Temperature Control in Practice

A UK retailer survey showed that the average temperature of retail storage was 4°-6°C. (CCFRA, 2004). Microbiological surveys by the PHLS (now HPA) in the UK have reported ranges of product temperatures on sale. On average, 94% of the 10,596 retail samples taken in three recent reports were found to be at ≤8°C. However, the reports did not detail the actual temperatures, simply that they were lower than 8°C (Table 4.4). This is perhaps an area overlooked in such surveys, giving actual temperatures and not simply noting < or > value would be a useful addition and give a greater value to the reports.

Table 4.4: UK PHLS/HPA Retail product temperatures

Product	Storage temperature (°C)	% Samples held at temperature	Number of samples	Outlet types	Ref.
Cold meat and pate	≤8	95	3,766	Supermarkets, butchers, delis, market stalls, other retail	Elson *et al.* (2004)
	>8	5	215		
	Total		3,981		
Bagged prepared RTE salad vegetables	≤8	94	3,511	Retail outlets	Sagoo *et al.* undated (2001 survey)
	>8	6	229		
	Total		3,740		
VP and MAP cooked RTE meats at the end of shelf life	<5	71	2,030	Supermarkets, corner shops, butchers, greengrocer, delis, market stalls, farm shops	Sagoo *et al.* (2006)
	>5-8	24	685		
	>8	6	160		
	Total		2,875		
Overall totals	**≤8**	**94**	**9,992**		
	>8	**6**	**604**		
	Total		**10,596**		

The Food Safety Authority Ireland, in surveys of the microbiological quality of certain retailed foods, reported on product temperatures at the time of sampling from points of sale:

- The core temperature of 168 samples of pre-packed sandwiches obtained from retail premises which had been prepared and packed on-site or which had been delivered from off-site manufacturers was measured, and 57% of samples were at >5°C, 26% of samples were at >10°C and 8% were at >15°C (Table 4.5).
- The temperature displayed in storage units of retailed pre-packed cooked sliced ham (MAP) for 14% of samples was above 5°C, and for 2% of samples the core temperatures >10°C (Table 4.6). The mean core temperature in the ham was 5.7°C, and the range was 1.5°-12.7°C.

Table 4.5: Measured core temperatures of pre-packed retailed sandwiches in Ireland (FSAI, 2002)

Measured temperature (°C)	Number of samples	% samples
0-5	74	43
>5-10	51	30
>10-15	30	17
>15	13	8
Total >5	94	57
Total >10	43	26
Total	168	100

Table 4.6: Storage unit temperature and measured core temperatures of MAP pre-cooked sliced ham in Ireland (FSAI, 2003)

Storage Unit Temperature (°C)		Product Core Temperature (°C)			
Temperature displayed	Number (%) of samples within temperature range	Number of samples for which core temperature was taken	Min	Mean	Max
<0	9 (6)	7	2.2	4.2	5.4
0-5	134 (81)	73	1.5	5.6	12.7
>5-10	20 (12)	9	3.7	6.2	9.2
>10-15	3 (2)	3	9.5	10.6	11.3
Total	**166 (100)**	**92**	**1.5**	**5.7**	**12.7**
>5°C	**23 (14)**	**12**			
>10°C	**3 (2)**	**3**			

A French study carried out in winter 2001 and spring 2002 (Cemagref/Ania, 2004) found that around 90% of yoghurts were kept at <6°C, and 66% of meat products were kept at <4°C. However the weakest link in the chain was the consumer (53% of yoghurts at <6°C, and 25% of meat products at <4°C). It was also found that 57-67% of product shelf life was spent in commercial refrigeration (Table 4.7).

Table 4.7: Average Duration in Different Environments related to types of Products in the French Commercial Chill Chain (Cemagref/Ania, 2004)

Chain Segment	Dairy Products	Delicatessen & Charcuterie	Pre-packed meat
Refrigerated transport	6h (2d)*	6h (21h)	5h (22h)
Wholesaling	2d (10d)	1d (7d)	9h (4d)
Display cabinet	4d (21d)	5d (18d)	4d (13d)
Transportation after purchase	58 min (7h)	75 min (13h)	66 min (6h)
Domestic refrigeration	3d (26d)	4d (23d)	3d (15d)

*The number is the average, with the maximum duration in parenthesis

The average minimum, mode and average maximum temperature of commercial refrigerated transportation were -2°C, 3-4°C, and 8°C (Cemagref/Ania, 2004). It was found that 30% of commercial transportation in France was above the legal maximum temperature of 4°C, with 4% above 6°C (Table 4.8).

Table 4.8: Distribution of Average Temperatures during Refrigerated Transport in the French Commercial Chill Chain (Derens *et al.*, 2004)

Average Temperature (°C)	Number of lorries at specified temperature	Percentage of lorries at specified temperature	Cumulative percentage
-2 to -1	7	0.9	0.9
-1 to 0	19	2.5	3.4
0 to 1	60	7.7	11.1
1 to 2	98	12.6	23.7
2 to 3	166	21.4	45.2
3 to 4	196	25.3	70.5
4 to 5	143	18.5	88.9
5 to 6	57	7.4	96.3
6 to 7	22	2.8	99.1
7 to 8	7	0.9	100

Total number of samples = 775

In Sweden, Rosengren and Lindblad (2003) in 2001 sampled six groups of RTE (ready-to-eat) foods from retail stores, food production plants, restaurants and other mass catering establishments and found that about 40% of fish samples were stored at temperatures higher than 4°C and 15% were stored above 8°C.

The Finnish National Food Agency (2006) in its study of the microbiological quality of prepared salads found that the average storage temperature was 5.4°C, and 10% of the 116 samples were above 10°C. In a 2000 National Food Agency survey of 315 retail cold storage units, 52% of VP fish products were held at 3°C, 44% between 3-8°C and 4% above 8°C. A 1995 study found a temperature of 8°C was exceeded in 20% of cases.

Various surveys have shown the temperature of foods in US chilled food distribution channels are frequently in the range of 45-55°F (7.2°-12.8°C) (Food Spectrum, 2002). Jol *et al.* (2005) report that “While major manufacturers and retailers operate a constant and effective cold chain, surveys in the US have revealed that 20% of domestic and commercial refrigerators operate at a temperature of >10°C”. A further US survey found that 48% of product temperatures in retail refrigerators were >5.0°C and 17% were >8.3°C (Table 4.9).

Table 4.9: Frequency Distribution of USA Retail Refrigerator Product Temperatures (Audits International, 1999)

Product temperature (°C)	Percentage of refrigerators at specified temperature
0	6
0.5-1.6	5
2.2-3.3	15
3.8-5.0	27
5.5-6.6	21
7.2-8.3	10
8.8-10.0	10
10.5-11.6	3
12.2-13.3	2
13.8-15.0	0.8
15.5-18.3	0.9

An Australian government survey (Adams and Ashton, 2002) of ham sold through butchers and delicatessens found that only one of the 27 premises sampled (4%) was able to provide a sample with a surface temperature of less than 5°C. All other samples were recorded with temperatures ranging from 6-15°C, mean 8.5°C.

An ANZFA (2001) study of 483 Australian food businesses reported that 90% of businesses sampled stored potentially hazardous food at or below 5°C. Displayed potentially hazardous food were stored at or below 5°C in 82% of businesses sampled, but 10% of the businesses did not have a method for ensuring the displayed food remained safe.

Numerous other surveys have been carried out on the microbiological quality of chilled foods in various countries, but storage/product temperatures (and allocated shelf lives) are not recorded as a matter of course, raising questions regarding the usefulness of such work as potential tools for effecting positive change.

4.4 Temperatures from Shop to Home

The frequency of shopping governs the length of time chilled food is stored in the home. It must be noted that shopping patterns have changed in the UK over the last 15 years; new developments include the introduction of online ordering from major multiples with home delivery by the retailer or a third party using refrigerated vehicles. The transportation, storage and temperature profiles reported in the 1991 and 1992 UK surveys cannot therefore be taken to be relevant to the UK today in relation to online shopping from major multiples.

In a UK survey of consumers in the early 1990s (James and Evans, 1992a), 99% of the survey population were reported to shop at least once a week; 24% shopped for chilled food on two days a week, 34% shopped on 3-4 days per week, and 26% shopped on 5-7 days per week. Generally shopping was divided into trips for large quantities (defined as greater than one bag) and small amounts of food (less than one bag). The majority of households (85%) shopped for small quantities of chilled food on a variable basis, as required.

A majority of participants in this UK survey carried out their main shopping between 1 and 5 miles from their homes and most (85%) used a car to transport their main shopping home (James and Evans, 1992a). Unprotected chilled food will warm up during transportation. The survey results showed that consumers took on average 43 minutes to bring meat, fish or dairy items home from the shops and place them in a refrigerator. Although most people brought food home well within 60 minutes there were a number of items that took far longer to be brought home (up to 2 days) and placed in a refrigerator. Although insulated bags and boxes are widely sold only a small percentage (13%) used them to transport some of their food home. The vast majority (87%) of people did not use any means of protecting food from temperature gains during transportation.

A survey of French domestic chilled storage and transport practices found mean product transportation times by consumers from the retailer to home storage ranging from 58-75 min, with the overall average across the products studied being 66 min (Table 4.7).

Increases in product temperatures during transportation can be considerable. In UK investigations, the temperatures of 19 different types of chilled product (including a variety of fish products) were monitored during a simulated journey from the supermarket to home (James and Evans, 1992a). One sample of each product was placed in a pre-cooled insulated box containing eutectic ice packs and the second left loose in the boot of the car. The car was then driven home and the product removed and placed in a domestic refrigerator after a total journey time of one hour. Initial temperatures of the chilled products measured when the food reached the car ranged from 4°C to over 20°C (Table 4.10). Some

of the fish product temperatures in samples placed in the boot rose to around 38°C during the one hour car journey, whilst most of the samples placed in the insulated box cooled during the car journey except for a few at the top of the box which remained at their initial temperature. Thin sliced chilled products showed the highest temperature changes during transport. After being placed in the domestic refrigerator, "warm'" chilled products required approximately 5 hours before the temperature at the surface was reduced below 7°C.

Table 4.10: Maximum temperatures measured in UK fish products after being transported for 1 hour in the boot of a car without protection or within a cooled insulated container (James and Evans *et al*., 1992a)

Product	Maximum temperature (°C)	
	Unprotected transport	Transported in cool box
Trout	28	5
Prawns	37	14
Smoked salmon	38	18

4.5 Mail Order/Website Postal Sales

A UK (MAFF, 1991) survey of mail order foods found that the temperature of a simulated food product (sterile agar in water gel) was >8°C for 70% of the distribution time (Table 4.11). In this study the average temperature of mail order foods (smoked salmon and smoked salmon trout) recorded on receipt was 15°C, with a minimum of 11°C, and a maximum of 19°C (Table 4.12). All were the fish at > 8°C.

Table 4.11: Temperatures of Simulated Food Products - UK Mail Order (MAFF, 1991)

Temperature (°C)	Percentage of time at temperature
<5	10
5-8	20
>8	70

Table 4.12: Distribution of Temperatures of Mail Order Products on Receipt (MAFF, 1991)

Temperature (°C)	Number (%) of packs at temperature on receipt
11	1 (2)
12	4 (9)
13	7 (16)
14	4 (9)
15	7 (16)
16	5 (12)
17	11 (26)
18	2 (5)
19	1 (2)
Total	43 (100)

The time taken for packages to arrive at their destinations was usually 2-3 days, the maximum being 10 days. It is not known whether these data are a valid indicator of current temperature profiles during postal delivery of chilled foodstuffs.

Owing to an exemption for food "which, as part of a mail order transaction, is being conveyed to an ultimate consumer" there is no legal requirement in England, Wales and Northern Ireland to keep chilled foods at 8°C or cooler. However, the food temperature must be maintained at a safe level. MOFFA (2006) state that "if it is likely to rise in transit above 8°C, the mail order operator should be confident that this is safe by reference to supporting technical or other data. Long established practices that have proved safe over many years are relevant in this context."

The Food Standards Agency (2006) states on its website that foods sent through the post requiring refrigeration, including vacuum-packed products such as smoked fish, must be kept cool while they are being transported. Consumers are advised to "check with the supplier what they do to keep it cool until delivery".

In the USA perishable foods must not be held between 40°-140°F (4.4°-60°C), including those distributed by the mail order industry and foods prepared and mailed from home (FSIS, 2003). However, data demonstrating the level of compliance could not be located. USDA (FSIS, 2003) advises consumers as follows regarding the receipt of perishable mail order foods:

- Make sure the company sends perishable items, like meat or poultry, cold or frozen and packed with a cold source. It should be packed in foam or heavy corrugated cardboard.
- The food should be delivered as quickly as possible – ideally overnight. Make sure perishable items and the outer packaging are labelled "Keep refrigerated" to alert the recipient.
- When you receive a food item marked "Keep Refrigerated" open it immediately and check its temperature. The food should arrive frozen or partially frozen with ice crystals still visible. Even if a product is smoked, cured and/or fully cooked, it is still a perishable product and must be kept cold. If perishable food arrives warm, notify the company. Do not consume the food. Do not even taste suspect food.
- Tell the recipient that the company has promised a delivery date. Or alert the recipient that "the gift is in the mail" so someone can be there to receive it. Don't have perishable items delivered to an office unless you know it will arrive on a work day and there is refrigerator space available for keeping it cold.

4.6 Farmers' Markets

National rules apply but little specific guidance is available to operators.

FSA Scotland (2005) states that:

- Chilled food should be kept at a temperature of 5°C or less (range 0-8°C)
- Chilled food should be transported by temperature controlled vehicle to and from the market and stored on site under temperature controlled conditions. However, small traders may use icepacks in insulated containers, provided the temperature is kept at 5°C or below.
- In the case of fish, ice should be provided for keeping the temperature at 5°C or below.

No significant data are available to demonstrate the level of compliance with these recommendations. Recent PHLS/HPA surveys (Elson *et al.,* 2004; Sagoo *et al.,* 2006) included sampling from "market stalls" but data are not separated in the reports (Table 4.4).

4.7 Catering

UK national legislation is applicable, and a study carried out in 2002 assessed the level of compliance (Table 4.13). The data from this single study show that just over three quarters of the 3,709 samples of open, ready to eat salad vegetables taken from catering premises were at or below 8°C.

Table 4.13: UK/PHLS Study – Catering Premises Selling Open Ready to Eat Salad Vegetables (Sagoo *et al.*, undated)

Location	Product temperature (°C)	Number of samples	% Samples
Refrigeration in foodservice and preparation area	≤8	1,246	65
	>8	681	35
Refrigeration post-sale display (in foodservice and preparation area)	≤8	583	98
	>8	11	2
Total: Refrigeration in foodservice and preparation area	**≤8**	**1,829**	**73**
	>8	**692**	**27**
Display in customer self-service area	≤8	754	81
	>8	180	19
Post-sale display in customer self-service area	≤8	249	98
	>8	5	2
Total: Display in customer self service area	**≤8**	**1,003**	**84**
	>8	**185**	**16**
Grand total	**≤8**	**2,832**	**76**
	>8	**877**	**24**
	Total	**3,709**	

4.8 Domestic Refrigerators

Worldwide there are about 10^9 domestic refrigerators and freezers (Billiard, 2002). According to the Office of the Deputy Prime Minister (ODPM, 14.3.06) in 2003 there were 2.1×10^7 households in the UK. UK household refrigerator/fridge-freezer penetration is >99% (AMA Research, 2003). Household numbers are projected to rise to 2.6×10^7 by 2026, of which 150,000 are projected to be due to a higher number of single people living alone (ODPM, 14.3.06).

A total of 1.9×10^7 domestic refrigerator/fridge-freezers were sold between 1999 and 2005 (Table 4.14), which implies that that on average a UK household replaces its refrigerator/fridge-freezer every 7.75 years. The improved energy efficiency of UK domestic refrigeration equipment is documented (MTP, 2006) but it is not known how the replacement of equipment has affected, if at all, UK domestic refrigerator temperature performance. The last UK domestic refrigerator survey (1990) is therefore two generations out of date and needs to be re-run.

Table 4.14: UK Domestic Refrigerator and Fridge-Freezer Sales (1999-2005) [MTP (2006)]

Year	**Unit sales per year**
1999	2,833,625
2000	2,762,962
2001	2,227,753
2002	2,697,797
2003	2,757,137
2004	2,783,235
2005	2,809,656
Total	18,872,165

In January 2006, ISO/DIS 15502 ('Household Refrigeration appliances – characteristics and test methods') was published in the UK as BS EN ISO 15022: 2005, and includes temperature performance standards including for chilled compartments, which few fridges sold in the UK have (Table 4.15). Compliance with these standards would ensure better domestic temperature control of chill foods, and contribute to microbiological food safety.

Table 4.15: ISO 15502 Specified Domestic Refrigerator Storage Temperatures

Temperature within specified compartment of domestic fridge			
Fresh food storage compartment		Chill compartment (Instantaneous)	Cellar compartment
Range	Mean		Range
0-8°C	≤4°C	-2 to +3°C	+8 to +14°C

The various compartments are defined as follows:

- chill compartment is intended specifically for the storage of highly perishable foodstuff in which the above specified storage temperature can be maintained.
- fresh food storage compartment: is intended for the storage of unfrozen food at the temperature specified.
- cellar compartment is intended for storage of particular foods and beverages at a temperature warmer than that of the fresh food compartment.

There is no legal requirement in the UK for domestic refrigerators to have chill compartments. However, it is noteworthy that the French Government issued Decree 2002-478 concerning "domestic refrigerators and thermometers designed to indicate the temperature inside these appliances". It specified that as of 10 September 2002, all domestic refrigerators must incorporate a chill compartment (with a mean temperature of ≤4°C), a temperature-indicating device and a temperature-regulating device. The adoption of such an approach in the UK would ensure better domestic temperature control of chill foods, and contribute to microbiological food safety.

It should also be noted that domestic refrigerators are designed to perform to the above temperature standards according to the climate (ambient temperature) in which they are intended to be operated (Table 4.16). It is therefore important that the correct class of refrigerators is being supplied and used by consumers according to the local climate.

Table 4.16: ISO 15022 Domestic Refrigerator Climate Classes

Class	Symbol	Range of ambient temperatures in which the appliances are intended to be used and for which the required storage temperatures shell be fulfilled
Extended temperate	SN	+10 to +32
Temperate	N	+16 to +32
Subtropical	ST	+16 to +38
Tropical	T	+16 to +43

4.9 Domestic Food Refrigeration Temperatures in Practice

Surveys of consumer storage and handling of refrigerated foods (Table 4.17) indicate that performances are similar throughout the world.

Table 4.17: Surveys of Domestic Storage/Handling of Refrigerated Foods

Country	Reference
USA	VanGarde & Woodburne, 1987
China	Shixiong & Jing, 1990
UK	Evans *et al*., 1991; Evans, 1992; James & Evans, 1992a; James & Evans, 1992b
Northern Ireland	Flynn *et al*., 1992
France	Victoria, 1993
New Zealand	O'Brien, 1997
Greece	Sergelidis *et al*., 1997
The Netherlands	Notermans *et al*., 1997 (data from 1994 study)
USA	Daniels, 1998
UK	Johnson *et al*., 1998
Australia	Jay *et al*., 1999
USA	Audits International/FDA, 1999
USA	CFSAN/FSIS, 2001
France	Laguerre *et al*., 2002
Northern Ireland	Jackson, 2003
US	Redmond & Griffith, 2003
Sweden	Marklinder *et al*., 2004
Ireland	Kennedy *et al*., 2005
Portugal	Azevedo *et al*., 2005
Greece	Koutsoumanis & Taoukis, 2005

The temperature at which a refrigerator operates is critical for the safe storage of chilled food. A recommendation made in 1991 in the UK concerning the microbiological safety of foods advised that the maximum temperatures in domestic refrigerators should not exceed 5°C (Richmond, 1991). In view of the period since the last survey was carried out, it is not clear whether this has been achieved.

Awareness of the correct refrigerator temperature has been reported to be variable. An Irish study found 22% of consumers aware of the correct refrigerator temperature (Kennedy *et al*., 2005). While a recent Swedish survey (Marklinder *et al*., 2004) found a much better level of awareness amongst its survey group, with 85% of respondents knowing the recommended refrigeration temperature (in this case 8°C). However, not all of those consumers put their knowledge into practice; the Swedish survey found 40% of food storage temperatures exceeded the maximum recommended temperature for the food being stored. Only 25% knew, or regularly measured, the temperature of their refrigerator. In the Irish study, 23% of those asked had a refrigerator thermometer.

A US Government report (CFSAN/FSIS, 2001) reported that 73% of domestic refrigerators were found to be at ≤5°C and 4% at >8.3°C (Table 4.18). Jol *et al.* (2005) report that while major manufacturers and retailers operate a constant and effective cold chain, surveys in the US have revealed that 20% of domestic and commercial refrigerators operate at a temperature of >10°C.

Table 4.18: Frequency Distribution of Home Refrigerator Temperature from a Survey of 939 U.S. Refrigerators (Adapted from Table 111-8 FDA *Listeria* Risk Assessment, CFSAN/FSIS, 2001)

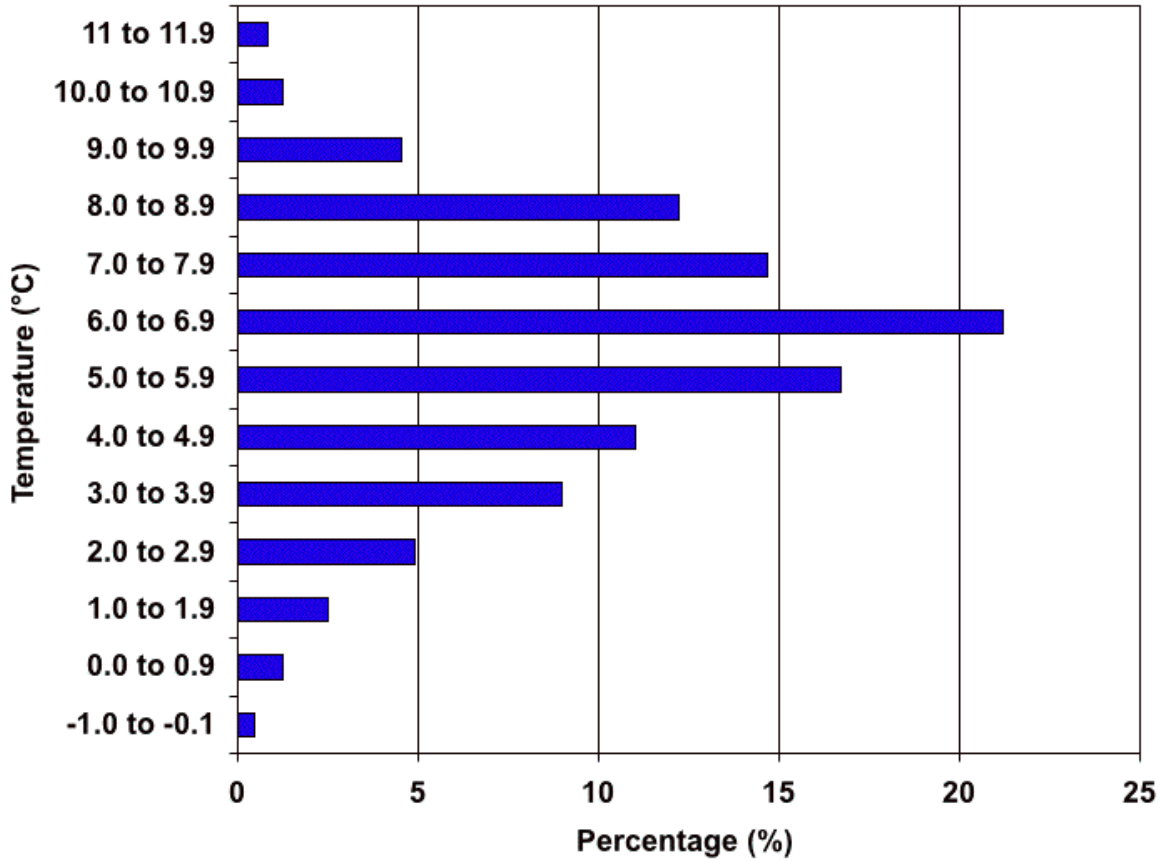
Refrigerator Temperature (°C)	Percentage of refrigerators at specified temperature
0.0	9
0.5-1.6	10
2.2-3.3	25
3.8-5.0	29
5.5-6.6	18
7.2-8.3	5
8.8-10.0	3
10.5-11.6	0.4
12.2-13.3	0.5
13.8-15.0	0.4
15.5-17.2	0.1

An Australian telephone survey (Jay *et al*., 1999) found only 16% of respondents knew the temperature of their refrigerator. A 2004 New Zealand survey of domestic refrigerator temperatures found that 16 out of 53 fridges tested (30%) were operating above 5°C (NZ Foodsafe Partnership, 2004). Twenty six (49%) showed temperatures ranging from 5°C-7°C. Four of the 53 fridges (7%) had average air temperatures above 7°C, and the warmest average air temperature recorded was 9.9°C. The lowest recorded temperature at any one time was -2.5°C. Almost 72% of the fridges surveyed recorded higher temperatures on the top shelf than on the bottom shelf. Of all the fridges surveyed, 23 (43%) had average air temperatures between 1°C and 5°C.

A survey of 2001/2002 found that 47% of yoghurt samples in French domestic refrigerators were at >6°C, and more than 75% of meat product samples were at >4°C (Berens *et al*., 2004). In addition, 5% of domestic refrigerators were operating at >10°C.

In the last major public UK survey (Evans *et al*, 1991) results showed that the mean temperature over 7 days (evaluated from top, middle and bottom sensors) ranged from -1°C to 11°C. The overall mean air temperature for all the refrigerators in the survey was 6°C, with 70% of refrigerators operating at average temperatures above 5°C (Figure 4.1). [Note, the earlier comments regarding the current validity of this work given the renewal rates of domestic refrigeration equipment in the UK].

Figure 4.1: Overall mean temperatures for all refrigerators in UK survey (Evans *et al*., 1991)



A recent review of all European studies showed that overall the average air temperature in European refrigerators would appear to be 6.64°C (Nauta *et al*., 2003).

A detailed survey of food temperatures of various products stored in Swedish consumers' refrigerators found that 83-94% were at >5°C, 22-44% were at >8°C, and 5-19% were at >10°C 22-44% (Table 4.19). Marklinder *et al.* (2004) also found that mean food temperatures were not related to the age or type of refrigerator in Sweden.

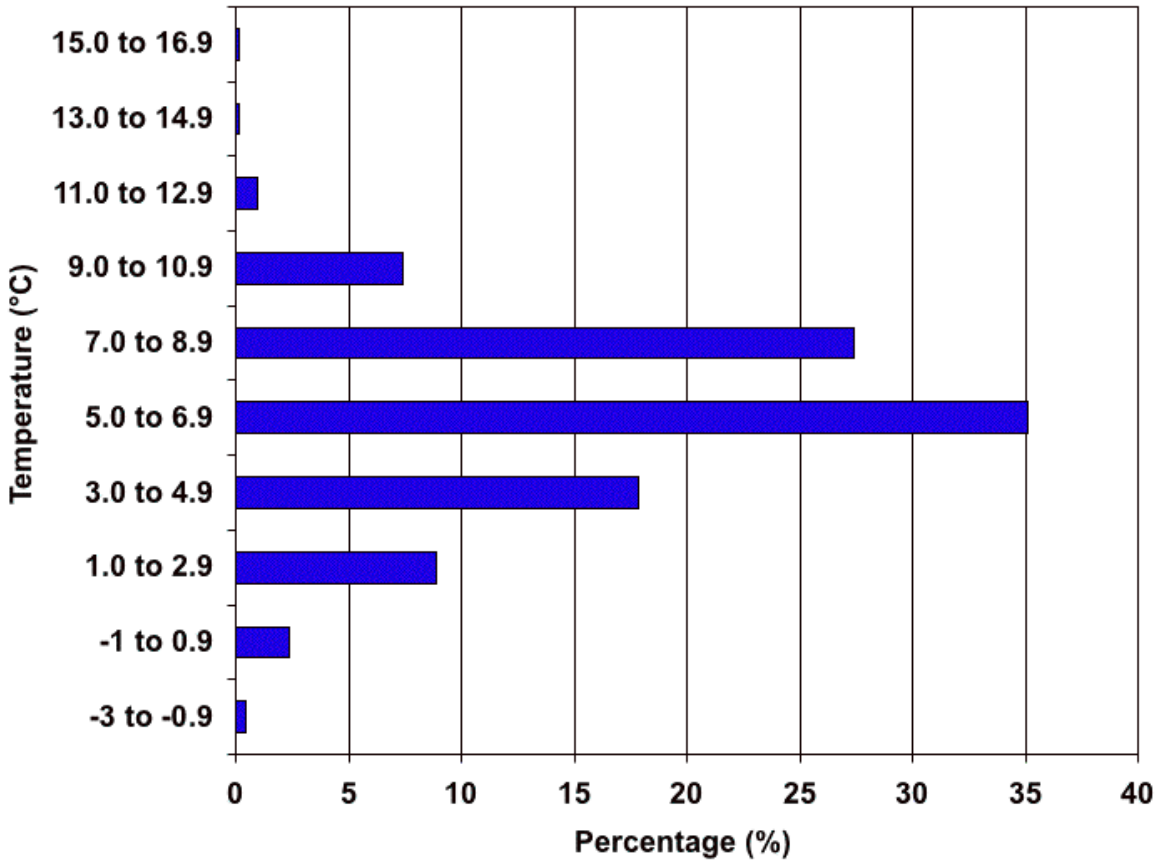
A figure of 60-70% of domestic refrigerators operating at an average temperature >5°C appears to be relatively common to many studies throughout the world (Table 4.19).

Table 4.19: Temperatures Measured in Surveys of Domestic Refrigerators

Reference	Country	Number of samples	Measurement	T_{min}	T_{mean}	T_{max}	% >x°C
Rose *et al.,* 1990	UK	75 (air)	unknown		<5	15	6%>5°C
Evans *et al*., 1991	UK	252 (air)	Data logger (3 levels: T, M, B)	0.9	6.0	11.4	70%>5°C
Flynn et al., 1992	Northern Ireland	150 (air)	Thermometer (3 levels: T, M, B)	0.8	6.5	12.6	71%>5°C
Victoria, 1993	France	102 (air)	Thermometer (3 levels: T, M, B)			14	70%>6°C
Notermans *et al*., 1997	The Netherlands	125 (air)	Thermometer				70%>5°C
O'Brien, 1997	New Zealand	50 (air)	Thermometer (2 levels: T, B)	0	4.9	11	60%>4°C
Sergelidis *et al*., 1997	Greece	136 (air)	Thermometer				50%>9°C
Daniels, 1998	USA	106 (air)	unknown				69%>5°C
Johnson *et al.*, 1998	UK	645 (air)	Thermometer	-2	7	13	70%>5°C
CFSAN/FSIS, 2001	USA	939	unknown				73% ≤5°C; 4% >8.3°C
Laguerre *et al*., 2002	France	119 (air)	Data logger (3 levels: T, M, B)	0.9	6.6	11.4	80%>5°C
Jackson, 2003	Northern Ireland	30	Data logger (1 level M)	-5	4.5	13.0	53%>5°C
Marklinder *et al*., 2004	Sweden (Jan-Mar and Sept-Nov 2002)	102 households, 705 food samples	Data logger	0.2 (VP salmon)	7.1	12.3	88%>4°C; 38%>8°C; 11%>10°C
				0.2 (fresh herring fillets)	6.5	12.8	83%>4°C; 24%>8°C; 7%>10°C
				0.6 (milk)	6.9	13.2	92%>4°C; 31%>8°C; 11%>10°C
				0.8 (minced meat)	6.2	11.3	85%>4°C; 22%>8°C; 6%>10°C
				1.1 (sliced cooked ham)	7.2	12.3	90%>4°C; 44%>8°C; 10%>10°C
				1.8 (RTE green salad)	7.4	18.2	94%>4°C; 39%>8°C; 19%>10°C
				2.4 (soft cheese)	6.8	13.6	93%>4°C; 27%>8°C; 5%>10°C
Cemagref/Ania, 2004	France	314 product samples/ fridges	Data logger	yoghurt			47%>6°C; 5%>10°C
				meat			75%>4°C; 5%>10°C
Kennedy *et al*., 2005	Ireland	100	Data logger (1 level M)	-7.9	5.4	20.7	59%>5°C
Azevedo *et al.,* 2005	Portugal	86	Digital thermometer				70%>6°C
Koutsoumanis & Taoukis, 2005	Greece	250	Data logger (3 locations)	-2.5			85%>5°C
Terpstra *et al*., 2005	Netherlands	31	Glass thermometer	3.8		11.5	21%>7°C

While an increasing number of refrigerators are sold with a single point temperature display, Laguerre *et al*. (2002) found that the temperature measured using a thermometer does not represent the "true operating conditions of the refrigerator". Indeed temperatures in refrigerators are not static. Various studies such as Koutsoumanis and Taoukis (2005) note major temperature variations throughout a refrigerator. An analysis of percentage time spent between certain temperatures carried out by Evans *et al.* (1991) in the UK, calculated for all refrigerators that the greatest proportion of time (80%) was spent between 3.0°C and 8.9°C. Approximately 28% of the time was at <5°C, 35% of the time was at 5.0-6.9°C, 28% of the time was at 7.0-8.9°C, and 9% of the time was spent above 9.0°C (Figure 4.2). Only four refrigerators (2%) in the whole survey operated below 5°C during all the monitoring period, and 33% of refrigerators spent all their time above 5°C.

Figure 4.2: Frequency distribution of temperatures in all refrigerators (UK study) (Evans *et al*., 1991)



The mean temperature range within a refrigerator was found to vary between refrigerator types. Ice box refrigerators had the smallest range (average 1.8°C); whereas the range in temperature in fridge-freezers and larder refrigerators was nearly twice as great (average of 3.4°C in fridge-freezers and 3.7°C in larder refrigerators).

A survey carried out in China found higher temperature ranges within domestic refrigerators with only 2% of the refrigerators surveyed operating with a temperature range of less than 6°C. It was found that 34% of fridges had a temperature range 8°C -12°C, and that 64% had a temperature range greater than 12°C (Shixiong & Jing, 1990).

From analysing the data from the various surveys reported in Table 4.19, it can be ascertained that 39% of the 3,607 domestic refrigerators worldwide were below 5°C, and that 80% were below 8°C (Table 4.20).

Table 4.20: International Domestic Refrigerator Temperature Performance

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

Raw table in Table 4.19

Selecting for UK survey data from Table 4.19, it can be seen that more than 65% of UK refrigerators were at or above >5°C (Table 4.21). The mean reported temperature is 6.6°C. This is comparable with the overall international position.

Table 4.21: UK Domestic Refrigerator Performance

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

Raw table in Table 4.19

4.10 Domestic Chilled Food Storage Practices

Marklinder *et al.* (2004) found in Sweden that in most cases, perishable foods were not stored for more than 3 days (Table 4.22). A UK consumer study (Evans, 1992) in which the majority of respondents thought that most chilled food should be stored for 2 days or less.

Table 4.22: Stated Domestic Storage Times for Short Shelf Life Products – Swedish Consumers (Marklinder *et al.* 2004))

	Domestic storage times for short shelf life products in Sweden (% of time)*		
Storage time	Minced Meat	Herring fillet	RTE green salad
<1 day	27	23	17
1 day	42	33	37
2 days	17	25	24
3-4 days	4	2	5
5-6 days	0	2	0
7 days	0	2	0
Consume by/best before date	10	10	6
Own judgement	0	2	0
Other/no opinion	0	2	12

*There were 52 observations for minced meat and herring fillets and 102 observations for RTE green salads

A French survey (Table 4.7) found that mean domestic storage times of all purchased product types ranged from 3 to 26 days, but in the case of pre-packed meat ranged from 3 to 15 days. The overall mean storage time was 3 days.

All three studies (France, Sweden, UK) therefore found that chilled food domestic storage times were on average short (in the order of 3 days) or that consumers believed they should be.

Marklinder *et al.* (2004) found that respondents opinions about long shelf life products storage times varied depending whether the packs were opened or not (Table 4.23).

Table 4.23: Swedish Consumers' Stated Domestic Storage Times for Opened or Unopened Packs of Long Shelf Life Chilled Products (Marklinder *et al*., 2004)

Storage time	Domestic storage times for long shelf life products in Sweden (% of time)*			
	Salmon (%)		Ham (%)	
	Opened	Unopened	Opened	Unopened
1 day	10	2	2	0
2 days	21	10	5	0
3-4 days	23	2	32	2
5-6 days	6	2	12	0
1 week	10	10	25	12
2 weeks	0	0	2	6
3 weeks	0	0	0	0
'Best before' date*	12	44	7	54
Judgement	10	10	13	15
Other/no opinion	10	21	2	12

* VP salmon shelf life commonly 3-5 weeks after packing. Ham ~3 weeks; there were 52 observations for salmon opened, salmon unopened and ham unopened, and 101 observations for ham opened

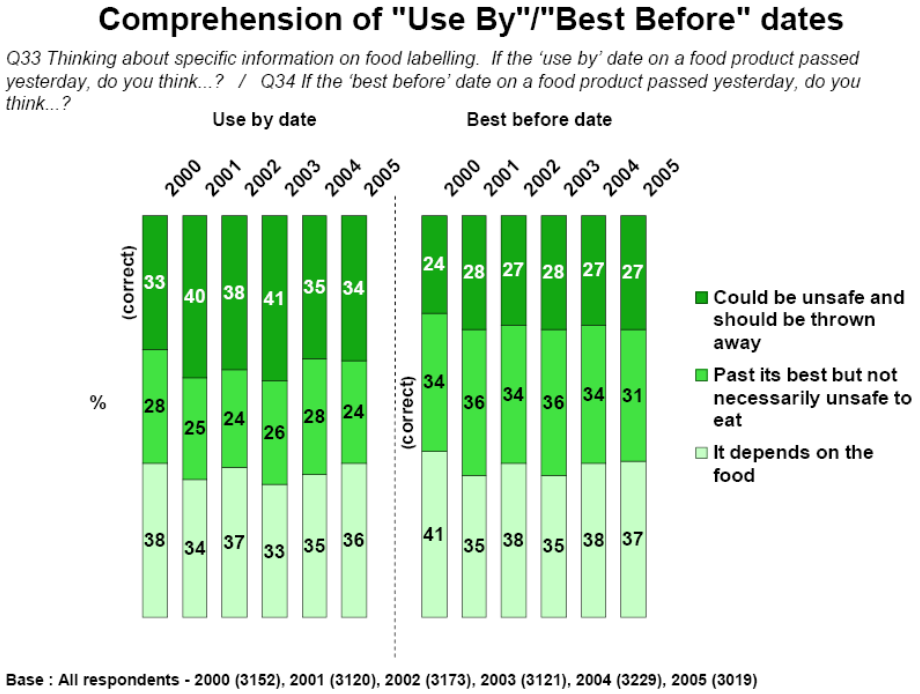
Shorter storage times were stated for opened than unopened packs, and for unopened packs it was more common to base the decision of storage time on the use by/best before date. Most respondents did not store an opened VP salmon pack for more than a few days, whereas half of them said that unopened packs could be stored up to the "best before" date. In principle this might lead to storage times of 5 weeks, 38% of the time at temperatures >8°C. Similarly, most respondents determined storage time of unopened ham packs on the basis of the "best before" date (reported to be about 3 weeks from packing) but did not store opened packs for more than 1 week. It was found that 44% of the time storage was at >8°C.

A UK study (Evans *et al.*, 1991) found that:

- 84% consumers 'always' checked product shelf life in shops
- 50% 'always' look for longest shelf life pack
- 74% used their own judgement regarding consumption of the product if the shelf life had expired
- 19% 'very rarely' threw food away if it was past its labelled shelf life
- 17% of products in fridges were past their shelf life
- 26% products would be eaten after expiry of their use by dates
- 49% would ignore shelf life if a time temperature indicator read 'OK'

Annual FSA consumer attitude surveys since 2000 have shown that the level of comprehension of "use by" and "best before" dates has been consistent since the start of the study in 2000. However, only one-third of UK respondents correctly interpret each term (Figure 4.3).

Figure 4.3: Comprehension of 'Use by' and 'Best Before' Dates (FSA, 2005)



The US FDA is reportedly concerned that consumers interpret the labelling terms "consume by" and "best if used by" as synonymous with "safe to consume until". At the January 2002 meeting of the National Advisory Committee on the Microbiological Criteria for Food, the Committee was asked to provide assistance to the FDA in developing a scientific framework for the establishment of safety-based "use by" date labels. Specifically, the Committee was asked to consider five questions, their conclusions on which are shown below (NACMCF, 2005):

1. What are the scientific parameters for establishing safety-based "use by" date labels for refrigerated RTE foods?

- The pathogen of concern must be able to grow at refrigerated temperatures in the food in question to a level that will be likely to cause illness in the host
- Scientific evidence that a safety based date label will reduce the risk of foodborne illness for that food must be available
- Identification of safety-based end points is necessary for establishing a safety based date label
- Determination of temperature to use for establishment of a safety based date label

2. What effects do the multiple factors that influence the growth and survival of *L. monocytogenes*, i.e. strain differences, food matrices, production and distribution systems, consumer susceptibility, etc., have on the establishment of safety-based "use by" date labels for refrigerated foods?

- Strain differences
- Food matrices
- Competing microflora and packaging
- Production, distribution and handling practices

3. What data needs to be acquired to scientifically validate and verify the adequacy of a proposed safety-based "use by" date label for a refrigerated RTE food?

- Ultimately the success of the safety based date label concept depends on how the consumer

interprets and uses the information measured both before and after implementation

4. Should safety-based "use-by" dates for refrigerated RTE foods be established using mathematical modeling techniques? If so, what modeling approaches are best suited to the development of safety-based "use-by" date labels for refrigerated RTE foods?

- The relevance and validity of a model must be carefully evaluated when determining the degree of confidence that can be given to a model's predictions

5. What impact would safety-based "use by" date labels likely have on the control of other foodborne pathogens in RTE foods?

- The impact will depend on the specific food-pathogen combination. Based on the epidemiological information the vast majority of cases of foodborne illness are due to pathogens that are incapable of growth in food at refrigeration temperatures. Thus a safety based date label for refrigerated products would have no impact on foodborne illnesses caused by these pathogens.
- For pathogens that can multiply in refrigerated ready to eat foods, the factors of primary concern are the likelihood of contamination with a particular psychrotrophic pathogen, the level of contamination, the rate of growth at refrigeration temperatures, storage and handling practices, and the level required to cause illness.
- The Committee's hazard analysis led to the conclusion that the duration of refrigerated storage is not a major factor in foodborne illness caused by *Y. enterocolitica*, *B. cereus* and non-proteolytic (psychrotrophic) *C. botulinum*. Therefore, the Committee believes that a safety based date label to limit the potential for growth of *L. monocytogenes* would have little or no impact on diseases related to these pathogens.

NACMCF (2005) concludes that educational efforts focusing on safety based date labels should also emphasise the importance of refrigeration temperature control.

4.11 Commercial Shelf Life Determination Protocols

The aim of a shelf life testing protocol is to ensure that a product is exposed to the time and temperature conditions it is likely to experience during manufacture, retail distribution, retail storage, purchase and storage by the consumer.

There are many different protocols in use throughout the food industry internationally to represent specific and general chill chains. It is believed that in practice however, it is likely that most industrially-produced chilled foods are produced, distributed and stored in retail and domestic refrigerators under similar conditions.

The selection of shelf life determination protocol is critical to the allocation of product shelf life since there are many differences regarding assumed storage temperatures and times at these temperatures.

Table 4.24 shows the agreed testing protocol intended to represent manufacture of most current chilled foods in the UK, which was developed by a working group of major UK manufacturers and retailers (CCFRA, 2004).

Table 4.24: Recommended UK Shelf Life Evaluation Protocol for Chilled Foods (CCFRA, 2004)

Manufacturing stage	Storage temperature*	Time
Under commercial control: In-house storage at manufacturer	5°C[a] or 7°C[b]	To be defined by the manufacturer and/or retailer
Distribution vehicles storage depot	5°C[a] or 7°C[b]	
Retail display	5°C[a] or 7°C[b]	
Outside commercial control: consumer purchase	22°C	2h
consumer storage	7°C	Remainder of life

* Temperatures stated assume a typical deviation of ±1°C

[a] The use of 5°C should be supported by evidence that these temperatures can be maintained throughout distribution and retail. If this is not the case 7°C should be used

[b] 7°C is recommended for use where there is insufficient data to show that a lower temperature can be maintained throughout the chill chain, or where there are concerns about the growth of pathogens such as *B. cereus* which has maximum acceptable levels within products.

For the majority of chilled prepared foods the UK major multiples' chill chains should be able to be maintained at 5°C throughout distribution and retail display. Indeed, agreed retailer own label chilled prepared food temperature on delivery to retailers' Regional Distribution Centres is commonly set at 5°C maximum through commercial agreements.

However, the CCFRA protocol notes that the England, Wales and Northern Ireland legislated temperature currently applicable to the majority of chilled foods is 8°C and states that if there is insufficient evidence to support the use of 5°C then the higher temperature 7°C±1°C should be used to give a safety margin. The rationale for the choice of storage times and conditions in this protocol is:

- 5°C being representative of on-site storage at most manufacturing sites (note usage of 'deep chill' in an increasing number of operations in the UK)
- A Working Party survey of retail storage believed that the average temperature was 4-6°C
- Purchase and transportation by the consumer was found by a Working Party survey to be a maximum average of 1 hour in the shop and a further hour for loading the shopping into the car, driving home and unloading shopping into the refrigerator
- Storage in a domestic refrigerator was found in a MAFF survey (Evans *et al.,* 1991) to be at 6°C on average, with the majority (74%) of UK refrigerators surveyed operating at <8°C. A temperature of 7°C±1°C was believed by the Working Party to cover the maximum operating ranges of the majority of domestic refrigerators in the UK.

A French national protocol for the determination of shelf life of perishable refrigerated foods was published by AFNOR (2003). It sets out different approaches for the determination of shelf life dependent on the chill chain (Table 4.25).

Table 4.25: French Shelf Life Determination Protocol in Relation to Chill Chains (AFNOR, 2003)

Chill Chain Type	Storage Regime
Insufficiently known or controlled, storage temperature believed to be somewhat long at t_1	t_1 for one third of the estimated shelf life t_2 for two thirds of the estimated shelf life
Partially controlled chill chain	t_1 for two thirds of the estimated shelf life t_2 for one third of the estimated shelf life
Totally controlled chain	t_1 for the whole of the estimated shelf life

Where:
t_1 is the fixed storage temperature by legislation (4°C for chilled prepared foods) or the temperature fixed by the manufacturer; and
t_2 is a representative temperature of a reasonable breach of the chill chain or a modification of the storage temperature (e.g. in the home). t_2 is in practice taken to be 8°C (based on the findings of a survey of consumer behaviour carried out in France (Cemegref/Ania, 2004).

The standard application practice of the French protocol is for the distribution chain to be treated as "partially controlled chill chain", since storage at 4°C by consumers seems unlikely. A "totally controlled chill chain" would for example be on-site production for on-site consumption, e.g. hotel/restaurant catering.

The US Refrigerated Foods Association (2002) has proposed a draft 'Protocol for Determining the Shelf Life of Refrigerated Foods', comprising:

- Shelf Life Verification: covering microbiological, chemical and sensory analysis.
- Challenge Studies (with respect to *Listeria monocytogenes* and *C. botulinum*): in products when changes in formulation, packaging etc. are made. *Clostridium sporogenes* will be used as a surrogate organism for *C. botulinum*. Only products which have a pH $\geq$4.6 and have been produced under reduced oxygen conditions, including MAP, should be challenged with *C. sporogenes*.
- Testing Protocol for Refrigerated Food Shelf Life: to validate that the shelf life that has been established for a specific product is appropriate. All products will be tested at six different ages regardless of length of expected shelf life. Shelf life analysis is dependent upon examination of batches of samples until the shelf life becomes unacceptable. Sampling intervals should be determined at 20% of product shelf life, which comprise six different ages from fresh to full shelf life. For example, if a product with an extended shelf life of 30 days is analysed, sampling intervals should consist of day 0, 6, 12, 18, 24 and 30.
- For the purpose of verification, the criteria shall be strictly applied on the product(s) held at 4°C. For product(s) held at 10°C the results shall be used to help the manufacturer understand any inherent weaknesses that may exist as well as recognize the reality of the distribution system.

However, it is important to note that *C. sporogenes* is more commonly used as a surrogate for proteolytic *C. botulinum* (e.g. in thermal tests). Since it does not grow below 10°C it is unlikely to be a good surrogate for non-proteolytic *C. botulinum* in these tests if the chill chain is maintained. The protocol therefore appears to be designed primarily to assess the growth potential in relation to significant temperature abuse conditions (i.e. >10°C).

4.12 Conclusions

Within the UK, when held and distributed by the manufacturer, it is likely that chilled food is maintained at no more 5°C, and probably lower. Indeed, agreed retailer own label chilled prepared food temperature on delivery to retailers' Regional Distribution Centres is commonly set at 5°C maximum, through commercial agreements.

The maximum temperature specified in legislation for retail of chilled food is 8°C in England, Wales and Northern Ireland. In Scotland the requirement is to keep chilled food in “a refrigerator, or refrigerating chamber, or a cool ventilated place”. Neither is there is not a harmonised approach to legislated temperature rules within the EU, with temperatures of 0°C to 8°C specified in different countries. There can also be different requirements for different food groups.

In practice, surveys of all types of chilled food outlets (including major multiple retailers, farmers markets, small stores and other outlets) indicate that in the UK, the average temperature at retail was 4°C-6°C, with only 6% of samples at >8°C. The position appears similar in many other European countries.

In the UK, a 1990 study showed that transportation of food from the point of purchase to the domestic refrigerator took an average of 43 min, with most achieved in 60 min. The majority of people (87%) did not chill the food during transport, and in some cases the food reached temperatures in excess of 20°C, albeit for a short period of time. It took several hours for the food to cool to below 7°C. The increased use of insulation bags or boxes would help consumers maintain the chill chain.

Chilled food purchased through mail order is exempt from legislation in England, Wales and Northern Ireland, although the temperature should be maintained at a “safe level”. A MAFF study in 1991 reported that mail order chilled foods spent 70% of their time at 8°C or higher, and that the average temperature on receipt was 15°C. MOFFA (Mail Order Fine Foods Association) state that “if the temperature is likely to rise in transit above 8°C, the main order operator should be confident that it is safe by reference to supporting technical or other data”.

Domestic refrigerators are present in >99% of households in the UK, and on average are replaced every 8 years. Refrigerators provide a key food safety device within the domestic kitchen, their correct operation will reduce the risks of the growth of food poisoning organisms in foods stored within them. Unfortunately there is a lack of recent published data on the temperatures of domestic refrigerators, with the last UK domestic refrigerator survey carried out in 1990. In view of the elapsed time, there would be merit in repeating this survey. This would provide up to date information on current practice.

The 1990 UK survey found that the mean domestic fridge temperature ranged from -1°C to 11°C over a 7 day period, and that the overall mean temperature was 6.6°C, with 65-70% of fridges at more than 5°C. There was variation in performance between fridges, and within each fridge over time. Different temperatures were also recorded in different parts of single fridges. Overall, for all domestic fridges the time spent at various temperatures were as follows; 28% of the time at <5.0°C, 35% of the time at 5.0-6.9°C, 28% of the time at 7.0-8.9°C, and 9% of the time at >9°C. The position appears similar in other countries, and an average temperature of 6.64°C has been reported for European fridges. In 1991, Richmond recommended that the maximum temperature of domestic fridges in the UK should not exceed 5°C, in view of the period since the last survey was carried out, it is not known whether this has been achieved.

A survey of consumer behaviour in France established that for short shelf life chilled products, approximately 60% of the shelf life was spent in commercial refrigeration, and 40% in domestic refrigeration. The general applicability of this to other countries is not known, given different practices in various countries.

UK consumer understanding of the “use by date” or “best before date” is poor, and UK consumer handling of chilled foods in practice needs to be more widely studied. Recent FSA data indicate that 27-34% of consumers believe that food past the “use by date” or “best before date” should be thrown away, 24-31% of consumers believe that such food might be past its best but not

necessarily unsafe to eat, and 36-37% believe that the action would depend on the food. An earlier survey found that 26% of consumers would eat products after the expiry of their use by date.

It is suggested that the UK continues to strive for better temperature control throughout the chill chain (including domestic storage), and that 5°C is adopted as a target for best practice. This could be aided by new domestic refrigerators including chill compartments and a temperature measuring device to assist consumers in assuring the appropriate chilled storage of foods. This proposal is made in order to further extend the margins of safety of chilled foods with respect to psychrotrophic foodborne pathogens (since their growth is slower at 5°C than 8°C), rather than on the basis of any specific problems.

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Chapter five: Summary of recent outbreaks of foodborne botulism

5.1 Introduction to foodborne botulism

Botulinum neurotoxin is the most potent substance known, and is the agent responsible for botulism. Consumption of as little as 30 ng of neurotoxin is sufficient to cause illness and even death (Lund and Peck, 2000). The consumption of even a minute quantity of food in which a neurotoxin-producing clostridia has grown can result in botulism. As an example, in Canada in 2002, a 35-year old man ate a mouthful of foil-wrapped baked potato, found it to be foul tasting and spat it out, but he had consumed sufficient neurotoxin to require extensive medical treatment that included more than six months in hospital (Bhutani *et al*., 2005). Since botulism is such a severe disease, a considerable effort is dedicated to ensuring the safe production of food with respect to this hazard, and that botulism outbreaks are rare. Also, because of the severity, single cases are generally treated as outbreaks.

5.2 Method for collection of data on foodborne botulism

An extensive literature search was carried out in March 2006 to collect literature data on outbreaks of foodborne botulism. References were retrieved from Web of Knowledge (1970-2006), BIOSIS (1969-2006), and SCOPUS (1992-2006), and combined with articles held in the personal libraries of the authors of this report. Also, a search was carried out of the internet. Based on a review of titles and abstracts, several hundred references (refereed publications, book chapters and conference proceedings) were identified that appeared to contain suitable information. The entire article of each of these references was then individually assessed for relevance, and appropriate information on outbreaks of foodborne botulism extracted.

5.3 Recent outbreaks of foodborne botulism

At the present time most outbreaks of foodborne botulism are associated with home-prepared foods, where control measures have not been implemented by those preparing foods in the domestic kitchen. While outbreaks involving commercial processing are uncommon, they can be associated with significant medical and economic costs. It has been estimated that in the USA the cost per case of botulism associated with commercial food is approximately $30 million, 3000-times more than for each case of listeriosis or salmonellosis (Setlow and Johnson, 1997).

Foodborne botulism is most frequently associated with proteolytic *C. botulinum* or with non-proteolytic *C. botulinum*, with occasional outbreaks associated with neurotoxigenic strains of *C. butyricum* and *C. baratii* (Anniballi *et al*., 2002; Harvey *et al*., 2002). Proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are physiologically and genetically distinct organisms. Proteolytic *C. botulinum* is a mesophile, with a minimum growth temperature of 10°C-12°C, while non-proteolytic *C. botulinum* is a psychrotroph that is able to grow and form toxin at 3.0°C (Table 5.1). In view of its ability to grow and form toxin at 3°C, non-proteolytic *C. botulinum* is a larger concern in chilled foods. Since proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are physiologically distinct, it is appropriate to consider their association with foodborne botulism independently.

Table 5.1 Characteristics of the two physiologically and genetically distinct clostridia most frequently associated with foodborne botulism

	Proteolytic *C. botulinum* (mesophilic)	Non-proteolytic *C. botulinum* (psychrotrophic)
neurotoxins formed	A, B, F	B, E, F
minimum growth pH	4.6	5.0
minimum growth temperature	10-12°C	3.0°C
maximum growth NaCl	10%	5%
spore heat resistance ($D_{100°C}$)	>15 min	<0.1 min

Botulinum toxins of types, A, B, and E, and more rarely type F toxin, are involved in cases of foodborne botulism. It should be noted that one limitation of many investigations of foodborne botulism outbreaks is that only the toxin type is identified, but not the type of *C. botulinum* involved. For example, in the case of strains forming type B toxin, it is often not clear whether the responsible organism is proteolytic *C. botulinum* or non-proteolytic *C. botulinum*. In some foodborne botulism outbreaks, the type of toxin formed and/or the responsible food are not identified.

5.3.1 Recent outbreaks of foodborne botulism involving proteolytic *C. botulinum*

Recent outbreaks of foodborne botulism associated with proteolytic *C. botulinum* have often involved inadequately canned foods (Table 5.2). There are two circumstances in which proteolytic *C. botulinum* has resulted in outbreaks of botulism associated with commercially-produced chilled foods. These are:

1) If products that are designed to be stored chilled, but are held at ambient by either the retailer or purchaser

or

2) If ingredients containing preformed botulinum toxin, are added to a chilled product that is correctly stored

Examples of the first circumstance include garlic-in-oil, black bean dip, clam chowder, and a fish soup (Table 5.2). Chopped garlic in soybean oil was purchased by a restaurant and stored unrefrigerated for eight months (and not refrigerated as indicated on the label). Proteolytic *C. botulinum* type B grew and formed toxin in one bottle of garlic, which was used in sandwiches (St. Louis *et al*., 1988). Two separate outbreaks in California in 1994 also involved temperature abuse of chilled foods, and associated growth and toxin formation by proteolytic *C. botulinum* type A (Anon, 1995). The first, in June, was associated with vacuum packed clam chowder purchased from the refrigeration section of a supermarket. The chowder was labelled “keep refrigerated” but stored at room temperature for one month prior to consumption. The second outbreak, in September, was associated with a black bean dip also purchased from the refrigeration section of a store. The container was labelled “perishable, keep refrigerated”, but was held at room temperature for three weeks prior to consumption. The chowder and bean dip were consumed despite seeming spoiled (Anon, 1995). One case reported in France in 1999 involved a commercial chilled fish soup in a carton. This soup was temperature abused in the home, and this allowed proteolytic *C. botulinum* type A to grow and form toxin (Carlier *et al*., 2001). Chilled storage was the only barrier to controlling neurotoxin formation by proteolytic *C. botulinum* in these four foods.

Examples of the second circumstance noted above, include the large outbreaks associated with hazelnut yoghurt in the UK and *skordalia* in the USA (Table 5.2). The largest recorded botulism outbreak in the UK was caused by type B toxin, with 27 cases and one death, and involved yoghurt prepared with toxin-contaminated hazelnut conserve (O'Mahony *et al.*, 1990). Heat-processing of the hazelnut conserve was inadequate to destroy spores of proteolytic *C. botulinum* type B. The spores subsequently germinated, leading to growth and neurotoxin formation. A large restaurant-associated outbreak in the USA was caused by type A toxin, with 30 cases, and involved *skordalia* prepared with neurotoxin-containing potato (Angulo *et al.*, 1998). Potatoes wrapped in foil were baked, and then stored at ambient temperature. Temperature abuse during storage permitted growth and toxin formation by proteolytic *C. botulinum* type A. The neurotoxin-containing potatoes were then added to yoghurt to give *skordalia,* a potato-based dip.

Table 5.2 Examples of recent incidents of foodborne botulism involving proteolytic *Clostridium botulinum*

Year	Country	Product	Toxin type	Cases (deaths)	Factors contributing to botulism outbreak	Reference
1985	Canada	Commercial garlic-in-oil	B	36	bottled; no preservatives; temperature abuse	St Louis *et al.,* 1988
1987	Canada	Bottled mushrooms	A	11	Underprocessing and/or inadequate acidification	CDC, 1987; McLean *et al.,* 1987
1989	UK	Commercial hazelnut yoghurt	B	27(1)	hazelnut conserve underprocessed	O'Mahony *et al*., 1990
1993	USA	Restaurant, commercial process cheese sauce	A	8 (1)	Contaminated after opening, then temperature abused	Townes *et al*., 1996
1993	Italy	Commercial canned roasted eggplant in oil	B	7	Insufficient heat treatment; improper acidification	CDC, 1995
1994	USA	Restaurant; potato dip ("skordalia") and aubergine dip ("meligianoslata")	A	30	Baked potatoes held at room temperature	Angulo *et al*., 1998
1994	USA	Commercial clam chowder	A	2	no secondary barrier; temperature abuse	Anon, 1995
1994	USA	Commercial black bean dip	A	1	no secondary barrier; temperature abuse	Anon, 1995
1996	Italy	Commercial mascarpone cheese	A	8(1)	no competitive microflora; pH >6, temperature abuse	Franciosa *et al*., 1999 Aureli *et al*., 2000
1997	Italy	Home-made pesto/oil	B	3	pH 5.8, a_w 0.97	Chiorboli *et al*., 1997
1997	Germany	Home-prepared beans	A	1	Poor preparation	Gotz *et al*., 2002
1997	Iran	Traditional cheese preserved in oil	A	27(1)	Unsafe process	Pourshafie *et al*., 1998
1998	Thailand	Home canned bamboo shoots	A	13(2)	Inadequate processing (?)	CDC, 1999
1998	Argentina	Meat roll ("matambre")	A	9	cooked and heat-shrink plastic wrap; temperature abuse	Villar *et al*., 1999
1998	UK	Home bottled mushrooms in oil (imported from Italy)	B	2(1)	Unsafe process	CDSC, 1998; Roberts *et al*., 1998
1999	France	Commercial chilled fish soup	A	1	Temperature abuse at home	Carlier *et al*., 2001
2000	France	Home-made asparagus soup	B	9	Inadequate processing (?)	Abgueguen *et al*., 2003
2001	USA	Commercial frozen chilli sauce	A	16	Temperature abuse at salvage store	Kalluri *et al*., 2003
2002	South Africa	Commercial tinned pilchards	A	2(2)	Corrosion of tin, permitted secondary contamination	Frean *et al.,* 2004
2002	Canada	Restaurant, baked potato in aluminium foil	A	1	Baked potato held at room temperature?	Bhutani et al., 2005
2005	UK	Travel from Georgia	A	1	Not known	K. Grant, pers. com.
2006	Thailand	Home canned bamboo shoots	A	163	Inadequate processing (?)	CDC, 2006

5.3.2 Recent outbreaks of foodborne botulism involving non-proteolytic *C. botulinum*

Outbreaks of foodborne botulism associated with non-proteolytic *C. botulinum* have been most frequently associated with (Table 5.3):

- salted, smoked or dried fish
- home-made foods prepared by the peoples of Alaska and north Canada (e.g. fermented beaver tail and paw, fermented seal or walrus, fermented salmon roe, "muktuk")
- meat (e.g. Bologna sausage, home-cured ham, reheated chicken)

A majority of botulism outbreaks have involved home-made foods, some of which are attributed to inadequate temperature control. Outbreaks of foodborne botulism involving commercial chilled foods and non-proteolytic *C. botulinum* have occurred when the shelf-life or storage temperature of the food have been abused (Table 5.3). For example, an outbreak in Germany in 1997 was associated with suspected temperature abuse of commercial hot-smoked vacuum-packed fish. An outbreak in Germany in 2004 was associated with consumption of commercial vacuum packed salmon after the use by date. Outbreaks of botulism have been associated with temperature abuse of commercial products intended for frozen storage in France in 1998 (Table 5.3). It should be noted that no reports could be identified of foodborne botulism that involved toxin formation by non-proteolytic *C. botulinum* in commercial chilled foods for which the shelf-life and storage temperature were maintained at levels specified by manufacturers.

While most reported outbreaks of botulism linked to non proteolytic *C. botulinum* are associated with type E toxin (Table 5.3), it is likely that many outbreaks in Europe associated with type B neurotoxin are due to non-proteolytic *C. botulinum* type B (Lucke, 1984; Hauschild, 1992). In other recent botulism outbreaks, the responsible organism (and sometimes toxin) has not been identified, and it is likely that those involving smoked, dried or salted fish are associated with non-proteolytic *C. botulinum* (Table 5.4).

Table 5.3 Examples of recent incidents of foodborne botulism involving non-proteolytic *Clostridium botulinum*

Year	Country	Product	Toxin type	Cases (deaths)	Factors contributing to botulism outbreak	Reference
1981	USA	Uneviscerated salted, air-dried fish ("kapchunka")	B	1	Poorly controlled salting, lack of refrigeration	California State Health Department, 1981
1982	Madagascar	Commercial pork sausage	E	60 (30)	Inadequate preservation	Viscens et al., 1985
1985	USA	Uneviscerated salted, air-dried fish ("kapchunka")	E	2 (2)	Poorly controlled salting, lack of refrigeration	CDC, 1985
1987	USA and Israel	Commercial uneviscerated salted, air-dried fish ("kapchunka")	E	8(1)	Poorly controlled salting, lack of refrigeration	Slater *et al*., 1989
1991	Sweden	Vacuum-packed hot-smoked rainbow trout	E	?	?	Korkeala *et al*., 1998
1991	Egypt	Commercial uneviscerated salted fish ("faseikh")	E	>91(18)	Putrefaction of fish before salting	Weber *et al*., 1993
1992	USA	Commercial uneviscerated salted fish ("moloha")	E	8	Insufficient salt	CDC, 1992
1994	Sweden	Vacuum-packed hot-smoked rainbow trout	E	?	?	Korkeala *et al*., 1998
1995	Canada	"Fermented" seal or walrus (4 outbreaks)	E	9	Unsafe process	Proulx *et al.,* 1997
1997	France	fish	E	1	?	Boyer *et al.,* 2001
1997	Germany	Commercial hot-smoked vacuum-packed fish ("Raucherfisch")	E	2	Suspected temperature abuse	Jahkola and Korkeala, 1997; Korkeala *et al.,* 1998
1997	Argentina	Home cured ham	E	6	?	Rosetti *et al*., 1999
1997	Germany	Home smoked vacuum-packed fish ("Lachsforellen")	E	4	Temperature abuse	Anon, 1998
1998	Germany	Commercial smoked vacuum-packed fish	E	4	?	Therre, 1999
1998	France	Commercial frozen vacuum packed scallops	E	1	Temperature abuse (?)	Boyer *et al.,* 2001
1998	France	Commercial frozen vacuum packed prawns	E	1	Temperature abuse (?)	Boyer *et al.,* 2001
1999	Finland	Whitefish eggs	E	1	Temperature abuse	Lindström *et al*., 2004
1999	France	Salmon or fish soup	E	1	?	Boyer *et al.,* 2001
1999	France	Grey mullet	E	1	Temperature abuse (?)	Boyer *et al.,* 2001
2001	Australia	Reheated chicken	E	1	Poor temperature control	Mackle *et al*., 2001
2001	USA	Home-made fermented beaver tail and paw	E	3	Temperature abuse	CDC, 2001
2001	Canada	Home-made fermented salmon roe (2 outbreaks)	E	4	Unsafe process	Anon, 2002
2002	USA	Home-made "muktuk" (from Beluga whale)	E	12	Unsafe process	McLaughlin *et al*., 2004
2003	Germany	Home salted air-dried fish	E	3	Temperature abuse (?)	Eriksen *et al*., 2004
2004	Germany	Commercial vacuum-packed smoked salmon	E	1	Consumed after "use-by date"	Dressler, 2005

Table 5.4 Examples of recent incidents of foodborne botulism where responsible toxin/organism not reported

Year	Country	Product	Toxin type	Cases (deaths)	Reference
1990	USA	Barbequed [fresh] surgeon fish (palani)	B*	3	CDC, 1991
1993/4	Switzerland	Commercial dry-cured ham	B	12	Troillet and Praz, 1995
1994	Georgia	Fish consumed at wedding	nk†	173	Varma *et al*., 2004
1997	USA	Commercial burrito	B	1	Sobel *et al*., 2004
1998	Algeria	Rotten poultry and processed meat dish (kashir)	nk	1400 (17)	www.promedmail.org (24/07/1998)
1998	Croatia	Ham	B	20	Pavic *et al*., 2001
1999	Morocco	Commercial Mortadella sausage	B	78 (20)	Ouagari *et al*., 2002
1999	Azerbaijan	Restaurant, contaminated fish	nk	90 (4)	www.promedmail.org (21/12/1999)
2001	Austria	Home canned fruits/vegetables (?)	B	1	Allerberger, 2001
2002	Denmark	Commercial garlic in chilli oil dressing	B	1	Krusell, 2003
2002	UK	Homemade sausage (from Poland)	B	1(1)	K. Grant, personal communication
2003	France	Halal sausage	B	4	Espie *et al*., 2003
2003	Germany	Homemade smoked sparsely cured ham	B	1	Merz *et al*., 2003
2003	South Korea	Commercial canned sausage	nk	3	www.promedmail.org (29/06/2003)
2003	Ukraine	Home canned corn	nk	6	www.promedmail.org (21/11/2003)
2004	UK	Commercial chilled organic hummus	nk	1	K. Grant, personal communication
2004	Italy	Restaurant, preserved green olives in saline	B	16	Cawthorne *et al*., 2005
2004	Russia	Commercial dried fish	nk	4 (1)	www.promedmail.org (26/05/2004)
2004	Russia	Smoked fish	nk	10 (1)	www.promedmail.org (25/08/2004)
2004	Ukraine	Commercial dried fish	nk	6	www.promedmail.org (30/10/2004)
2004	Kyrgyzstan	Home canned aubergine	nk	5 (1)	www.promedmail.org (03/12/2004)
2005	UK	Home preserved pork (from Poland)	B	1	K. Grant, personal communication
2005	Russia	Smoked fish	nk	9	www.promedmail.org (18/01/2005)
2005	Ukraine	Commercial dried fish	nk	3	www.promedmail.org (20/02/2005)
2005	Russia	Home canned cucumbers	nk	16	www.promedmail.org (26/02/2005)
2005	Kyrgyzstan	Home canned salad	nk	6	www.promedmail.org (06/04/2005)
2005	Russia	Home canned food	nk	5	www.promedmail.org (05/07/2005)
2005	Russia	Canned food	nk	15 (1)	www.promedmail.org (12/07/2005)
2005	Russia	Home salted fish	nk	6	www.promedmail.org (14/10/2005)
2005	Kazakhstan	Home dried fish	nk	25 (1)	www.promedmail.org (17/10/2005)
2005	Kyrgyzstan	Home canned salads	nk	5	www.promedmail.org (19/12/2005)
2005	Kyrgyzstan	Home canned salads	nk	3 (1)	www.promedmail.org (19/12/2005)
2006	Armenia	Fish or canned food?	nk	4	www.promedmail.org (16/02/2006)

Note that many of these reports are of a preliminary nature
* only toxin identified, unclear whether proteolytic *C. botulinum* type B or non-proteolytic *C. botulinum* type B
† not known, toxin reported as present, but type not reported

5.4 Review of incidence of foodborne botulism in various countries

5.4.1 An overview of the position in different countries

The extent of reporting and investigating cases of foodborne botulism varies from country to country (Therre, 1999). For example, botulism is a reportable disease in some countries, while in other countries it is not. It is likely, therefore, that the reported data are far from complete. Table 5.5 summarises the reported incidence of foodborne botulism in various countries. Between 20 and 40 cases of foodborne botulism are recorded annually in France, Georgia, Germany, Italy and USA, with a higher incidence in China and Poland (Table 5.5).

Recent data indicate that in Europe, more than 2,500 cases of foodborne botulism were reported in 1999/2000 (Table 5.6). Countries with a high reported incidence include Armenia, Azerbaijan, Belarus, Georgia, Poland, Russia, Turkey and Uzbekistan (Table 5.6). Georgia has one of the highest nationally reported rates of foodborne botulism in the world, with more than 80% of the cases attributed to home-preserved vegetables (Varma *et al*., 2004). One large outbreak at a wedding in 1994 affected 173 people and was associated with consumption of contaminated fish (Table 5.4). Recent cases of foodborne botulism in Russia have been attributed to smoked, salted and dried fish and to canned vegetables, often prepared in the home (Table 5.4).

Table 5.5 Recorded foodborne botulism in different countries

Country	Period	Number of cases	
		Total	Average per year
Belgium	1982-2000	32	2
Canada	1971-2005	439	13
China	1958-1983	4377	168
Denmark	1984-2000	18	1
France	1971-2003	1286	39
Georgia	1980-2002	879	40
Germany	1983-2000	376	22
Italy	1979-2000	750	34
Japan	1951-1987	479	13
Poland	1971-2000	9219	307
Spain	1971-1998	277	10
Sweden	1969-2000	13	1
UK	1971-2005	38	1
USA	1971-2003	934	28

Table compiled from information in: Hauschild and Gauvreau (1985); Hauschild (1992); Galazka and Przbylska (1999); Therre (1999); Lund and Peck (2000); Varma et al. (2004); CDC (2005); Popoff and Carlier (2004); K. Grant (Personal communication); J. Austin (Personal communication); WHO (www.bfr.bund.de/internet/7threport/7threp_fr.htm (accessed 08/03/2006) and www.bfr.bund.de/internet/8threport/8threp_fr.htm (accessed 12/10/2005)). Note: May include cases of human botulism that are not foodborne.

Table 5.6 Recorded foodborne botulism in European countries in 1999/2000

Country	Number of cases	Country	Number of cases
Albania	0	Kyrgyzstan	94
Armenia	176	Latvia	7
Austria	0	Lithuania	36
Azerbaijan	181	Netherlands	3
Belgium	0	Norway	5
Belarus	344	Poland	169
Bosnia and Herzegovina	0	Portugal	33
Bulgaria	18	Moldova	32
Croatia	16	Romania	0
Czech Republic	7	Russia	887
Finland	1	Slovakia	4
France	60	Slovenia	7
Georgia	122	Sweden	0
Germany	30	Switzerland	3
Greece	0	Tajikistan	4
Estonia	1	Turkey	114
Iceland	0	United Kingdom	0
Italy	21	Uzbekistan	131

Source: www.bfr.bund.de/internet/8threport/8threp_fr.htm (accessed 12/10/2005)

5.4.2 Foodborne botulism in the UK

Foodborne botulism is a rare disease in the UK. Fifteen outbreaks have been reported in total, with eight of these in the last forty years (Table 5.7). There have been 62 cases of foodborne botulism, of which 20 were fatal (Table 5.7). There is no consistent vehicle associated with this

disease. Eight of the 15 outbreaks involved home prepared food, and five outbreaks involved commercial foods. The largest outbreak involved commercially produced hazelnut yoghurt, where an inadequate heat treatment was given to the hazelnut conserve. Proteolytic *C. botulinum* then grew and formed toxin in the hazelnut conserve that was subsequently added to the yoghurt. Non-proteolytic *C. botulinum* type E has been involved in one outbreak that was associated with post-process contamination of shelf-stable canned salmon. Proteolytic *C. botulinum* type A or B has been associated with seven outbreaks.

There have been five botulism outbreaks since 1998 (Table 5.7); three of these have involved homemade foods brought into the UK (home bottled mushrooms (from Italy), homemade sausage (from Poland) and home preserved pork (from Poland)). Type B toxin and proteolytic *C. botulinum* type B were isolated from the mushrooms/serum/faeces of patients associated with the mushroom outbreak. Type B toxin was detected in the serum of the patient that consumed the homemade sausage, and a strain forming type B toxin was isolated from the home preserved pork (K. Grant, personal communication). The hummus case in 2004 was mild, and associated with consumption of commercially-produced organic hummus at a party. The hummus was opened at the party but was several weeks out of date and had been stored at room temperature rather than chilled, as indicated on the label. As the hummus tasted off, only a small amount was consumed by the patient, and no-one else ate it. Within one day, the patient developed botulism symptoms, and was diagnosed as suffering from botulism by a physician. He went on to receive ventilation support in hospital. Botulinum anti-toxin was not administered as it was too long since the hummus had been consumed. The case was suspected and not confirmed, as it was too late for a laboratory confirmation of the diagnosis and no hummus was available for testing (K. Grant, personal communication). One case reported in 2005 involved a patient returning from a family visit to Georgia. Proteolytic *C. botulinum* type A was isolated from the faeces of this patient.

It should be noted that non-proteolytic *C. botulinum* has not been associated with any outbreak of foodborne botulism in the UK involving chilled foods. The only outbreak in which non-proteolytic *C. botulinum* has been implicated was associated with post-process contamination of shelf-stable canned salmon (Table 5.7).

Table 5.7 Foodborne botulism in the UK

Year	Food vehicle	Home prepared food	Number of cases (deaths)	Toxin type
1922	Duck paste	No	8 (8)	A^P
1932	Rabbit and pigeon broth	Yes	2 (1)	?
1934	Jugged hare	Yes	1 (0)	?
1935	Vegetarian nut brawn	Yes	5 (4)?	A^P
1935	Minced meat pie	Yes	1 (1)	B
1947	Macaroni cheese	Yes	5 (1)	?
1955	Pickled fish (from Mauritius)	?	2 (0)	A^P
1978	Canned salmon (from USA)	No	4 (2)	E^n
1987	Rice and vegetables (Kosher airline meal)	No	1 (0)	A^P
1989	Commercial hazelnut yoghurt	No	27 (1)	B^P
1998	Home bottled mushrooms in oil (from Italy)	Yes	2 (1)	B^P
2002	Homemade sausage (from Poland)	Yes	1 (1)	B
2004	Commercial chilled organic hummus	No	1 (0)	*
2005	Travel from Georgia	?	1 (0)	A^P
2005	Home preserved pork (from Poland)	Yes	1 (0)	B

Source: Brett (1999), K. Grant (HPA, personal communication)
^{P} = proteolytic *C. botulinum*, ^{n} = non-proteolytic *C. botulinum*
* Case is suspected and not confirmed

5.4.3 Foodborne botulism in France

Between 1971 and 2003, there were 662 outbreaks of human botulism recorded in France, with 1286 cases, of which more than 30 were fatal (Popoff and Carlier, 2004). These were mostly foodborne botulism, with very few cases of infant or wound botulism recorded. Between 1956 and 1979, 97% of botulism outbreaks involved type B toxin, with only 3% of cases associated with other toxin types (Delbos *et al*., 2005). This has now changed, however, and between 1997 and 2002, 78% of outbreaks were associated with type B toxin, and 22% with other toxin types (including type E) (Haeghebaert *et al*., 2003).

Incriminated foods have been identified in 76% of the foodborne botulism outbreaks. Ham and other pork products account for 59% of the outbreaks, canned vegetables for 10%, and fish and shellfish for 4% (Popoff and Carlier, 2004). Until recently, home-made foods were responsible for virtually all outbreaks of botulism in France. As a recent example, an outbreak was associated with the consumption of home-made asparagus soup, and resulted in nine cases of botulism in the Loire region in 2000 (Abgueguen *et al*., 2003). There has, however, been an increase in the association of botulism outbreaks with commercial food or restaurants. In 1956-1979, commercial food or restaurants were associated with only 7% of outbreaks, in 1991-1995 this became 10% of outbreaks, in 1996-2000 this was 24% of outbreaks, and in 2001-2003 it was 30% (Popoff and Carlier, 2004).

It is suspected that two botulism outbreaks reported in 1998 were associated with imported commercial frozen vacuum-packed prawns, and with imported commercial vacuum-packed frozen scallops (Boyer *et al*., 2001). Non-proteolytic *C. botulinum* type E was responsible for these two outbreaks, and has also been responsible for seven other outbreaks involving fish/shellfish, and one involving ham (Boyer *et al*., 2001). In view of the change in the aetiology of foodborne botulism in France (an increased association with commercial food/restaurants and also with toxin formed by non-proteolytic *C. botulinum* type E) these authors comment that "changes in the eating habits of people in France, as in the rest of Europe, with the increased consumption of vacuum-packed fish from endemic areas and decreased consumption of local foodstuffs, could explain the occurrence of the most recent cases" (Boyer *et al*., 2001). Popoff and Carlier (2004) later made a similar comment "new methods of food preservation such as refrigerated, vacuum-packed food without heat treatment and sufficient preservative factors represent new vehicles of botulism". It is important to recognise that the suspected foods in the two non-proteolytic *C. botulinum* type E outbreaks in 1998 were frozen and not chilled. One case reported in 1999 involved a commercial chilled fish soup in a carton that was temperature abused in the home. Proteolytic *C. botulinum* type A was involved (Carlier *et al*., 2001). It is important to note that in some cases of foodborne botulism the incriminated food has not been identified despite a careful investigation (Lamboley *et al*., 2001).

In conclusion, foodborne botulism is more prevalent in France than in the UK. There has been a change in the aetiology of foodborne botulism in the last few years, with an increased association with commercial food/restaurants and also with toxin formed by non-proteolytic *C. botulinum* type E. This has led to speculation that this change may be associated with refrigerated, vacuum-packed foods, although a link with commercial minimally heated, chill foods has not been established. It should be noted that in France, non-proteolytic *C. botulinum* has not been associated with outbreaks of botulism involving correctly stored chilled foods.

5.4.4 Foodborne botulism in other European countries

Many outbreaks of foodborne botulism in Europe are associated with type B toxin, and it is likely that many of these are due to non-proteolytic *C. botulinum* type B (Lucke, 1984; Hauschild, 1992).

In Italy, 750 cases of botulism were recorded between 1979 and 2000 (Table 5.5). Between 1992 and 1996, 103 cases were associated with vegetables in oil, 20 with other vegetable

products, 35 with meat or fish products, seven with cheese, and 27 were unknown (Squarcione *et al*., 1999). A majority of cases (111) involved home-prepared foods, with commercially-produced foods and restaurants less frequently implicated (54 cases). One outbreak in 1996 involved commercial chilled mascarpone cheese that was temperature abused. Toxin was formed by proteolytic *C. botulinum* type A (Table 5.2).

Between 1983 and 2000, 376 cases of foodborne botulism were reported in Germany (Table 5.5). Many outbreaks involve home-prepared meat (Lucke, 1984), and home-prepared and commercial fish (Table 5.3). Temperature abuse of home-prepared fish, and time/temperature abuse of commercial chilled fish has been associated with foodborne botulism.

Countries with a high reported incidence in 1999/2000 include Armenia, Azerbaijan, Belarus, Georgia, Poland, Russia, Turkey and Uzbekistan (Table 5.6). The Republic of Georgia has one of the highest nationally recorded rates of botulism in the world, with 879 cases recorded between 1980-2002, of which 58 were fatal (Varma *et al*., 2004). A majority of cases were associated with home preserved vegetables (80% of cases), and smoked fish (12% of cases). Most cases were associated with type B toxin. The largest recorded outbreak, at a wedding in 1994, was associated with fish and affected 173 people (Varma *et al*., 2004).

More than 9,000 cases were reported in Poland between 1971 and 2000 (Table 5.5), of which more than 150 were fatal (Galazka & Przybylska, 1999). The number of cases recorded per year has declined significantly since 1982, when 738 cases were recorded (Galazka & Przybylska, 1999). A majority of cases (74% in 1990) are associated with home-prepared foods, in particular the practice of home-preserving meat and, to a lesser extent fruits and vegetables, in glass jars (weckglas). In 1990, 26% of all cases were associated with commercial foods, most frequently canned meat and fish. Most cases are associated with type B toxin, although the proportion of cases associated with type E toxin has increased in recent years (Galazka & Przybylska, 1999).

Non-proteolytic *C. botulinum* has not been associated with outbreaks of botulism involving correctly stored commercially produced chilled foods in Europe.

5.4.5 Foodborne botulism outside Europe

In recent years, an average of 13 cases of foodborne botulism has been recorded annually in Canada, and 28 cases recorded annually in the USA (Table 5.5). In Canada, 439 cases of foodborne botulism were recorded between 1971 and 2005, of which at least 32 were fatal (Hauschild and Gauvreau, 1985; J. Austin (Personal communication)). A majority of cases have involved non-proteolytic *C. botulinum* type E and foods, such as “fermented” seal and walrus “fermented” salmon roe, prepared at home by native people of northern Canada (Table 5.3).

In USA, 934 cases of foodborne botulism were recorded between 1971 and 2003. Between 1990 and 2000, there were 263 foodborne botulism cases; 92 cases involved home-prepared “fermented” foods in Alaska, and 97 cases involved home-canned foods and other home-prepared foods outside Alaska. Ten cases involved commercial foods and 25 cases involved restaurants. Examples are given in Tables 5.2-5.4.

In Canada and USA, foodborne botulism has been associated with commercial refrigerated foods that were time/temperature abused. These include garlic-in-oil, clam chowder and black bean dip (Table 5.2). These outbreaks all involved proteolytic *C. botulinum.* An interesting outbreak in Hawaii in 1990 was associated with consumption of barbequed fresh surgeon fish. Type B toxin was involved, but it is not apparent whether the producing organism was proteolytic *C. botulinum* or non-proteolytic *C. botulinum* (Table 5.4). Temperature abuse of the fish at the point of sale may have contributed to this outbreak. The fish was purchased from a local market, where other fish were found to have an internal temperature of 11°C (Anon, 1991). A 1989 CDC analysis of botulism outbreaks in USA indicated that no cases due to non-proteolytic *C.*

botulinum were obviously due solely to growth and toxin formation at refrigeration temperatures. In 2005, the NACMCF concluded that the epidemiological picture had not changed since 1989 (NACMCF, 2005).

In Japan, 114 outbreaks (more than 500 cases) of foodborne botulism were recorded between 1951 and 2005. Nine outbreaks involved strains forming type A or type B toxin, 104 outbreaks involved strains forming type E toxin, and one outbreak is unknown (Monma *et al*., 2005). Most Japanese outbreaks of botulism are associated with fish (99% in the period 1951-1987), and with home-prepared foods (98% in the period 1951-1987) (Lund and Peck, 2000). Large outbreaks of foodborne botulism have also been reported elsewhere in the world. For example an outbreak in Madagascar in 1982 affected an estimated 60 people, an outbreak in Egypt in 1991 affected more than 91 people, an outbreak in Algeria in 1998 affected 1400 people, and an outbreak in Thailand in 2006 affected 163 people (Tables 5.2-5.4).

Non-proteolytic *C. botulinum* has not been associated with outbreaks of botulism involving correctly stored commercially produced chilled foods outside Europe.

5.5 Conclusions

Foodborne botulism is a severe and deadly disease, with most outbreaks associated with home-made foods where known control measures have not been implemented. More rarely, outbreaks of foodborne botulism have been associated with commercial foods and with restaurants. Very large costs are associated with botulism outbreaks involving commercial foods, and are orders of magnitude greater than those associated with other foodborne pathogens (e.g. *Salmonella*, *Listeria*).

Foodborne botulism is most frequently associated with proteolytic *C. botulinum* or with non-proteolytic *C. botulinum*. Proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are physiologically and genetically distinct organisms. Proteolytic *C. botulinum* is a mesophile, has a minimum growth temperature of 10°C-12°C, and forms toxins of types A, B and F. Non-proteolytic *C. botulinum* is a psychrotroph that is able to grow and form toxin at 3.0°C, and forms toxins of types B, E and F. In view of its ability to grow and form toxin at 3.0°C, non-proteolytic *C. botulinum* is a larger concern in chilled foods.

In Europe, more than 2,500 cases of foodborne botulism were reported in 1999/2000. In the UK, 62 cases of foodborne botulism were reported between 1922 and 2005. Twenty of these cases were fatal. In the last twenty years there have been 34 cases of foodborne botulism in the UK, three of these have been fatal. Non-proteolytic *C. botulinum* has been associated with one outbreak of botulism in the UK (four cases and two deaths) that involved post-process contamination of ambient stable canned salmon in 1978.

Non-proteolytic *C. botulinum* has not been associated with outbreaks of botulism involving correctly stored commercially produced chilled foods.

Outbreaks of foodborne botulism have only been associated with commercially produced chilled foods that have been time/temperature abused, and also when botulinum toxin has been inadvertently added (with a food component) to a correctly chilled food product. There have been outbreaks associated with each of these scenarios in the UK, and in other countries. Twelve examples are given in Table 5.8.

No cases of foodborne botulism can be attributed to non-proteolytic *C .botulinum* and correctly stored commercial chilled foods in the UK or overseas. There is, however, speculation that the recent change in aetiology of foodborne botulism in France may be associated with refrigerated vacuum-packed foods, although a link with commercial refrigerated foods has not been established.

Table 5.8 Examples of foodborne botulism involving commercial chilled foods

Country (year)	Product	Organism and toxin type	Cases (deaths)	Factors contributing to outbreak	Reference
Canada (1985)	Garlic-in-oil	Proteolytic *C. botulinum* B	36	temperature abuse	St Louis *et al.,* 1988
UK (1989)	Hazelnut yoghurt	Proteolytic *C. botulinum* B	27(1)	Toxin added with hazelnut conserve to correctly chilled yoghurt	O'Mahony *et al*., 1990
USA (1990)	Surgeon fish (palani)	*C. botulinum* B*	3	temperature abuse	CDC, 1991
USA (1993)	Cheese sauce	Proteolytic *C. botulinum* A	8(1)	Recontamination and temperature abuse	Townes *et al*., 1996
USA (1994)	Potato dip ("skordalia") and aubergine dip ("meligianoslata") Restaurant;	Proteolytic *C. botulinum* A	30	Toxin added with potatoes to correctly chilled yoghurt	Angulo *et al*., 1998
USA (1994)	Clam chowder	Proteolytic *C. botulinum* A	2	temperature abuse	Anon, 1995
USA (1994)	Black bean dip	Proteolytic *C. botulinum* A	1	temperature abuse	Anon, 1995
Italy (1996)	Mascarpone cheese	Proteolytic *C. botulinum* A	8(1)	temperature abuse	Franciosa *et al*., 1999; Aureli *et al*., 2000
Germany (1997)	Hot-smoked vacuum-packed fish ("Raucherfisch")**	Non-proteolytic *C. botulinum* E	2	Suspected temperature abuse	Jahkola and Korkeala, 1997; Korkeala *et al.,* 1998
France (1999)	Chilled fish soup	Proteolytic *C. botulinum* A	1	temperature abuse	Carlier *et al*., 2001
Germany (2004)	Vacuum-packed smoked salmon**	Non-proteolytic *C. botulinum* E	1	Consumed after "use-by date"	Dressler, 2005
UK (2004)	Organic hummus	Not known	1	Time/temperature abuse	K. Grant, personal communication

* Not clear whether proteolytic *C. botulinum* or non-proteolytic *C. botulinum*
** It is likely that only the outbreaks in Germany in 1997 and 2004 involve VP/MAP foods

5.6 References

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Chapter six: Summary of data on growth and toxin formation by non-proteolytic *C. botulinum* at ≤10°C

6.1 Outputs from predictive models

Non-proteolytic *C. botulinum* is a psychrotrophic foodborne pathogen. The minimum temperature at which growth and toxin formation has been reported is 3.0°C (Graham *et al*., 1997). Growth and toxin formation are slow near to the minimum temperature for growth, and have been reported after seven weeks at 3.0°C, six weeks at 3.1°C, and five weeks at 3.2°C-3.3°C (Peck and Stringer, 2005). Several predictive models have been developed for growth of non-proteolytic *C. botulinum*. Each model is based on a different dataset and gives a slightly different prediction but, in general, the models are constructed to deliver a fail-safe prediction of time to toxin formation when other factors are not limiting. Predictions from four models are shown in Fig. 6.1 and Table 6.1. The four models are:

- Combase Predictor. The model in ComBase Predictor was developed and validated by Graham *et al.* (1996b), and is based on growth curves done in a microbiological broth medium. Most of the growth curves were at ≤10°C, and the model is most robust in this region (Graham *et al*., 1996b). This model is freely available in ComBase Predictor (www.combase.cc). In Fig. 6.1, time to toxin formation is taken as the predicted time to a 1000-fold increase in viable count.
- PMP (Pathogen Modeling Program). A probability model developed by Whiting and Oriente (1997) is freely available in PMP (www.arserrc.gov/mfs/PMP6_CurMod.htm). This probability model is also based on tests done in a microbiological broth medium, but time to turbidity is used as the measure of toxin formation (Whiting and Oriente, 1997). In Fig. 6.1, time to toxin formation is taken as the time to turbidity from a starting concentration of 10^4 spores/ml.
- Baker/Genigeorgis model. This model was developed by Baker and Genigeorgis (1992). It is based on tests carried out in more than 17,000 raw fish homogenates (Baker and Genigeorgis, 1992), and is a lag time model (where lag time is taken as the last time that all replicate samples were negative for toxin in a time series). This model is freely available in the Pathogen Modeling Program (PMP) site (www.arserrc.gov/mfs/PMP6_CurMod.htm).
- Skinner/Larkin. The Skinner/Larkin model is a more conservative version of the Baker/Genigeorgis model, modified to take account of observations of growth in other experiments (Skinner and Larkin, 1998).

All four models predict that toxin formation will occur in less than 10 days at 8°C (Fig. 6.1, Table 6.1). The different predictions of time to toxin formation reflect the different datasets on which the models were based. The model in ComBase Predictor is designed to be most robust at ≤10°C, and may give more reliable predictions in this region than the other two original models. The Skinner/Larkin model is designed to be an ultimate failsafe model. It may be that the prediction of toxin formation in 5-6 days at 8°C is the most reasonable fail-safe prediction from the models, and the prediction of toxin formation in 4 days at 8°C is the most conservative failsafe prediction. It is important to recognise, however, that these models are designed to represent various worst-case scenarios, and the issue that must be addressed is how closely predictions from these models relate to toxin production in actual chilled foods sold in the UK and elsewhere.

Just as models developed using microbiological broth media predict toxin formation in less than 10 days at 8°C (Table 6.1), tests in microbiological broth media have also reported growth/toxin formation in 10 days or less at 8°C (e.g. Jensen *et al*., 1987; Graham *et al*., 1997). In this report it is accepted that in microbiological broth media, growth and toxin formation can occur in 10 days or less at 8°C, and in this project no further data have been collected on tests carried out in microbiological broth media.

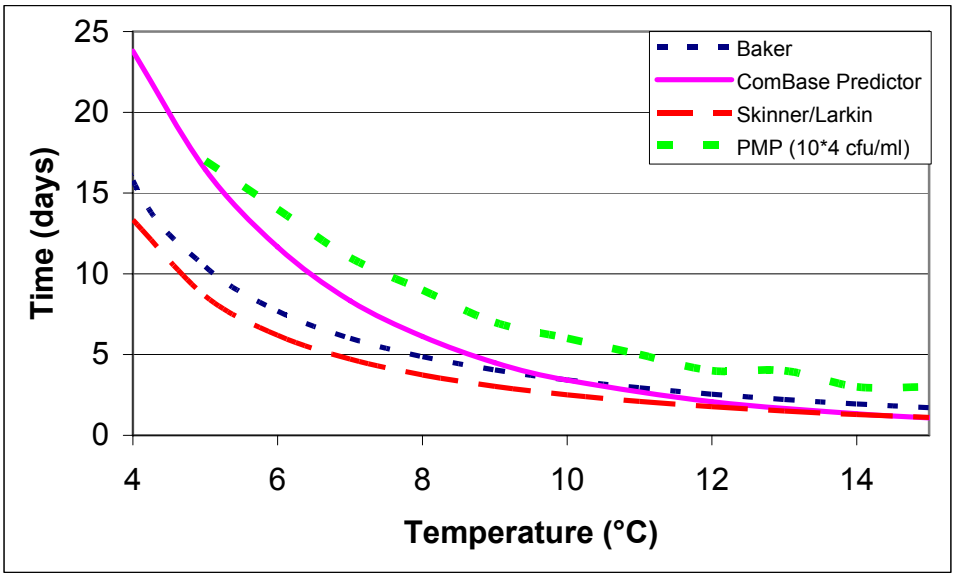

Fig. 6.1 Effect of incubation temperature on the time to toxin predicted by four mathematical models

Table 6.1 Example predictions of time to toxin formation at 4°C-12°C from four predictive models

model	Predicted time to toxin formation (d) at specified temperature								
	4°C	5°C	6°C	7°C	8°C	9°C	10°C	11°C	12°C
ComBase Predictor	24	16	12	8	6	5	3	3	2
PMP	--	17	14	11	9	7	6	5	4
Baker/Genigeorgis	16	10	8	6	5	4	3	3	3
Skinner/Larkin	13	9	6	5	4	3	3	2	2

6.2 Observations of time to toxin formation in chilled foods/food materials

6.2.1 Method for data collection

In order to collect literature data on toxin formation in chilled foods, an extensive literature search was carried out in January 2006 for articles referring to growth of non-proteolytic *C. botulinum* at temperatures of 10°C or less. References were retrieved from Web of Knowledge (1970-2006), BIOSIS (1969-2006), SCOPUS (1992-2006) and Agricola (to 2006) and combined with articles held in the personal libraries of the authors of this report. Also, members of the food industry were contacted with a request to donate any relevant confidential data. Based on a review of titles and abstracts, this search identified approximately 400 references (refereed publications, book chapters and conference proceedings) that appeared to contain suitable data. The entire article of each of these references was then individually assessed for relevance, and appropriate data extracted. After an initial examination of the data, the following relevance criteria were adopted for data:

Growth matrix - Only studies in food matrices or food materials should be included. Tests in microbiological broth media were excluded.

Temperature - The incubation temperature must be stated and ≤10°C.

Food properties – The type of food or food material must be recorded. Where possible information on pack size, pH, Aw, NaCl (% salt-on-water) and other preservative factors should be noted. It should be recorded whether the food or food material was vacuum packed (VP) or

modified atmosphere packed (MAP). If MAP, the gas mixture should be noted. Details of any heat process should be noted, and whether applied before or after addition of spores.

Clostridium botulinum – Only data for non-proteolytic *C. botulinum* should be included. The type of toxins formed by the tested strains should be noted, and the inoculum concentration recorded (both per g and per pack). The inoculum concentration must not exceed 10^5 spores/g or 10^6 spores/pack.

Growth and toxin formation – Where possible, data on toxin formation should be included. If growth data are included, the criteria for being positive/negative should be noted. In most of these tests, replicate samples (often duplicate or triplicate) have been removed at time intervals and tested for toxin (or growth). The last time when all the replicate samples were negative, and the first time when one of the replicate samples was positive for toxin (or growth) must be recorded in days. It should be noted that this approach leads to the omission of some negative data for the replicate samples (e.g. if two of the replicates are negative and one is positive, then the sample is recorded as positive). If the time between the last negative (last time when all replicates were negative) and first positive sample is four days or less, the time to toxin (TTT) should be calculated, where TTT = $\sqrt{}$ (last time when all samples were negative x time to first positive sample). If the period between sampling is greater than four days, then TTT should be recorded as uncertain. The results from all studies should be included, even if samples at all the time points are negative for toxin/growth.

Reference – Full details should be included.

In view of the substantial quantity of data available, it was necessary to select which data to include. Most of the data on pasteurising-irradiation are absent, as this process is not used in the UK (but see comments in section 6.4), and not all data on foods packed in air are included as this is not a low oxygen atmosphere.

Although high oxygen packs are excluded it should be noted that toxin formation can be as rapid (or in some circumstances more rapid) in air as under VP or low-oxygen MAP (presumably because there is no oxygen in the food, i.e. the food is reduced [possibly by aerobic organisms]). For example, at 12°C flounder became toxic in 11 days in air, in 10-14 days in various anaerobic modified atmospheres, and in 15 days in VP. It was also noted that fish packed in air frequently spoiled more rapidly than when packed in MAP/VP (Post *et al*., 1985). Eklund (1982) reported that salmon stored for 7 days at 10°C was more likely to become toxic when packed in air than when packed under CO_2 atmospheres. Additionally, Reddy *et al*. (1996, 1997a, 1997b) noted that toxin formation could be more rapid when salmon, tilapia and catfish were packed in air than under N_2:CO_2 (25:75). Packaging under air or a similar oxygen-containing atmosphere is therefore not a guarantee that toxin formation by non-proteolytic *C. botulinum* will be prevented.

Altogether, data were extracted from 61 literature publications. These publications yielded 887 independent tests of time to toxin formation. Additionally, members of the food industry were contacted with a request to donate any relevant confidential data. Altogether, a total of 27 confidential datasets were kindly donated by members of the food industry. These datasets contained 420 independent tests of time to toxin formation, giving a total of 1307 independent tests. One independent test would typically be one product at one temperature sampled a number of times. Replicate samples would be removed and tested for toxin at various time points (often in duplicate or triplicate), and the last time when all the replicate samples were negative, and the first time that one of the replicate samples was positive noted. Toxin would typically be detected using the mouse test, although some data were based on growth tests. It is important to recognise that this approach records whether the test at each sampling time was either negative or positive for toxin/growth. All the information collected is included in Appendix two, and is to be deposited in ComBase (www.combase.cc) to allow continued access.

6.2.2 Summary of tests of toxin formation by non-proteolytic *C. botulinum* at 10°C

A total of 389 independent tests carried out at 10°C have been identified. The full dataset is included in Appendix 2 and all the data collected at 10°C are summarised in Table 6.2. At day 5, 24 tests were positive (7%); at day 10, 132 tests were positive (36%); and at day 15, 166 tests (50%) were positive. A substantial number of these tests were carried out with various raw/smoked fish (from lines 4-96 in Appendix 2), and these are summarised in Table 6.3. After 10 days, 41 tests were positive for toxin and 50 were negative.

Table 6.2 Summary of all data on toxin formation by non-proteolytic *C. botulinum* at 10°C

	Negative for toxin	Positive for toxin	Uncertain[a]
Individual results			
Day 5	82[b],40[c],31[d],22[e],144[f]	11,2,0,0,11	0,0,0,11,35
Day 10	50,33,31,18,106	41,8,0,15,68	2,1,0,0,16
Day 15	15,32,31,2,86	61,10,0,15,80	17,0,0,16,24
Summary of all the results			
Day 5	319 (93%)	24 (7%)	46
Day 10	238 (64%)	132 (36%)	19
Day 15	166 (50%)	166 (50%)	57

[a] The result is uncertain because of the low frequency of testing for toxin (sampling time greater than every 4 days).
Individual results: [b]Unpublished and published fish data (93 tests), [c]Carlin & Peck, 1996 (42), [d]Peterson *et al.*, 2002 (31), [e]Rodgers datasets (33), [f]other datasets (189). Total = 389 independent tests.

Table 6.3 Summary of 93 independent tests of toxin formation by non-proteolytic *C. botulinum* in various raw/smoked fish at 10°C (Appendix 2 lines 4-96)

	Number of tests in fish positive and negative for toxin on each indicated day at 10°C							
Day	3	4	5	6	7	8	9	10
Positive	0	4	11	16	28	36	36	41
Negative	93	89	82	77	65	57	55*	50*

* Two additional tests were negative at day 8 when testing was stopped

6.2.3 Summary of tests of toxin formation by non-proteolytic *C. botulinum* at 8°C

Approximately 75% of the data collected on growth of *C. botulinum* at 8°C comes from studies carried out in five laboratories. Comments on these studies, and other studies, follow.

Unpublished data from D. Baker

An unpublished study carried out by Dr. David Baker (Davis, University of California, USA) in 1988/1989 assessed the safety of up to 69 sous-vide foods at seven temperatures from 4°C-30°C, and the results have been kindly made available. In this study, various sous-vide foods (in packs of 100g) were inoculated with 10^4 spores per pack of a mixture of strains of non-proteolytic *C. botulinum*, heated at 60°C-70°C for 10 min, and then stored at 8°C. The data for samples stored at 6°C and 8°C are shown in Table 6.4. At 6°C, the first foods were toxic at 13 days. While at 8°C, two sous-vide foods (rack of lamb and salmon linguini) were toxic on day 9, three foods (chicken strips, caribbean fish ragout, chunk chicken stew) became toxic at day 11, and a further eight foods became toxic at day 12. It was not obvious from measurement of pH and NaCl content why some foods presented a greater risk of toxin formation than others. The

inoculum concentration used in these tests is not unreasonably high. These tests provide a good indication of the safety of sous-vide foods.

Table 6.4 Summary of D. Baker unpublished data on toxin formation by non-proteolytic *C. botulinum* at 6°C and 8°C in various sous-vide foods

	Number of sous-vide food samples positive and negative for toxin on each indicated day at 6°C and 8°C										
Day	8	9	10	11	12	13	14	15	16	17	18
Tests at 6°C											
Positive	0	0	0	0	0	2	3	10	10	10	12
Negative	49	49	49	49	49	47	46	39	39	39	37
Tests at 8°C											
Positive	0	2	2	5	13	13	13	14	16	*	*
Negative	69	67	67	64	56	56	56	55	53	*	*

* At 8°C, tests were stopped at 16 days

Data from Genigeorgis laboratory

A number of papers from the laboratory of Professor C. Genigeorgis (Davis, University of California) have assessed toxin formation in various species of raw fish, cooked turkey breast homogenate, and pre-cooked sous-vide foods (Table 6.5). Tests were carried out at 4°C-30°C. There were 174 independent tests conducted at 8°C, nine with sous-vide foods, 75 with cooked turkey and 90 with various species of raw fish. The raw data are on lines 377-550 in Appendix 2. In these tests, unheated spores (10^0-10^4 per pack) were added to the food (3-50g per test) that was either VP or MAP (100% CO_2, 70% CO_2/30% air), and held at 8°C. In some tests, sodium lactate was also added. Sixty out of 174 tests were positive for toxin in 10 days (4 tests were positive in 4 days, 17 in 7 days, 9 in 8 days, 6 in 9 days, and 24 in 10 days). The tests that were positive in 4 days were all VP salmon fillets (Garcia *et al*., 1987). Some patterns are discernable from the data (Table 6.6). For example; (i) salmon appears better able to support growth and toxin formation by non-proteolytic *C. botulinum*, than red snapper, than sole; (ii) a modified atmosphere of 70% CO_2/air supported the most rapid toxin formation by non-proteolytic *C. botulinum* in red snapper, but for salmon, VP supported the most rapid toxin formation. Samples positive for toxin were found amongst most foods and modified atmospheres tested (Table 6.6).

The inoculum concentration used in these tests is not unreasonably high. The gas atmospheres are not typical of those used in the UK, but are unlikely to have promoted the growth of *C. botulinum* compared with atmospheres used in the UK. It is interesting to note that the presence of 6% O_2 in the 70% CO_2/30% air gas mixture did not prevent toxin formation (see earlier comments on packing under air too).

The spores used in these tests were unheated, and it could be argued that in a minimally heated product the spores could be heat damaged and that the time to toxin formation might be delayed. On the other hand, the raw fish would have a background microflora that could compete and restrict the growth of non-proteolytic *C. botulinum*, whereas in a minimally cooked product the heat treatment might reduce growth by competitive flora allowing more rapid growth and toxin formation by non-proteolytic *C. botulinum*. The tests with cooked turkey breast homogenate and pre-cooked sous-vide foods are likely to mimic situation in the case of post-process contamination, but may be less typical of the likely situation if a food was contaminated prior to the heat treatment.

Overall, these studies provide evidence that, if present, non-proteolytic *C. botulinum* is able to grow and form neurotoxin within 10 days at 8°C given suitable conditions (60/174 tests were positive). Toxin formation will occur within raw fish under these conditions, while post-process contamination of turkey breast (and possibly also other meats) appears to also present a risk if

standard industry hygiene measures were not in place. It is not clear, however, how these observations relate to other chilled foods.

Table 6.5 Summary of 174 tests of toxin formation by non-proteolytic *C. botulinum* in raw fish, cooked turkey breast homogenate and pre-cooked sous-vide foods at 8°C (published data from Genigeorgis laboratory)

	Number of tests positive/negative for toxin on each indicated day at 8°C												
Day	3	4	5	6	7	8	9	10	11	12	13	14	15
Positive	0	4	4	4	21	30	36	60	61	61	74	75	79
Negative	174	170	170	170	153	144	138	114	113	113	100	99	95

Table 6.6 Effect of gas atmosphere and food on toxin formation by non-proteolytic *C. botulinum* in 10 days at 8°C (data from Genigeorgis laboratory)

Food	Number of spore concentrations positive for toxin in specified atmosphere[a]		
	VP	100% CO_2	70% CO_2/30% air
Red snapper homogenate	1	2	5
Red snapper fillet	1	2	4
Salmon homogenate	4	3	2
Salmon fillet	5	4	3
Sole homogenate	1	0	NT
Cooked turkey homogenate	3	NT	NT

[a] Five spore concentrations were tested (10^0, 10^1, 10^2, 10^3, 10^4 per pack)
Where the combination has been tested more than once, a mean number is given
Data where other preservatives were added are not included
NT = not tested

Data from Reddy laboratory
Dr. R. Reddy (FDA, Chicago) assessed toxin formation by non-proteolytic *C. botulinum* in various species of raw fish (100-130g) stored under different modified atmospheres (VP, CO_2:N_2 (75:25), air). An inoculum of approximately 10^4 spores per pack was used. Raw catfish became toxic in 6 days when stored in VP, and in 7 days when stored under air (Table 6.7; Appendix 2 lines 955-993). Raw salmon became toxic in 8 days when stored in VP. Other fish (including raw cod and tilapia) were not positive for toxin until day 15.

The inoculum concentration used in these tests is not unreasonably high. The gas atmospheres are not typical of those used in the UK, but are unlikely to have promoted the growth of *C. botulinum* compared with atmospheres used in the UK. The spores used in these tests were unheated, and the comments made in the preceding section also apply. This study provides further evidence of the ability of non-proteolytic *C. botulinum*, if present, to form neurotoxin within 10 days at 8°C in raw fish.

Table 6.7 Summary of 15 independent tests of toxin formation by non-proteolytic *C. botulinum* in raw fish at 8°C (published data from Reddy lab)

	Number of tests positive/negative for toxin formation on each indicated day at 8°C												
Day	3	4	5	6	7	8	9	10	11	12	13	14	15
Positive	0	0	0	1	2	3	3	3	3	3	3	3	6
Negative	14	14	14	14	13	12	12	10	10	10	10	9	6
Uncertain	1	1	1	0	0	0	0	2	2	2	2	3	3

Uncertain = The result is uncertain because of the low frequency of testing for toxin (sampling time greater than every 4 days).

Data from tests at IFR (Institute of Food Research)

A total of 97 independent tests have been carried out in sterile minced beef at IFR (Appendix 2; papers by Graham *et al*. 1996a, Fernandez and Peck, 1997; 1999; Peck and Fernandez, 1995; Peck *et al*., 1995). In each test, spores (10^6 per pack) were added to the sterile minced beef (20g) packed in an anaerobic atmosphere. Various heat treatments were then applied and cooled samples were stored at 8°C. Twenty-five of the tests were positive for toxin at 8°C in 10 days, and 72 were negative (Table 6.8).

The inoculum concentration used in these tests is high compared with expected levels of natural contamination but provides a measure of whether a 6-decimal process has been achieved. The gas atmospheres are not typical of those used in the UK for chilled foods, but are unlikely to have promoted the growth of non-proteolytic *C. botulinum* compared with gas atmospheres used in the UK. In these experiments, the spores were heated in the beef, and thus mimic the situation in minimally heated products. There was, however, no background flora to compete with non-proteolytic *C. botulinum*, so toxin formation might have been more rapid in these tests than in minimally heated chilled foods sold in the UK, although it should be noted that if a heat-treatment of 70°C/2 min or greater were applied, then most of the competing background would be eliminated. It would seem likely that these tests provide a fail safe indication of the possible time to toxin formation in chilled foods at 8°C. In view of the tremendous variety of chilled foods sold in the UK, it is possible that the observations made in these tests may be relevant to some chilled foods.

One merit of this work is that it identified combinations of heat treatment, storage temperature, and shelf-life that prevented growth and toxin formation from 10^6 spores of non-proteolytic *C. botulinum*. For example, for samples stored at 8°C, heating at 80°C for 23, 70 and 98 min prevented growth/toxin formation until days 20, 25 and 41, respectively. While heating at 85°C for 18 and 36 min prevented growth/toxin formation until days 42 and 47, respectively (Appendix two). These heat treatments are less than those advocated by the ECFF and ACMSF, but when combined with a restricted shelf-life and storage at 8°C provide a safety factor of 10^6 with respect to spores of non-proteolytic *C. botulinum* (Fernandez and Peck, 1999).

Table 6.8 Summary of 97 independent tests of toxin formation by non-proteolytic *C. botulinum* in sterile minced beef at 8°C (published data from IFR)

	Number of tests positive/negative for toxin formation on each indicated day at 8°C												
Day	3	4	5	6	7	8	9	10	11	12	13	14	15
Positive	0	2	7	10	15	17	24	25	26	26	28	29	29
Negative	97	95	90	87	82	80	73	72	71	71	69	68	68

Data from tests carried out at the University of Helsinki
A total of 44 independent tests have been carried in smoked and raw fish and sous-vide foods by workers at the University of Helsinki (Hyytia *et al*., 1997; 1999; 2000; Lindstrom *et al*., 2001; 2003). In 18 tests with smoked/raw fish, 4 were positive for toxin by day 10. All the positive tests were with VP hot-smoked fish that were inoculated with $10^{5.8}$ spores per pack (530-600g), hot-smoked at 85°C for 1.5-44 min, and then stored at 8°C. This is a good test of the safety of VP hot-smoked fish, although the spore concentration is at the top end of what might be reasonably included.

A further 26 tests with various sous-vide foods have also been performed. Foods were inoculated with $10^{5.5}$ spores per pack (pack size 1500g), subjected to various heat treatments, and stored at 8°C. None were positive for toxin at day 10, although the status of four tests is uncertain due to a low frequency of sampling. These tests provide a good assessment of the safety of sous-vide foods.

Data from tests carried out by other authors
Three other authors have published reports of growth and toxin formation by inoculated non-proteolytic *C. botulinum* in 10 days at 8°C (Table 6.9). Carlin and Peck (1996) reported a positive result in 10 days in cooked (sterile) mushroom puree, while Lucke *et al*. (1981) reported a positive result in bone in ham, cured in brine also in 10 days.

Post *et al.* (1985) inoculated 100g fillets of raw cod and whiting fillets with $10^{3.7}$ spores of non-proteolytic *C. botulinum* packed under different modified atmospheres. Cod and whiting packed under CO_2:N_2:O_2 (90:8:2) and CO_2:N_2:O_2 (65:31:4) became toxic in 4-8 days, while fish packed under VP, N_2 (100%) and CO_2 (100%) did not become toxic until day 16 at the earliest (Table 6.9).

Table 6.9 Other reports of toxin formation by non-proteolytic *C. botulinum* in 10 days at 8°C

Food	Inoculum (spores per pack)	Time to toxin (d)	Reference	Line number in Appendix 2
Cooked pureed mushroom	10^5	10	Carlin & Peck (1996)	612
Bone in ham, cured in brine	10^6 *	10	Lucke *et al*. (1981)	850
Raw cod fillets	$10^{3.7}$	7	Post *et al*. (1985)	943
Raw cod fillets	$10^{3.7}$	8	Post *et al*. (1985)	944
Raw whiting fillets	$10^{3.7}$	7	Post *et al*. (1985)	949
Raw whiting fillets	$10^{3.7}$	4	Post *et al*. (1985)	950

* This is an estimate (concentration of $10^{4.8}$ spores/g, pack size not stated)

Summary of data on all tests at 8°C
Altogether, 527 independent datasets on toxin production at 8°C have been collected (Table 6.10). The last time when all replicate samples were negative for toxin, and the first time when one of the replicates was positive for toxin have been recorded. Twelve of the tests were toxic at 5 days (500 not toxic), 100 were toxic at 10 days (414 not toxic), and 142 were toxic at 15 days (360 not toxic). This is ample evidence that non-proteolytic *C. botulinum* is able to produce toxin in foods at 8°C within 10 days. The more difficult issue is to assess the significance of these positive and negative results with respect to the safety of chilled foods currently sold in the UK.

Table 6.10 Summary of all data on toxin formation by non-proteolytic *C. botulinum* at 8°C

	Negative for toxin	Positive for toxin	Uncertain[a]
Individual results			
Day 5	69[b],170[c],14[d],90[e],36[f],121[g]	0,4,0,7,0,1	0,0,1,0,8,6
Day 10	67,114,10,72,35,116	2,60,3,25,4,6	0,0,2,0,5,6
Day 15	55,95,6,68,29,107	14,79,6,29,6,8	0,0,3,0,9,13
Summary of all the results			
Day 5	500 (98%)	12 (2%)	15
Day 10	414 (81%)	100 (19%)	13
Day 15	360 (72%)	142 (28%)	25

[a] The result is uncertain because of the low frequency of testing for toxin (sampling time greater than every 4 days).
Individual results: [b]D. Baker (69 tests), [c]C. Genigeorgis lab (174), [d]Reddy (15), [e]IFR (97), [f]University of Helsinki (44), [g]others (128). Total = 527

6.2.4 Evaluation of the significance of the positive results for toxin formation by non-proteolytic *C. botulinum* at 8°C by food type

One hundred independent tests out of 527, have indicated that non-proteolytic *C. botulinum* can form toxin in foods and food materials in 10 days or less at 8°C (Table 6.11). A total of 414 tests were negative for toxin formation in 10 days at 8°C. The day at which the tests became positive is partly a reflection of the experimental design, and this explains the disproportionately large number of samples that became positive on day 7 (Table 6.11). A number of food types have been studied.

Raw/smoked fish. Fifty six of the positive results are from studies concerned with raw or smoked fish (Table 6.11). Based on these data, there is a real possibility that if contaminated with spores of non-proteolytic *C. botulinum*, fish could become toxic within 10 days at 8°C. It should be noted that more non-proteolytic *C. botulinum* challenge studies have been carried out with fish than with other food types (Table 6.11).

Sterile/Pre-cooked food. Forty one positive results have been obtained with sterile or pre-cooked foods. This includes 25 tests with sterile minced beef, 13 tests with pre-cooked turkey breast homogenate, two tests with pre-cooked sous-vide foods, and one test with cooked (sterile) pureed mushroom (Table 6.11). The tests with sterile minced beef provide a fail safe indication of the possible time to toxin formation in chilled foods. It should be noted that these foods have no background microflora to provide competition to the inoculated organism, however, as there is a large range of chilled foods sold in the UK, it is possible that these observations of toxin formation may be relevant to some chilled foods. The tests with pre-cooked turkey breast and sous-vide foods provide a good indication of the risk presented if there were to be post-process contamination.

Sous-vide foods. Two tests in sous-vide foods (rack of lamb and salmon linguini) where spores were added before a heat treatment (60°C-70°C for 10 min) was applied, were positive for toxin at day nine. Three other foods became toxic at day 11, and a further eight foods became toxic at day 12. Fifty-six sous-vide foods were negative for toxin at day 12. From measurement of pH and NaCl content alone, it was not readily apparent why 13 of the foods had become toxic at day 12, while the other 56 remained negative for toxin. Tests with sous-vide foods at the University of Helsinki and by industry were negative for toxin at day 10 (Table 6.11).

Cured ham. Toxin formation has also been reported in 10 days at 8°C in bone in ham, cured in brine (Table 6.11).

Table 6.11 Summary of different food types in which toxin formation has been reported by non-proteolytic *C. botulinum* in 10 days at 8°C

Food type	Total number of positive tests (total number of all tests)	Number of positive tests at each indicated day							
		3d	4d	5d	6d	7d	8d	9d	10d
Raw/smoked fish	56 (169)	-	5	-	1	24	2	0	24
Sterile minced beef	25 (97)	-	2	5	3	5	2	7	1
Cooked turkey breast	13 (75)	-	-	-	-	-	7	6	-
Pre-cooked sous-vide foods	2 (15)	-	-	-	-	-	2	-	-
Sous-vide foods	2 (110)	-	-	-	-	-	-	2	-
Other foods	2 (61)	-	-	-	-	-	-	-	2
Total	100 (527)	-	7	5	4	29	13	15	27

6.2.5 Summary of tests of toxin formation by non-proteolytic *C. botulinum* at 4°C-7°C

Data have also been collected on time to toxin formation by non-proteolytic *C. botulinum* at less than 8°C. Altogether 391 independent pieces of data have been collected (Table 6.12). At 10 days, 382 tests were negative for toxin (98%), five were positive for toxin (1%), and four (1%) were uncertain because of the low frequency of sampling. Of the five positive results, one was in catfish fillets stored at 4°C which became toxic in 7 days (Appendix 2, line 563). The other four were in sterile minced beef at 6°C where an inoculum of 10^6 spores was added per pack. The spores received no, or a very small, heat treatment in the beef. Time to toxin was 7-10 days (Appendix 2, lines 875-877 and 881). Table 6.12 includes the results of tests with sous-vide foods that were inoculated prior to the delivery of a small heat treatment and then stored at 6°C (summarised in Table 6.4) and at 4°C (where 13 foods became toxic between days 26 and 35 days, while another 56 foods were negative at day 37; Baker and Genigeorgis, 1992).

Table 6.12 Summary of time to toxin formation by non-proteolytic *C. botulinum* at 4°C-7°C

Day	Tests at 4°C				Tests at 5°C				Tests at 6°C				Tests at 7°C				ALL tests			
	N	P	U	∑	N	P	U	∑	N	P	U	∑	N	P	U	∑	N	P	U	∑
5	287	0	0	287	30	0	1	31	68	0	1	69	4	0	0	4	389	0	2	391
10	285	1	1	287	29	0	2	31	64	4	1	69	4	0	0	4	382	5	4	391
15	276	4	7	287	29	0	2	31	51	18	0	69	4	0	0	4	360	22	9	391
20	263	18	6	287	25	4	2	31	47	20	2	69	4	0	0	4	339	42	10	391
25	231	18	38	287	23	5	3	31	16	20	33	69	2	0	2	4	272	43	76	391
30	214	24	49	287	22	5	4	31	10	22	37	69	2	0	2	4	248	51	92	391

N = Number of negative tests for toxin on the indicated day
P = Number of positive tests for toxin on the indicated day
U = Number of uncertain tests (because sampling time greater than every 4 days) for toxin on the indicated day
∑ = Total number of samples

6.2.6 Overall evaluation of tests of toxin formation by non-proteolytic *C. botulinum* in foods at ≤10°C and ≤10 days

It should be noted that most of the data included in this assessment had not been generated for the purpose of evaluating the potential for growth and toxin production by non-proteolytic *C. botulinum* in chilled foods sold in the UK within 10 days or less at 10°C or below. The relevance of these data to short shelf-life chilled foods sold in the UK is in some cases, therefore, limited. Also, the number and proportion of positive tests is to some extent a reflection of the

experimental design in the tests that have been carried out. For example, some tests have been carried out in sterile raw materials where conditions are very favourable for growth and toxin formation, while other tests have been carried in conditions not at all conducive to growth and toxin formation (e.g. preservatives added). The proportion of positive tests is a reflection of the balance between these extremes (and all intermediate positions). It is not easy to relate the number and proportion of positive tests to position with regard to chilled foods sold in the UK.

However, these results do provide evidence that non-proteolytic *C. botulinum,* if present, could form toxin in foods and food materials at ≤10°C within 10 days. All the data for toxin production within 25 days or less are summarised in Fig. 6.2, along with the prediction of time to toxin formation from ComBase Predictor. In total, 237 out of 1307 individual challenge tests were positive for toxin formation by day 10 (19%). At 10°C, 132 of the tests were positive at day 10 (36% of those tested); at 8°C, 100 of the tests were positive at day 10 (19% of those tested); and at 4°C-7°C, five of the tests were positive (1% of those tested). In 38 independent tests, time to toxin formation was more rapid than predicted by the model in ComBase Predictor (Table 6.13). These tests were from ten different publications, and the foods/food materials involved were raw fish (in 21 tests), cooked turkey breast (1 test), pre-cooked sous-vide beef with gravy (2 tests), cooked minced beef (12 tests), and sterile chicken skin and exudate (2 tests).

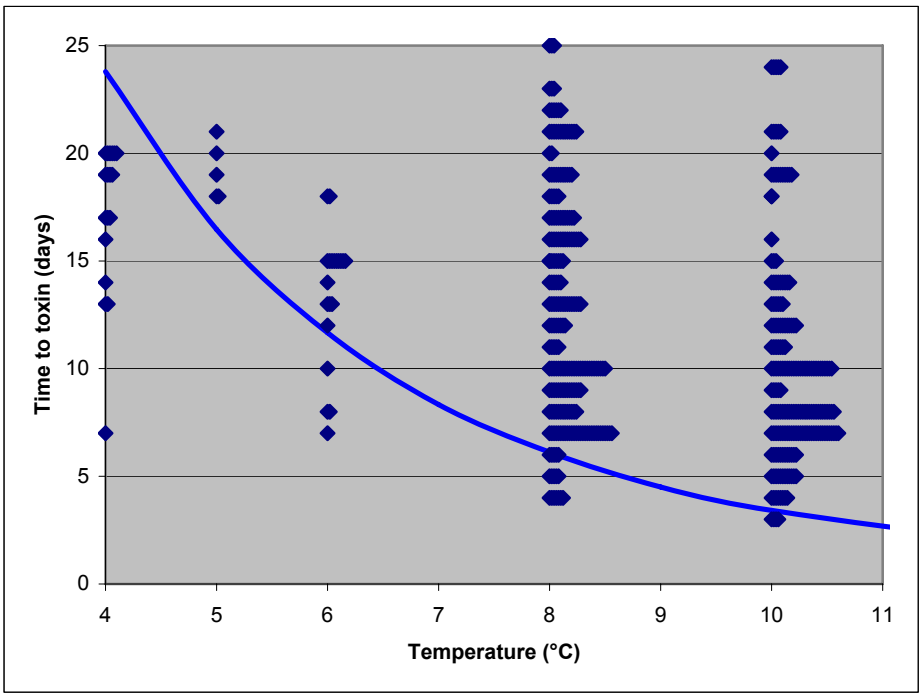

Fig. 6.2 Effect of incubation temperature on time to toxin formation by non-proteolytic *C. botulinum*. The curve is predicted time to toxin from ComBase Predictor. Observations of time to toxin formation in foods and food materials at 4°, 5°, 6°, 8° and 10°C are shown (raw data are in Appendix 2). Where there is more than one observation at each temperature/time, successive observations are plotted to the right (and give the "bars"). Many tests were negative for toxin formation at 25 days (especially at 4°C-7°C).

Table 6.13 Details of where toxin formation has been reported in foods or food materials more rapidly than predicted by ComBase Predictor at ≤10°C

Storage temperature	Time to toxin formation (days)	Number of examples	Food/food material (Line numbers in Appendix 2)
4°C	7	1	Raw fish (563)
	13	2	Raw fish (1103,1108)
	14	1	Cooked turkey breast homogenate (796)
	16	1	Raw fish (564)
	17	3	Pre-cooked sous-vide beef with gravy, raw fish (625,626,945)
	19	4	Raw fish (1104,1105,1109,1110)
	20	6	Raw fish (1073,1074,1088,1089, 1138,1139)
6°C	7	1	Cooked minced beef (881)
	8	2	Cooked minced beef (875,877)
	10	1	Cooked minced beef (876)
8°C	4	7	Raw fish, cooked minced beef (417,418,419,420,885,886,950)
	5	5	Cooked minced beef (710,711,719,720, 891)
10°C	3	4	Raw fish, sterile chicken skin and exudate (566,567,701,702)

Since the current recommended storage temperature for chilled foods in England, Wales and Northern Ireland is ≤8°C, most effort has been dedicated to analysing data at 8°C. A total of 527 independent datasets for storage at 8°C were considered, and 100 of these were positive for toxin at day 10. Of the 100 positive tests, 56 were with raw or smoked fish, 41 were with sterile or pre-cooked food, two with sous-vide foods, and with salted ham.

Based on these data there is a possibility that if contaminated with spores of non-proteolytic *C. botulinum*, raw or smoked fish could become toxic within 10 days at 8°C. Many of these positive tests provide a fail safe indication of the time to toxin formation in chilled foods, however in view of the large variety of chilled foods sold in the UK, it is possible that these observations of toxin formation within 10 days at 8°C may be relevant to some chilled foods. The difficult issue is to identify which chilled foods. It should be noted that two sous-vide foods that were inoculated with spores prior to the heat treatment became toxic in nine days at 8°C, with eleven other sous-vide foods becoming toxic at days 11 or 12.

Growth and toxin formation by non-proteolytic *C. botulinum* in chilled foods are likely to be influenced by a number of factors. These include:

a) Extent of contamination with spores of non-proteolytic *C. botulinum*.
A number of surveys have been carried out of the contamination of raw materials and foods with spores of non-proteolytic *C. botulinum*, but are subject to various limitations (Lund and Peck, 2000). For example, in some studies the heat treatments that have been used will inactivate spores of non-proteolytic *C. botulinum*, while many studies fail to include positive controls and the efficiency of detection is not known. Surveys of the contamination level of foods and food materials indicate that the spore concentration can approach 10^4 spores/kg, although most studies report lower concentrations (Lund and Peck, 2000). Fish and shellfish were the most heavily contaminated food group. Based on literature data, it is likely that most packs of chilled foods will contain few spores, some packs will contain medium spore concentrations, and rarely packs will contain high concentrations of spores. To ensure safety is maintained in all eventualities, the approach has always been to control *C. botulinum* by the application of processes that prevent toxin formation from defined number of spores (i.e. 12-decimal process for proteolytic *C. botulinum* in low acid canned foods, 6-decimal process for non-proteolytic *C. botulinum* in chilled foods).

b) Background microflora.
The background microflora may compete with non-proteolytic *C. botulinum*, and bring about an increase in time to toxin formation. Any effect will depend on the composition of the background microflora, and its physiological state. A heat process (or other process) may reduce this anti-*C. botulinum* effect of the background flora (see below). In other circumstances, the background flora may lower the oxygen concentration and redox potential, and thereby facilitate growth and toxin formation by non-proteolytic *C. botulinum* (see earlier comments on effect of air).

c) Heat process (or other process) delivered
A heat treatment may decrease the number of viable spores of non-proteolytic *C. botulinum* present, and/or damage the spores and thus increase the time to toxin formation (or prevent it altogether). Since much of the background flora will be comprised of vegetative organisms, it is possible that some mild preservative treatments (including various pasteurising treatments, e.g. heat, pressure, irradiation) may inactivate or damage the competing microflora more than spores of non-proteolytic *C. botulinum*, thereby diminishing its anti-*C. botulinum* effect. In these circumstances the process might actually facilitate growth and toxin formation by non-proteolytic *C. botulinum.*

d) Storage temperature
The storage temperature is very important. The model in ComBase Predictor gives a prediction of time to toxin as 3 days at 10°C, 6 days at 8°C, 12 days at 6°C, and 24 days at 4°C (Table 6.1). Slight fluctuations in storage temperature could have a big affect on the time required for toxin production.

e) Other preservative factors.
Reduced pH, low water activity, presence of NaCl or preservatives, modified atmosphere (or VP), or food matrix may individually or in combination restrict growth and toxin formation by non-proteolytic *C. botulinum*.

Relating this information on time to toxin formation at 10°C and below to the safety of chilled foods sold in the UK requires all of these factors to be taken into account. As there is a large range of chilled foods sold in the UK, it is reasonable to suppose that some represent a bigger risk of toxin formation than others. It may be possible to categorise foods as a high, medium or low risk. However, from the unpublished work of Baker, it is clear that this will not be easy.

It is important to note that the large quantity of chilled foods sold in the UK over the last two decades or more has not been associated with foodborne botulism and non-proteolytic *C. botulinum*, and the absence of foodborne botulism outbreaks is presumably a reflection that the following three factors have occurred in short shelf-life chilled foods simultaneously;
(iii) the foods have not contained a dangerous number of spores of non-proteolytic *C. botulinum*,
(iv) the foods cannot support growth and toxin formation by non-proteolytic *C. botulinum*,
(v) the time and temperature of storage are insufficient for toxin formation.

6.3 Effect of other environmental factors on toxin formation by non-proteolytic *C. botulinum* in chilled foods

6.3.1 General comments
Other intrinsic or extrinsic factors that influence growth and toxin formation by non-proteolytic *C. botulinum* include pH, low water activity, NaCl concentration, gas atmosphere and other preservatives. Storage temperature is very important, and has been discussed previously (Fig. 6.1, Table 6.1). Predictive models and other data may be helpful in assessing the effect of combinations of factors. For examples, the models in ComBase Predictor and the Pathogen Modeling Program, describe the effect of pH, NaCl and temperature on growth from spores

(www.combase.cc; www.arserrc.gov/mfs/PMP6_CurMod.htm). Predictive models have also been developed that describe the effect of heating time/temperature and incubation temperature on time to toxin formation from 10^6 spores of non-proteolytic *C. botulinum* (Fernandez and Peck, 1997, 1999). A dataset has been published that describes the effect of NaCl, pH, heating time/temperature and incubation temperature on time to toxin formation from 10^6 spores of non-proteolytic *C. botulinum* (Peck and Stringer, 2005). The effect of carbon dioxide concentration on time to toxin formation has also been quantified (Gibson *et al.*, 2000; Fernandez *et al*., 2001). The factors tested are, however, not exhaustive. For example, although some predictive models include water activity, this is based on the NaCl concentration. The minimum water activity at which organisms can grow is solute dependant so in foods where reduced water activity results from agents other than NaCl, growth may be greater than predicted. Using water activity predictions based on NaCl may not be appropriate for some chilled pasta products or gnocchi (indeed they might provide an unsafe or dangerous prediction). Also, models are not available that describe the effect of nitrite on growth and toxin formation by non-proteolytic *C. botulinum*. This could be addressed by future research (e.g. extending the model in ComBase Predictor to include nitrite)

6.3.2 Combinations of storage temperature and shelf life

The growth model for non-proteolytic *C. botulinum* within ComBase Predictor can be used to predict the effect of a single storage temperature on time to toxin (see Table 6.1, Fig. 6.1). The model can also be used to predict the effect of fluctuating temperatures, and a software tool could be developed for this purpose. The answer to a typical question that a software tool could address is shown in Table 6.14. Here it is assumed that the chilled food spends two days with the manufacturer/distributor, and then four days in retail storage. The question considered is "given these time-frames, and storage in a domestic refrigerator at 6.6°C (as found in various surveys), what effect do different storage temperatures during manufacture, distribution and retail have on the time to toxin". It is predicted that if storage is at 2°C to the end of retail then toxin formation is expected in 16 days, if storage is at 5°C to the end of retail then toxin formation is predicted in 12 days, while if storage is at 8°C to the end of retail then toxin formation is predicted in 6 days (Table 6.14). A number of different time temperature combinations could be entered into such a software tool. Indeed there would be merit in using temperature distributions (as described in Chapter 4). Account could also be taken of temperature abuse, for a short period, as the food is transported from the supermarket to the home. These results demonstrate the merit of storing the food at 5°C or below to the end of retail (Table 6.14).

Table 6.14 Effect of different storage scenarios on time to toxin by non-proteolytic *C. botulinum*

Storage during manufacture/distribution		Retail storage		Domestic storage		Total time to toxin (days)
Time (days)	Temp. (°C)	Time (days)	Temp. (°C)	Time (days)	Temp. (°C)	
2	2	4	2	10	6.6	16
2	2	4	4	8	6.6	14
2	2	4	5	7	6.6	13
2	2	4	8	3	6.6	9
2	5	4	5	6	6.6	12
2	5	4	8	2	6.6	8
2	8	4	8	0	6.6	6

6.3.3 Predictive models of combinations of heat treatment, storage temperature, shelf-life and other preservative factors on time to toxin formation that prevent toxin formation by non-proteolytic *C. botulinum*

Predictive models that could be incorporated in a software tool have also been developed, that describe the effect of heating time/temperature and incubation temperature on time to toxin formation from 10^6 spores of non-proteolytic *C. botulinum* (Fernandez and Peck, 1997, 1999). For example, the original data indicate that for samples stored at 8°C, heating at 80°C for 23, 70 and 98 min prevented growth/toxin formation until days 20, 25 and 41, respectively. While heating at 85°C for 18 and 36 min prevented growth/toxin formation until days 42 and 47, respectively (Appendix two). These heat treatments are less than those advocated by the Industry Code of Practice, ECFF and ACMSF, but when combined with a restricted shelf-life and storage at 8°C provide a safety factor of 10^6 with respect to spores of non-proteolytic *C. botulinum* (Fernandez and Peck, 1999). A further dataset describes the effect of NaCl, pH, heating time/temperature and incubation temperature on time to toxin formation from 10^6 spores of non-proteolytic *C. botulinum* (Peck and Stringer, 2005).

6.4 Effect of alternative and emerging processing technologies on toxin formation by non-proteolytic *C. botulinum* in chilled foods

A large number of new food processing technologies have been investigated over recent years. Only a few of these have been taken forward to commercial trial or full commercial use. In this review it is intended to briefly cover those techniques that have been commercialised, and consider their effects, if data permits, specifically on spores of non-proteolytic *C. botulinum.* If such data are not available, then general effects on bacterial spores are noted.

6.4.1 High Hydrostatic Pressure (HPP)

High hydrostatic pressure (HPP) treated high-acid foods (e.g. fruit juices, conserves, sauces) were first commercialised in Japan in the early 1980s. These foods were pressure-pasteurised, so that viable spores remained, but subsequent growth was prevent by an acidic environment, giving intrinsically safe products. More recently, the range of products has been extended to include products with a high pH and water activity. It is important that the risk of toxin formation by non-proteolytic *C. botulinum* is effectively controlled in these products. In contrast to vegetative cells, bacterial spores are particularly pressure-tolerant.

There have been a number of publications reporting on the effects of high pressure on bacterial spores. These tend to indicate that spores can be highly resistant to high pressures and can survive treatments of more than 1000 MPa (Patterson, 2005). It is clear that spores of different species exhibit different resistances to pressure, and that non-proteolytic *C. botulinum* forms pressure resistant spores.

Reddy *et al.* (1999b) examined the effects of pressure on spores of non-proteolytic *C. botulinum* type E. Pressures between 414 and 827 MPa were applied at temperatures of between 25°C and 60°C for up to 10 min. Their general conclusions were that pressures of less than or equal to 827 MPa may not alone inactivate spores of non-proteolytic *C. botulinum* type E unless used in combination with moderate heating (>40°C). Five log kills of spores of non-proteolytic *C. botulinum* type E resulted from the application of a relatively high pressure of 827 MPa at 40°C for 10 min, and from 827 MPa at 50°C for 5 min (Reddy *et al*., 1999b). In other work, the results of Gola and Rovere (2005) tended to suggest that spores of non-proteolytic *C. botulinum* type B were marginally more resistant to pressure than those of proteolytic *C. botulinum* types A and B (Table 6.15). It is also noted that these authors only used temperature greater than 80°C during their work.

Some authors have suggested that relatively low pressures can be used to germinate spores. Thus the application of HPP in two stages could be used to initially cause spore germination, then application of another high pressure process could kill germinated spores (Heinz & Knorr,

2001). Caution will be required with the use of a two stage process, however, as it has been noted that a small proportion of any spore population will always resist pressure induced germination.

In conclusion, it would appear that the use of pressures up to approximately 800 MPa, at temperatures below 35°C are not effective at killing of spores of non-proteolytic *C. botulinum*. Indeed such pressure must be combined with a higher temperature before a suitable spore kill is achieved. At pressures at or above 827 MPa and temperatures greater than 40°C a 5 log reduction appears to be possible within a 5 to 10 minute timescale. It should be noted that while the data included here could be used to give an indication of the type of process that may be required in a food product, if HPP was to be used as a control for non-proteolytic *C. botulinum* then a full validation of the process would be recommended.

Table 6.15 Effects of high hydrostatic pressure on spores of proteolytic *C. botulinum* and non-proteolytic *C. botulinum* in phosphate buffer

C. botulinum group and toxin type	Pressure (MPa)	Time (min)	Temperature (°C)	D Value (min)	Log reduction	Ref[a]
Non-proteolytic *C. botulinum*						
Type E	≤ 827	≤10	<35		0	1
Type E	827	5	55		1	1
Type E	827	10	40		5	1
Type E	827	5	50		5	1
Type E	827	5	55		5	1
Type B	800		90	3.45		2
Proteolytic *C. botulinum*						
Type A	600		100	1.38		2
Type A	800		90	3.10		2
Type A	800		88	3.25		2
Type A	600		100	0.91		2
Type B	800		90	2.66		2

[a]Reference: 1 = Reddy *et al.* (1999b), 2 = Gola & Rovere (2005)

6.4.2 Pulsed Electric Field (PEF)

Pulsed electric field (PEF) processing consists of the application of short duration (1 to 100μs) of high electric field pulses (10 to 50 kV/cm) to a food placed between two electrodes (Manas and Pagan, 2005). The mode of action of PEF on microbial cells is generally accepted to be structural or functional alteration of the cell membrane (Sale and Hamilton, 1967). Due to this mode of action, it is theoretically difficult to see how PEF used alone, would have any significant killing effect on bacterial spores. In their review of novel processes, Manas and Pagan (2005) indicate that spore inactivation by PEF is not possible. Early studies provided an indication of the difficulties of inactivating *Bacillus* spores with PEF (Sale & Hamilton, 1967; Hamilton & Sale, 1967), while later work reported no inactivation of spores of *B. cereus* and *C. tyrobutyricum* (Grahl & Markl, 1996). Work with *B. cereus* and *B. polymyxa* have indicated a resistance to PEF treatment at intensities greater than 30kV/cm, however once spore germination begins, sensitivity to the process increased. This observation of sensitivity to the PEF process beginning, only when spores germinate has also been reported for *B. cereus* spores (Pol *et al*., 2001). Some authors have reported significant inactivation of spores of *B. cereus*, *B. subtilis*, and *B. stearothermophilus* using PEF (Marquez et al., 1997; Dantzer, 1999). In conclusion there have been no reports of the effects of PEF on spores of non-proteolytic *C. botulinum*. Some work has been done with spores of other species, with an overall conclusion that the process has a very limited effect on spores unless they are in the process of germination or have germinated.

6.4.3 Irradiation pasteurisation

Spores of non-proteolytic *C. botulinum* are considered to be more sensitive to irradiation than those of proteolytic *C. botulinum*. Reported D-values range from 1.4 kGy (beef stew, room temperature) for non-proteolytic *C. botulinum* to 3.5 kGy (beef at 25°C) and 6.0 kGy (beef at -196°C) for proteolytic *C. botulinum* (Patterson and Loaharanu, 2000). The effect of irradiation on toxin formation by non-proteolytic *C. botulinum* in fish has been widely studied. The process used may be considered as "irradiation-pasteurisation" as it appears that the doses of irradiation used (1 to 3 kGy) has no major destructive effect on spores of non-proteolytic *C. botulinum* (Eklund, 1982). There are reports that in some circumstances, irradiation-pasteurisation can even lead to more rapid toxin formation by non-proteolytic *C. botulinum*. For example, in tests with VP haddock fillets inoculated with 10^2 spores/g of non-proteolytic *C. botulinum* type E, toxin formation was detected at 8°C in 48 days in unirradiated fillets, and in 40 days in fillets irradiated with 2 kGy (Eklund 1982). Toxin was not formed in 42 days in unirradiated sole fillets stored at 6°C, but was detected in 28 days in fillets irradiated with 2 kGy (Eklund 1982). These effects are presumably due to irradiation-pasteurisation inactivating or damaging the competing background flora. In conclusion, irradiation may inactivate spores of non-proteolytic *C. botulinum*, but any effect will depend on dose, food type, presence of other preservative factors, and the temperature of the product during the irradiation process. All of the work quoted would appear to have involved the use of Cobalt 60 irradiation, no reports of the effects of electron beam irradiation were found. It should be noted that the botulinum toxin itself is reported to be highly resistant to irradiation.

6.4.4 Pulsed Light

The microbial inactivation effects of high intensity light have been known for many years (Gould, 2000). The antimicrobial effects are reported to be due to either the ultraviolet portion of the light applied or to localised and transient rises in temperature that occur at the product surface during treatment. While there are no reports of effects of this process on spores of non-proteolytic *C. botulinum*, it has been reported to be effective against spores of other bacterial species (e.g. *B. subtilis*), however the antimicrobial effects of light based systems will only occur at the surface of foods. Light does not penetrate into food products, thus any microorganisms that are within food product or protected from the lights source by shadowing, will not be affected by the process.

6.4.5 Conclusions

When considering new processing technologies, it is apparent that we have very little information on the effect of these technologies compared to the wealth of data on the effect of heat. This results in a major issue when it comes to using a new process, while some of its effects may be predictable, the confidence in the prediction will be low, and the need for extensive validation is high. In conclusion, the effects of new processes on non-proteolytic *C. botulinum* will depend on the type of process applied, some do have a killing effect if properly applied, whilst others have a very limited effect. Thus, any use of new process technology to inactivate non-proteolytic *C. botulinum* will have to be extensively validated. The validation would need to take into account strain variation, the effects of the food type, composition and storage regime, and any effects on other food microflora. In establishing safe processes, it is important that at the very least, 'equivalence' with established safe technologies must be assured.

6.5 Lag time of non-proteolytic *C. botulinum*

The predictive model developed by Baker and Genigeorgis (1992) describes the effect of temperature on "lag time". It must be noted that in these studies lag time dose not represent the classical time to the start of growth as observed in growth curves, but refers to the last sampling time before the production of detectable levels of toxin. The predictive model developed by Graham *et al*. (1996b) is a growth curve model, consequently it is possible to derive predictions of population lag time from this model.

More recent studies have investigated the lag phase of growth of non-proteolytic *C. botulinum* (Stringer *et al*., 2005). Knowledge of distribution of growth times from individual spores and quantification of such biovariability is important if predictions of growth from spores in food are to be improved, particularly when, as for non-proteolytic *C. botulinum*, growth in chilled foods is likely to initiate from a low number of spores. Times to various stages of lag were quantified for individual spores using phase-contrast microscopy and image analysis. Times for germination, emergence, cell maturation and doubling were independent, and each showed considerable variability. Consequently, it was not possible to predict the total duration of lag phase from information on just one of the stages. For example, it was not possible to predict the duration of lag phase from germination time alone. In these tests, the first spore to germinate was not the first to give actively dividing cells, as the variability in post-germination stages was so great (Stringer *et al*., 2005).

6.6 Conclusions

Non-proteolytic *C. botulinum* is a psychrotrophic foodborne pathogen. The minimum temperature at which growth and toxin formation has been reported is 3.0°C. Several predictive models have been developed for growth of non-proteolytic *C. botulinum*. Each model is based on a different dataset and gives a slightly different prediction but, in general, the models are constructed to deliver a fail-safe prediction of time to toxin formation when other factors are not limiting. Four predictive models have been considered in this study:

- Combase Predictor model.
- PMP (Pathogen Modeling Program) model.
- Baker/Genigeorgis model.
- Skinner/Larkin model.

All four models predict that toxin formation will occur in less than 10 days at 8°C. The different predictions of time to toxin formation reflect the different datasets on which the models were based. The model in ComBase Predictor is designed to be most robust at ≤10°C, and may give more reliable predictions in this region than the other two original models. The Skinner/Larkin model is designed to be an ultimate failsafe model. It may be that the prediction of toxin formation in 5-6 days at 8°C is the most reasonable fail-safe prediction from the models, and the prediction of toxin formation in 4 days at 8°C is the most conservative failsafe prediction. It is important to recognise, however, that these models are designed to represent various worst-case scenarios, and the issue that must be addressed is how closely predictions from these models relate to toxin production in actual chilled foods sold in the UK.

Just as models developed using microbiological broth media predict toxin formation in less than 10 days at 8°C, tests in microbiological broth media also record growth/toxin formation in 10 days or less at 8°C. In this report it is accepted that in microbiological broth media, growth and toxin formation can occur in 10 days or less at 8°C, and in this project no further data have been collected on tests carried out in microbiological broth media.

In order to collect literature data on toxin formation in chilled foods/food materials, an extensive literature search was carried out, and combined with articles held in the personal libraries of the authors of this report. Data extracted from 61 literature publications yielded 887 independent tests of time to toxin formation. Additionally, 27 confidential datasets that contained 420 independent tests of time to toxin formation were kindly donated by members of the food industry. This gave a total of 1307 independent tests. One independent test would typically be one product at one temperature sampled a number of times. Replicate samples would be removed and tested for toxin at various time points (often in duplicate or triplicate), and the last time when all the replicate samples were negative, and the first time that one of the replicate samples was positive noted. It should be noted that most of the data included in this assessment

had not been generated for the purpose of evaluating the potential for growth and toxin production by non-proteolytic *C. botulinum* in chilled foods sold in the UK within 10 days or less at 10°C or below. The relevance of these data to short shelf-life chilled foods sold in the UK is in some cases, therefore, limited.

The results from 1307 independent tests demonstrate that non-proteolytic *C. botulinum,* if present, is able to form toxin in foods and food materials at ≤10°C within 10 days. In total, 237 individual tests were positive for toxin formation by day 10 (19%). At 10°C, 132 of the tests were positive at day 10 (36%); at 8°C, 100 of the tests were positive at day 10 (19%); and at 4°C-7°C, five of the tests were positive (1%) (Table 6.16).

Since the current recommended storage temperature for chilled foods in England, Wales and Northern Ireland is ≤8°C, much effort has been dedicated to analysing data at 8°C. A total of 527 independent datasets for storage at 8°C were considered, and 100 of these were positive for toxin at day 10. Of the 100 positive tests, 56 were with raw or smoked fish, 41 were with sterile or pre-cooked food, two with sous-vide foods, and one with salted ham. Based on these data there is a possibility that if contaminated with spores of non-proteolytic *C. botulinum*, raw or smoked fish could become toxic within 10 days at 8°C. Many of these positive tests provide a fail safe indication of the time to toxin formation in chilled foods, however in view of the large variety of chilled foods sold in the UK, it is possible some of these observations of toxin formation within 10 days at 8°C may be relevant to some chilled foods. The difficult issue is to identify which chilled foods. It should be noted that two sous-vide foods that were inoculated with spores prior to the heat treatment became toxic in nine days at 8°C, with eleven other sous-vide foods becoming toxic at days 11 or 12.

Table 6.16 Effect of storage conditions on toxin formation by non-proteolytic *C. botulinum*

Storage conditions		Number of samples (percentage) negative/positive for toxin under specified storage conditions[a]	
temperature	time	Negative for toxin	Positive for toxin
10°C	5 days	319 (93%)	24 (7%)
	10 days	238 (64%)	132 (36%)
	15 days	166 (50%)	166 (50%)
8°C	5 days	500 (98%)	12 (2%)
	10 days	414 (81%)	100 (19%)
	15 days	360 (72%)	142 (28%)
4-7°C	5 days	389 (100%)	0 (0%)
	10 days	382 (99%)	5 (1%)
	15 days	360 (94%)	22 (6%)
TOTAL (4-10°C)	5 days	1208 (97%)	36 (3%)
	10 days	1034 (81%)	237 (19%)
	15 days	886 (73%)	330 (27%)

[a] Results scored as uncertain are not included in this table

At a first glance, the reported ability of non-proteolytic *C. botulinum* to form toxin at 8°C and below within 10 days would appear to be in conflict with the observation of safe production and sale of large quantities of correctly stored chilled food in the UK and overseas without incidence of botulism. For example, approximately 4 x 10^9 chilled prepared meals have been produced following 8°C/10 days since 1990 in the UK. The fact that there has not been a single botulism outbreak associated with chilled foods (some of which may have been temperature abused) is presumably a reflection that one or both of the following controls has ensured safety;

(i) the foods contain no spores or only a low number of spores of non-proteolytic *C. botulinum*,

(ii) the foods do not support growth and toxin formation by non-proteolytic *C. botulinum* within the time and temperature of storage.

Short shelf-life chilled foods that have been sold in the UK would therefore appear to have "unknown controlling factors (unknown hurdles)" that have prevented growth and toxin formation by non-proteolytic *C. botulinum*. In more detail, these "unknown controlling factors" might include:

- low spore contamination
- a heat process that damages or decreases the number of spores
- an inhibitory background microflora
- a reduced pH, low water activity, presence of NaCl or preservatives, inhibitory modified atmosphere, an effect of food structure,
- storage at less than 8°C through part or all of the chill chain
- consumption of the food before the end of shelf-life
- the food is heated before consumption to inactivate any toxin formed

It is important to note however, that:

(i) different "unknown controlling factors" are likely to be important in different chilled foods
(ii) the magnitude and variability of these "unknown controlling factors" is not known and is likely to be different for different foods, consequently the safety margin is not known and will vary from chilled food to chilled food, and will also vary from pack to pack for each food.

Considering the large range of chilled foods sold in the UK, and the above comments, it is likely that some chilled foods are a bigger risk than others. It may be possible to categorise the foods as a high, medium or low risk, and for these different risk categories to have a different maximum shelf-life at 8°C. For example, raw fish is a high risk product, with numerous tests indicating the possibility of toxin formation in 10 days at 8°C. A difficult matter is to decide which foods fit into which category, and from the unpublished work of Baker on sous-vide foods, it is clear that this will not be straightforward. The designation of low, medium or high risk should be made only on the basis of sound scientific evidence.

It should be noted that great effort and investment goes into the application in commercial UK chilled food production of hygiene measures from raw material selection, handling and raw/cooked segregation, which are important control measures to prevent the presence of spores of *C. botulinum*.

It was reported in several studies that toxin formation can as rapid (or in some circumstances more rapid) in foods packed in air as under VP or low-oxygen MAP (presumably because there is no oxygen in the food, i.e. the food is reduced [in some cases possibly by aerobic organisms]). Packaging under air or a similar oxygen-containing atmosphere is therefore not a guarantee that toxin formation by non-proteolytic *C. botulinum* will be prevented.

In considering the effect of new processing technologies, it is apparent that there is very little information on the effect of these technologies compared to the wealth of data on the effect of heat. This results in a major issue when it comes to using a new process, while some of its effects may be predictable, the confidence in the prediction will be low, and the need for extensive validation is high. The effects of four "new" processes (high hydrostatic pressure (HPP), pulsed electric field, irradiation pasteurisation and pulsed light) on non-proteolytic *C. botulinum* have been considered, and the effectiveness is dependent on the type of process applied, some do have a killing effect if properly applied, whilst others have a very limited effect. Thus, any use of new process technology to inactivate non-proteolytic *C. botulinum* will have to be extensively validated. The validation would need to take into account strain variation, the effects of the food type, composition and storage regime, and any effects on other food

microflora. In establishing safe processes, it is important that at the very least, 'equivalence' with established safe technologies must be assured.

In conclusion, it is not easy to base a determination of the maximum shelf-life of chilled foods at 8°C (where other controlling factors are not known) on only the data from 1307 independent tests of toxin formation by non-proteolytic *C. botulinum*. It is clear that, given the correct circumstances, if present non-proteolytic *C. botulinum* could form toxin in less than 10 days at 8°C. That this has not happened with short shelf-life chilled foods sold in the UK must be due to presence of one or more "unknown controlling factors". The difficulty is that the magnitude, variability and nature of these "unknown controlling factors" are not known, and it is suspected that they are not the same for all chilled foods. The position is therefore that although short shelf-life foods have been produced safely in the UK and internationally for more than two decades, it is not known precisely what the safety margins are with respect to foodborne botulism. Research is needed to identify the magnitude, variability and nature of these "unknown controlling factors". This will aid the continued safe development of chilled foods in the UK and overseas.

This study noted a dramatic effect of storage temperature on toxin formation by non-proteolytic *C. botulinum*. For example from the model in ComBase Predictor, time to toxin is predicted as 3 days at 10°C, 6 days at 8°C, 12 days at 6°C, 16 days at 5°C, and 24 days at 4°C. Thus, if all chilled food could be maintained at 4°C/5°C (for example) throughout the chill chain (including in the home), the safety margin would be extended further. It is likely that there will also be a benefit with other psychrotrophic pathogens and shelf-life extension may also be possible. In order to further extend the margins of safety of chilled foods with respect to psychrotrophic foodborne pathogens, it is suggested the UK continues to strive for better temperature control throughout the chill chain (including domestic storage), and that 5°C is adopted as a target for best practice. It should be noted that this comment is not based on any particular outbreak of food poisoning. This suggestion re-iterates various recommendations made by Mark Richmond in 1991.

6.7 References

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Chapter seven: Re-packing VP/MAP chilled foods during the 10 day shelf-life

It has been brought to our attention that some chilled VP/MAP foods, such as meat, may be given a "rolling 10 day shelf-life". That is, the product is opened during the initial 10 day shelf-life, some is used, and then the remainder is repacked and given a further 10 day shelf-life. Thus, the shelf-life is extended beyond 10 days without the identification of other factors that control toxin formation by non-proteolytic *C. botulinum*. While we are not aware of this practice leading to outbreaks of botulism, this represents a significant divergence from the guidance and would appear to be a high risk practice.

It is therefore recommended that for foods where no other controlling factor can be identified, the maximum shelf-life is 10 days, and that this commences once the product is first vacuum or modified atmosphere packed. The shelf-life must not be restarted if the product is subject to a further packing under vacuum or modified atmosphere, unless other controlling factors (as described by the ACMSF) are applied. This approach is similar to that included in Australian guidelines (AIFST/ACCC, 2000).

For products that have been assigned a shelf-life greater than 10 days, other controlling factors (as described by the ACMSF) will have been identified. These products should only be repacked under vacuum or modified atmosphere after a thorough consideration of the HACCP. In particular, consideration should be given to the possibility of recontamination with non-proteolytic *C. botulinum* spores, and subsequent growth and toxin formation.

Reference:

AIFST/ACCC (2000) Guidelines for Chilled Food Production Systems including Food Safety Programs. Australian Institute of Food Science and Technology/Australian Cook Chill Council [www.aifst.asn.au/templates/aifst.aspx?edit=false&pageID=395 (accessed 7/1/06)]

Chapter Eight: Risk assessments on the control of non-proteolytic *Clostridium botulinum* in VP/MAP chilled foods

8.1 ALOPs and FSOs

Risk assessment is a term frequently used when attempting to define the potential of any object or system to cause harm. In foods safety the term is often applied to an assessment of the potential for any food to cause an adverse effect to the consumer. In taking the ideas of Risk Assessment forward, there has been a clear need to focus on public health and methods for establishing clear health targets within a population, these will usually be set at Governmental levels or by international bodies, and the term Appropriate Level of Protection (ALOP) has been formulated to describe the level of protection deemed appropriate by any country establishing a measure to protect human health (WTO, 1995).

ALOPs are public health goals and are set for populations rather than population sub-groups and food types. There has thus been a need to establish a link between improving public health goals and clear and achievable targets that can be used by those that produce, manufacture, and distribute foods. Food safety practitioners have used the concept of Food Safety Objectives (FSOs) as a link between an ALOP and performance or process criteria routinely used in production and manufacture. An FSO is defined as the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the ALOP (ICMSF, 2002). Using these tools an ALOP can be converted into an FSO that will detail the product/hazard combination concerned. The FSO itself may not always be able to be directly implemented, so can be converted into things that can be controlled and measured within the supply chain, such as specific control measures and performance criteria. The public health goal (ALOP) can thus be translated into the amount of hazard at the point of consumption (FSO) and can be used to set targets or criteria at suitable points in the food chain.

In order to establish an FSO it is necessary to do some form of Risk Assessment (see below for detail), there is debate as to how much detail is required at each step in a risk assessment in order to develop an FSO, indeed ICMSF (2002) have a view that a full risk assessment according to Codex principles may not be necessary to formulate an FSO.

8.2 Microbiological Risk Assessment

Microbiological Risk Assessment (MRA) is the procedure whereby the microbiological risks associated with particular foods or ingredients are formally assessed. The assessment must be thorough, well documented and transparent, with all data used in the formulation of its conclusions, all estimates made and all key data gaps recorded. There are recognised to be six parts to a formal (Codex style) MRA (Codex, 1999; Voysey, 2000):

1) Statement of Purpose, in which the scope and objectives of the MRA are clearly defined
2) Hazard Identification, which requires the identification of agents capable of causing adverse health effects
3) Exposure Assessment, requiring an evaluation of the degree of intake of the agent that is likely to occur
4) Hazard Characterisation, in which the nature of the adverse health effects associated with the microbiological hazard is evaluated
5) Risk Characterisation, requiring an estimation of the adverse health effects likely to occur
6) Reporting, in which a full report detailing the complete MRA, all data used and conclusions drawn is produced

A number of Risk Assessments have been published covering a range of foodborne hazards in various foods e.g. *Escherichia coli* in beefburgers (Cassin *et al*., 1998), *Bacillus cereus* in pasteurised milk (Notermans *et al*., 1997), *Salmonella* in eggs and broiler chickens (FAO, 2002), *Listeria* in Ready to eat Foods (FAO, 2004).

8.3 Risk Assessments for *Clostridium botulinum*

When considering specifically, the risks of non-proteolytic *Clostridium botulinum* in chilled foods packed under low oxygen conditions, a limited number of references are found. Carlin *et al.* (2000) researched factors that would allow a risk assessment of spore forming pathogens in cooked chilled foods containing vegetables. In this work, both *Bacillus cereus* and *Clostridium botulinum* were identified as potential hazards in these foods. Non-proteolytic *C. botulinum* was included as a high risk factor, and the authors also included proteolytic *C. botulinum* as a medium risk in refrigerated products subject to temperature abuse above 10°C. It is clear from the text of the paper, however, that the authors were able to consider the risk factors of *Bacillus cereus,* to a far greater extent those of *C. botulinum,* probably because of a greater amount of relevant published information about the former organism when compared to the latter group. The authors did conclude that there are a number of ways in which the future quality of risk assessments of this sort could be improved. These included: (1) continued research into areas in which the uncertainty is great (e.g. dose-response assessment, toxin production, contamination of foods with the relevant pathogen, growth and inactivation in food matrices and under changing environmental conditions, microbial interactions, effects of cell history and sub-lethal stress, and consumer behaviour); (2) the continued development of analytical tools to aid in risk assessment (e.g. Monte Carlo simulation and Bayesian belief networks for hazard analysis); (3) the reinforcement of collaborations between food microbiologists and risk assessors.

Research into the development and use of analytical tools to aid in *C. botulinum* risk assessments has been continued by other authors. Both Barker *et al*. (2002) and Barker *et al*. (2005) considered the use of Bayesian Belief Networks, whilst Jewell *et al.* (2004) used Monte Carlo simulations. The papers by Barker *et al*. (2005) and Jewell *et al*. (2004) both considered risks associated with very specific product types, namely a minimally processed potato product (gnocchi) and an MAP packed cooked sliced chicken product respectively. Both authors have taken different approaches to reach their conclusion. Jewell *et al.* (2004) considered the hazard to be outgrowth of spores, thus to present a hazard the product must contain a viable spore that has no residual lag (i.e. a spore that will germinate and give growth), whilst Barker *et al.* (2005) considered the hazard to be the presence of toxin, which the authors note will result from germination and cell division from a spore. Although the description of the hazard by both authors may appear at first to be different, in reality both recognise the same hazard. The reporting format of both authors is different, due to the work by Jewell, being written in the style of a formal risk assessment and covering the main 'Codex' principles, whilst the work of Barker is written as a research paper. It is clear however that the main principles of Risk Assessment are covered by both reports.

It is also apparent from both reports that the products being studied can be stored for longer than 10 days at 8°C, and remain safe with respect to risks associated non-proteolytic *C. botulinum.* In both cases, the work done to come to this conclusion has been extensive and involved detailed literature searches, the use of various forms of modelling and most critically has required considerable input from the food manufacturer involved. In the case of the work on gnocchi, extensive challenge testing work has been done with *C. botulinum* (Del Torre *et al*., 2004*).* Both these products were recognised by the authors as not complying with recommended control procedures for non-proteolytic *C. botulinum* (as described by the ACMSF (1992, 1995)). Challenge testing was done with eight strains of non-proteolytic *C. botulinum* in gnocchi, and toxin was not detected in samples stored for up to 75 days at 8°C or 12°C. This is a convincing verification of the risk assessment for this product, confirming that there is a high margin of safety for non-proteolytic *C. botulinum.*

Microbiological risk assessment can be used to assess the risks associated with non-proteolytic *C. botulinum* in chilled MAP/VP foods. To do this, however, is not a simple task and requires close interaction between microbiologists, risk assessors and critically food manufacturers. To yield information that is useful, the assessment should be limited to very specific product types,

about which there is extensive knowledge of both intrinsic food factors (water activity, pH, levels of contaminating microorganism including spores, presence of other preserving factors), and extrinsic conditions (process temperatures, holding times/temperatures, factory process flows, distribution conditions and potentially consumer related conditions). Even when this has been done, the question must be raised as to whether there would be full confidence by a manufacturer to use the result, without validation by a suitable challenge test. In principle, this would provide a suitable final confirmation that the company was being duly diligent in response to a known food safety risk.

A number of other organisations have commented upon the risk of growth and toxin formation of *C. botulinum* in chilled products that may have a modified atmosphere and hence done 'informal' risk assessments that do not exactly follow the Codex procedure.

A technical review by the International Fresh Cut Produce Association (Anon, 2003a) includes an assessment of the botulism risk in fresh cut produce (fresh fruit and vegetable products). This view recognised that fresh produce items do harbour spores of proteolytic *C. botulinum* (particularly those of toxin types A and B), and that with the exception of mushrooms, MAP packed fresh produce has not been confirmed as a cause of botulism, even though in excess of 1.5×10^{12} kg of chilled MAP packed fresh produce have been produced for the food service and retail sectors since 1991. There have been incidences where both MAP packed cabbage (Solomon *et al*., 1990) and garlic stored in oil, have been linked to cases of botulism and proteolytic *C. botulinum*, however in both of these the products appear to have been stored for extended periods at ambient temperature. This review recognises that there are no direct controls (process, pH, water activity) that can be implemented in these products, however a combination of low storage temperature, an in-pack environment that contains some oxygen, and the presence of a substantial and active competitive microbial population, does constitute an environment that is not well suited to the growth of *C. botulinum*, with overt product spoilage occurring before *C. botulinum* toxin could be formed. Various challenge test studies tend to support this view, with toxin only being found in products stored at temperatures of 12°C and above, and usually accompanied by overt product spoilage. Larson *et al*. (1997) concluded that in MAP fresh produce, the consumer risk is not increased over that in non-MAP produce, due to the lack of toxin production before gross organoleptic spoilage had occurred. However, some products such as butternut squash and onions have been demonstrated under temperature abuse conditions to have the potential of being acceptable after detection of *C. botulinum* toxin (Austin *et al.*, 1998).

Health Canada has considered the risks of non-proteolytic *C. botulinum* in smoked fish and fish roe caviar products, in documents that have not yet been published (J. Farber, personal communication 2000). In fish roe caviar products, it is considered that, unless the product is given a sporicidal treatment, the presence of *C. botulinum* spores should be assumed, and growth of and toxin production by *C. botulinum* must be prevented. The key control factors identified were a product Aw of <0.97, or a product pH of <5.0. No maximum life is given if these parameters are not reached. It should be noted that in Canada, chilled storage is legally described as 'exposure to a temperature of 4°C or less, but not frozen' (Anon, 2003b). For smoked fish products, the suggestion is they should be on sale in refrigerated storage (i.e. 4°C or less), if the pH is >5.0 or the Aw is >0.97. It should be noted, however, that these parameters are suggested specifically for products packaged to exclude air, and this is defined as any packaging that has an oxygen transmission rate of < $2000 cm^3/m^2/24h$ at 1 atmosphere. Again as for fish roe caviar products, there is no maximum life given if these parameters are not met.

In the USA the Institute of Food Technologists (IFT) have produced an 'Evaluation and Definition of potentially hazardous foods' for the United States Food & Drug Administration (Anon, 2003c). This document covers risks and controls in all types of potentially hazardous foods and thus will cover chilled VP/MAP products. It gives no definite control parameters for these products, but instead has developed a framework based on a decision tree type approach, to help establish if

time/temperature control is required. The decision tree begins by asking if the product was processed to destroy vegetative cells, it then goes on to consider critical pH and Aw values to control other organisms including spores. Depending on the pH and Aw values, the food is placed into either a 'Time/Temperature Controlled' (TCS) category or not. If foods fall into the TCS category, the report gives no information on possible shelf life or storage temperature, instead it considers the use of predictive microbiology, and finally challenge testing to ultimately define these parameters.

8.4 Final Safety Assessment

An interesting approach to a risk assessment with respect to *C. botulinum* was published by Hauschild and Simonsen (1985, 1986). These authors considered a safety assessment of shelf stable canned cured meats. These authors noted that there were few published data sets on *C. botulinum* in these products and this made a reliable safety assessment difficult to achieve. They did note that if the wealth of data generated by the food industry themselves could be used, then a much better safety assessment could be made. The authors went onto describe a way of using industry data (numbers of individual packs of product produced over many years, together with recorded cases of illness attributed to those products) to calculate Safety Units. This would appear to be a novel way of using the very large data sets available from industry to obtain a meaningful assessment of actual risks from those products in the past. As long a product production and preservation parameters (i.e. control factors) for these products are known, then this could indeed show that the controls used are valid and lead to a safe product.

The final assessment of the safety of a VP/MAP chilled food, will be dependent on the process parameters applied to the product, and the inherent intrinsic parameters within the food. The parameters that have been applied in the past in the UK as a part of the FSO for these products have been (CCFRA, 1996):-

1) A heat process sufficient to give a 6 log reduction of non-proteolytic *C. botulinum*, when combined with storage at a temperature of no greater than 8°C
OR

2) A combination of intrinsic properties which prevent the growth and toxin formation of non-proteolytic *C. botulinum* (i.e. individual defined Aw or pH values of the product, or a recipe that has been shown by experimentation to prevent the growth of and toxin formation from non-proteolytic *C. botulinum*)
OR

3) Storage at a temperature of no greater than 8°C for a maximum of 10 days

The use of a defined storage temperature (8°C or less for a maximum of 10 days) achieves control through knowledge of the growth of non-proteolytic *C. botulinum.* It must be acknowledged that if spores of non-proteolytic *C. botulinum* are present in products that do not have intrinsic control factors (e.g. pH or Aw) and have not had a sufficient heat process, then growth and toxin formation is possible. Such products must have a limited life that can be extended only by reducing the storage temperature.

It is clear that organisations in other countries also follow a similar approach. In some cases the same cardinal Aw and pH values are used as in the UK, in other cases a decision has to be made as to whether the combination used in the product will prevent growth. Observation of industry data (number of packs produced over a given time period combined with the number of cases of botulism from these products) can be used to build a safety assessment of this product grouping, and gain an impression of whether current process controls are working. There will, however, always be products which do not conform to the recognised process controls. These products required a separate assessment. One way to approach this is by use of predictive

microbiology, although again many models may not include key anti-botulinal factors which will make them much less useful to users. As an example the work of Roberts *et al.* (1976), clearly shows the anti-botulinal effects of sodium nitrite, and an interaction between sodium chloride, nitrite and storage temperatures. These effects, however could not be predicted using current model systems, as nitrite is not included as one of the models variables. Therefore a prediction could be obtained (for salt and temperature), but this could indicate that growth of *C. botulinum* was possible, even though the product was perfectly safe due to presence of nitrite. This leaves the final assessment tool as a challenge test, where the actual food product is inoculated with a culture, stored under defined conditions and tested, either for growth of *C. botulinum,* or presence of toxin.

In the course of their work on the safety assessment of shelf stable canned cured meats, Hauschild and Simonsen (1985, 1986) encountered problems of comparing published experimental data with commercial data. Whilst it is acknowledged that the product types they were considering, were very different to the chilled MAP/VP products being considered in this review, and the hazard was proteolytic *C. botulinum* rather than non-proteolytic *C. botulinum*, the issue of comparing experimental data with commercial information is similar. These authors noted that the safety record of shelf stable canned cured meats with respect to proteolytic *C. botulinum* was almost unblemished, however they noted that research data on safety and stability of the product was very sparse, thus they based their assessment on commercial practices and experience. There is considerable commercial information available on the production of chilled VP/MAP products that can be used in a safety assessment, according to the method used by Hauschild and Simonsen (1985, 1986) for canned meats. These authors describe how the protection (Pr) of canned meats from proteolytic *C. botulinum* can be expressed as :

$Pr = \log 1/P$

Where:
P = probability of individual spore to survive, grow and produce toxin.
Pr = protection from proteolytic *C. botulinum*

They go on to note that whilst log 1/P relates to the number of spores controlled, the number of cans protected is expressed as:

Number of cans protected = $\text{Log } 1/(P_{x}i)$

Where:
i=incidence of *C. botulinum* in the raw material

So if a product had a Protection (Pr) of 8, then:
$8 = \log 1/P$
or
$1x10^{8} = 1/P$
or
$\mathbf{P = 1/1x10^{8} = 1x10^{-8}}$

Also if there was 0.1 *C. botulinum* spores per can, the number of cans protected would be:
$\text{Log } 1/(P_{x}i)$
or
$\text{Log } 1/\ 1x10^{-8}x\ 0.1 = 9$

Thus the number of cans protected would be 9 (i.e. one from every 10^9 cans produced would be expected to allow toxin formation).

These authors use data on annual volumes of products manufactured over given years, combined with recorded cases of illness attributed to these products, to estimate the decimal number of products marketed per number causing illness. This unit they define as Safety Units (SU). As an example if 10^9 cans produced, and no cases of botulism resulted then the SU would be:

SU = number of cans produced/number of cans causing botulism

SU = 10^9/<1
or
SU = >9

There are some qualifications to this approach that must be noted. Commercial products may have a fast turnover and will be consumed before the end of their commercial life, whereas experimental product will tend to be tested at the end of their commercial life. Also commercial product in which growth has occurred may show signs of spoilage and be rejected by the consumer before consumption, whereas experimental product will always be tested. Finally although the probability of botulism incidents remaining undetected is very low, reporting of the illness cannot be guaranteed to be complete.

In order to use this approach with MAP/VP products, an understanding of the control factors for non proteolytic *C. botulinum*, is required. As consideration is being given in this review to products that contain no specific single controls (pH, Aw, salt etc.), then the only controls applicable are storage temperature and shelf life. Consideration must be given, however, to any interactions between these and non-controlling levels of pH, Aw, salt and pack atmosphere that may have an additive effect, to slow or prevent *C. botulinum* growth. We must also be aware that although control over a 10 day life at 8°C or lower is specified, some of this product will be consumed before the end of its 10 day life, and for some of its life the storage temperature may be less than 8°C (although once the product is move from commercial distribution to consumer storage, it is possible that some product is stored at temperatures in excess of 8°C). Finally some of the product is cooked or reheated before being consumed, if toxin had been formed in such product and sufficient heat applied, then it is possible that the toxin will have been denatured before consumption.

If this type of approach were to be used with MAP/VP products, then data presented within this document could be used give approximate safety units (SU) for different product types (Table 8.1). The table gives an example of how the procedure could be used, and it estimated that the safety units for cooked chill foods, sliced cooked meats and raw red meats are all >9.8, while for smoked fish it is estimated that the safety units are >8.0 (i.e. 1 in >$10^{9.8}$ packs are associated with botulism) while for smoked fish it is estimated that the safety unit is >8.0 (i.e. 1 in >$10^{8.0}$ packs are associated with botulism). It should be noted that none of these commercial foods have been associated with foodborne botulism when correctly stored.

Table 8.1 Estimate safety units for four chilled foods and foodborne botulism

Product	Country	Time period	VP, MAP, air	Temp (°C)	Max shelf life (days)	No. packs produced	Cases of botulism	SU
Cooked Chilled Meals	UK, France & Finland	1986 - 2005	Air	≤8°C	10	$8.3x10^9$	0	>9.9
Sliced cooked meats	UK	1999 - 2005	MAP	≤8°C	10	$7.2x10^9$	0	>9.8
Smoked Fish	UK	2003 - 2005	VP	≤8°C	10	$9.9x10^7$	0	>8.0
Raw red meats	UK	1999 - 2005	MAP (high O_2)	≤8°C	10	$7.9x10^9$	0	>9.9

8.5 Conclusions

Risk assessment is a frequently used term that can have many definitions. Formal Codex type risk assessments are well defined, require considerable data and are formally recorded, in order to provide a transparent assessment of the risks associated with the production of particular products. In this review formal risk assessments considering risks of growth and toxin formation from non-proteolytic *C. botulinum*, in a cooked sliced meat and gnocchi have been considered. Both assessments used slightly different approaches, but came to the conclusion that the products (which were not given a 90°C/10 min process and did not have pH or Aw controls), could be stored for longer than 10 days at 8°C, and remain safe with respect to the risks from non-proteolytic *C. botulinum* . In the case of the gnocchi the results from the risk assessment were confirmed in a challenge test.

The term risk assessment is often used to describe less formal determinations of product risk, however these less formal and structured assessments provide useful information in specific product categories. Work done on fresh produce packed in MAP, suggests that whilst there may be risks of growth from *C. botulinum* , this may only occur in temperature abused product (>12°C) and will usually be preceded by gross product spoilage that would render that product organoleptically unacceptable to the consumer. Assessment of various fish products has considered that unless a sporicidal heat process is given, the presence of *C. botulinum* spores should be assumed, and suitable controls (pH or Aw) put in place. These assessments have not however recommended any shelf life requirements (times or temperatures) if the controls are not in place. In these situations where no specific controls are recognised, recommendations to employ predictive microbiology or challenge testing are often given.

One issue that is frequently noted when considering risks of non-proteolytic *C. botulinum* in chilled MAP/VP products, is that whilst some challenge testing data may indicate the potential for growth and toxin formation to occur, it is known that many thousands of millions of packs of product of this type have been sold around the world, with little evidence of instances of botulism having occurred, and on the rare occasions when issues have arisen, these have been linked closely to product that has been temperature abused. The use of these data from this large commercial production would enable an assessment of the risks arising from these products. This has indeed been done for proteolytic *C. botulinum* in canned meats, and it is possible that this type of approach could add value to any assessment done on non-proteolytic *C. botulinum* in chilled MAP/VP products. Some very preliminary data assessment indicates that the safety units for cooked chill foods, sliced cooked meats and raw red meats are all >9.8, while for smoked fish it is estimated that the safety units are >8.0. These of the same order as for canned meats.

8.6 References

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Glossary

a_w (Water activity)
A measure of the free moisture in a food, is the quotient of the water vapour pressure of the substance divided by the vapour pressure of pure water at the same temperature. Pure water has an a_w of 1.0. This value reduces as the availability of water is reduced.

ACCC
Australian Cook Chill Council

ACMSF
Advisory Committee for the Microbiological Safety of Foods. The independent body that advises the FSA on the microbiological safety of foods.

AIFST
Australian Institute of Food Science & Technology

ALOP (Acceptable Level of Protection)
ALOPs are public health goals and are set for populations rather than population sub-groups and food types. ALOPs are made into achievable goals by use of Food Safety Objectives (FSOs).

AFGC
Australian Food & Grocery Council

AMI
American Meat Institute

ANZFA
Australia and New Zealand Food Authority (now known as FSANZ)

AQIS
Australian Quarantine Inspection Service

ASI
Australian Supermarket Institute

Balut
An embryo inside a fertile egg that has been incubated for a period sufficient for the embryo to reach a specific stage of development after which it is removed from incubation before hatching.

CCP
Critical Control Point. A point in a food production plant that has been identified by a HACCP plan to require monitoring and control, to assure food safety.

CFA
Chilled Food Association

CFIA
Canadian Food Inspection Agency

Case-Ready
Case-ready technology eliminates the need for in-store processing. Meat is packaged in rigid foam or plastic box-shaped containers at a packing plant or processor and can be displayed in that format in the retail store.

CCFRA
Campden & Chorleywood Food Research Association

CFSAN
Center for Food Safety and Nutrition, part of the FDA

Chilled food
A prepared food that for reasons of safety and/or quality is designed to be stored at refrigeration temperatures (at or below 8°C as defined by the England, Wales and Northern Ireland temperature control legislation; in Scotland legislation requires chilled food to be held in a refrigerator, or refrigerating chamber, or a cool ventilated place) throughout its entire life.

Challenge testing
Deliberate experimental inoculation of relevant microorganisms into a food product to determine the product's ability to support survival, growth or inactivation of the organism during storage at defined temperature(s).

Chilling
Rapid reduction of temperature, normally to a specified value.

Clean Labels
The production of foods that do not include artificial preservatives, colours etc.

CODEX
An International body that produces guidelines and information on safe methods of food production.

Cold Smoking:
Curing by smoking at an air temperature not higher than 30°C to avoid cooking the flesh or coagulating the protein.

ComBase
A database of response of foodborne bacteria (pathogens and spoilage bacteria) to environmental conditions in food. (website www.ComBase.cc)

ComBase Predictor
A predictive microbiology modelling package of response of foodborne bacteria (pathogens and spoilage bacteria) to environmental conditions in food. (website www.ComBase.cc)

Controlled Atmosphere Packaging (CAP):
Packaging in an atmosphere where the composition of gases is continuously controlled during storage. This technique is primarily used for bulk storage.

Cook-chill foods:
UK definition: Foods produced by a catering system based on cooking followed by chilling, storage in controlled low temperature conditions (0°C to 3°C) and subsequent reheating immediately before consumption.

Non-UK definition: Cooked food hot filled into impermeable bags which have the air expelled and are then sealed or crimped closed. The bagged food is rapidly chilled and refrigerated at temperatures that inhibit the growth of psychrotrophic pathogens.

Core Temperature
Temperature of food measured at its centre i.e. the coldest point in a product during the heating process.

Cured
Meat/protein products that have been preserved by addition of various salts e.g. nitrite, sodium chloride.

Deep chill:
No more than 3°C, but not frozen.

ECFF
European Chilled Food Federation

EVI
Elintarvikevirato. Finnish Food Agency

FDA
Food and Drug Administration of the USA

Food Safety Objectives
An FSO is defined as the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of health protection (ALOP).

Fresh Produce
Fresh fruit and/or vegetables

FSANZ
Food Safety Australia and New Zealand

FSA
UK Food Standards Agency

FSAI
Food Safety Authority of Ireland

FSIS
Food Safety Inspection Service. A part of USDA (US Department of Agriculture).

Gravad
Raw fish seasoned with salt and often dill. [Swedish : grava, to bury (from the original process of curing it in the ground)]

Hazard:
A biological, chemical or physical agent in, or condition of, food (or feed) with the potential to cause an adverse health effect.

Hazard Analysis:
The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

Hazard Analysis Critical Control Point (HACCP)

A system that identifies, evaluates, and controls hazards that are significant for food safety. HACCP is the primary risk management tool used in the food industry.

HACCP plan
A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain (e.g. chilled foods) under consideration.

Hermetically sealed container
Containers that are designed and intended to protect the contents against the entry of viable microorganisms after closing.

High Acid Food
Food with a pH at or below 4.5

High Oxygen Packaging:
A pack in which the internal gas composition contains oxygen at a level in excess of 20%.

Hot smoking
Curing by smoking at a temperature of 70-80°C at some stage of the process in order to cook the flesh.

HPA
Health Protection Agency (formally PHLS)

Hurdle
A factor which will limit or prevent microbial growth.

Hurdle technology
The use of a combination of factors to effect control of microbial growth.

IFPA (International Fresh-Cut Produce Association)
A USA based association representing organisations with an interest in prepared fresh produce.

IFR (Institute for Food Research)
An independent food research organisation based in the UK

kt (kilotonnes)
A unit of thousands of tonnes (1 tonne = 1000kg)

LACOTS (LACORS)
Local Authorities Coordinating Office on Regulatory Services (LACORS)
Local Authorities Coordinating Body on Food & Trading Standards (LACOTS)

Lethal rate
An expression of the rate at which a target organism is killed at any given temperature, relative to the rate at which it is killed at a reference temperature.

Low Acid Food
Food with a pH greater than 4.5

Low Oxygen Packaging
A pack in which the internal gas composition contains oxygen at a level lower that 20%.

M

Million

MAFF
Ministry of Agriculture Fisheries and Foods. UK ministry responsible for food safety prior to the establishment of the Food Standards Agency (FSA)

MOFFA
Mail Order Fine Foods Association

Modified Atmosphere Packaging (MAP)
Atmosphere in a packaged product that differs from the ambient atmosphere. The three main gases used in MAP are O_2, CO_2 and N_2, which together comprise air when in the proportion 20%, <1%, 79%. The choice of gas used in MAP is dependent upon, for example, the food's fat content, microbiological character and respiration rate (horticultural products). The proportion of each gas used is fixed when the mixture is introduced into the pack, but no further control is exercised during storage.

The science behind modified atmosphere as a food preservation technique was first researched at the end of the 19th Century by Pielsticker. It has been used to preserve foods for many decades. MAP of fish was first reported in the 1930s. Commercially produced packs appeared on the market in 1973 in Germany, 1974 in France, 1978 in Denmark and in the UK in 1981.

Pasteurisation
A heat process lower than that required to render a food commercially sterile. Usually designed to kill vegetative microorganisms in a food.

Pasteurisation value
The length of time at a given temperature required to obtain a specified level of destruction of a microorganism whose heat resistance characteristics are known. The heat resistance of a microorganism is characterised by D and z values defined as follows:
- D = time (in minutes) to achieve a 90% or one log reduction of a microbiological population at a given temperature;
- z = the number of degrees required for the thermal destruction curve to transverse one log cycle (expressed in degrees Celsius).

PHLS
Public Health Laboratory Service. Now a part of the HPA

PMP
Pathogen Modeling Programme. A predictive microbiology modelling package of response of foodborne bacteria (pathogens and spoilage bacteria) to environmental conditions in food. It was developed by the USDA.

Prepared Produce
Produce that has been processed to a point at which it can be directly used by the consumer (e.g. bagged ready to eat salad products).

Primal cut
In the meat industry, a primal cut is major section into which carcasses and sides of meat are separated, such as a beef round, pork loin, lamb flank, or veal breast.

Produce
Fruit or vegetables.

Protein Foods
Foods in which the primary constituent is protein e.g. meat, poultry, fish, vegetarian alternatives etc.

Rapid cooling
Lowering the temperature of the food in a way such that the critical zone for microbiological proliferation (60°C-10°C) is passed through as rapidly as possible and the specified temperature is attained.

Ratite
A flightless bird such as an emu, ostrich, or rhea.

Ready-to-eat (RTE) Food
Food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing, effective to eliminate or reduce to an acceptable level microorganisms of concern (e.g. a chilled cooked meat such as ham).

Ready-to-reheat (RTRH) Food
Food designed by the producer or manufacturer as suitable for direct human consumption without the need for cooking, but which may benefit in organoleptic quality from some warming prior to consumption. (e.g. a ready meal).

Reduced Oxygen Packaging
Pack type in which the level of oxygen is reduced to below 20%. This can include vacuum packed, low oxygen modified atmosphere packed, or other controlled atmosphere packs.

Refrigerated food
Food that is kept at cold storage temperatures to maintain its safety, quality and suitability, for the intended shelf life.

Reheating
Heating by the consumer to a temperature suitable for organoleptic purposes, where its application is not required to assure safety of the product.

REPFED
Refrigerated Packaged Foods of Extended Durability

Retail
The handling and/or processing of food and its storage at the point of sale or delivery to the final consumer, and includes distribution terminals, catering operations, factory canteens, institutional catering, restaurants and other similar food service operations, shops supermarkets distribution centres and wholesale outlets.

RWTAA
Refrigerated Warehouse and Transport Association of Australia

SVAC
Sous Vide Advisory Council

SCOOP
Scientific Cooperation Project of the European Union.

Shelf life
The period after its production, during which the product maintains its microbiological safety and sensory qualities at a specific storage temperature. It is based on identified hazards for the

product, heat or other preservation treatments, packaging method and other hurdles or inhibiting factors that may be used.

Shelf life testing
Experimental determination of the shelf life of a product. This will usually involve the storage of a product at a defined temperature for a defined time (a storage trial), whilst undertaking microbiological and organoleptic testing. On occasion challenge testing may also be used during a shelf life trial.

Sous vide
Cooking food within a hermetically sealed, impermeable bag, which is rapidly chilled after cooking. This procedure prevents recontamination of the cooked product after cooking.

Storage trial
Storing a product at predetermined times and temperatures as part of shelf life determination.

SYNAFAP
Syndicat National de Fabricants des Plats Préparés, the French trade organisation representing chilled food producers.

TNO
An independent food research organisation based in the Netherlands

UNIPI
Unione Industriale Pastai Italiani, the Italian Pasta Producers Association.

USDA
United States Department of Agriculture

Use By Date
The date after which the product should not be consumed. It is determined from the date of production by the manufacturer, utilising the product shelf life and building in a margin of safety.

Vacuum Packaging (VP)
Partial or total removal of air/gas from packaging.

Validation (of HACCP)
Obtaining evidence that the elements of the HACCP plan are effective.

Verification (of HACCP)
The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan.

Appendix two – Data on growth and toxin formation by non-proteolytic *C. botulinum* in foods at 10°C and below

Altogether 1307 independent tests of growth/toxin formation by non-proteolytic *C. botulinum* in chilled foods have been recorded, and the following excel spreadsheet contains details of 1238 independent tests. There is insufficient information to include the data of Baker and Genigeorgis (1992) in the spreadsheet (data collected at 4°C, 13 foods became toxic between days 26 and 35 days, and 56 foods were negative at 37 days).

The following information is recorded in the spreadsheet (if known):

- Food properties (food materials or food, pack size (g), pH, Aw, NaCl (% salt-on-water), storage temperature (°C), MAP or VP (and details), heat-process (including whether applied to spores in the food), other comments (including preservatives)). Note that in some cases the food materials are a component of chilled foods sold in the UK.
- *Clostridium botulinum* (type of toxins formed by the test strains of non-proteolytic *C. botulinum*, log of spore inoculum concentration (both per g and per pack).
- Growth/toxin formation (last time when all replicate samples were negative (days) [column headed "-ve"], the first time when one of the replicate samples was positive for toxin or growth (days) [column headed "+ve"]. If none of the samples were positive at the end of the test, this is indicated as "NP" [not positive] for each test. If the time between the last negative and first positive sample is four days or less, the time to toxin (TTT) should be calculated, where TTT = $\sqrt{}$ (time to last negative sample x time to first positive sample), whether data are of toxin formation (T) or growth (G))
- Additional comments
- Reference (includes confidential industry data)

It should be noted that in most of these tests, replicate samples (often duplicate or triplicate) have been removed at time intervals and tested for toxin (or growth). In this Appendix, two times are recorded with respect to toxin formation/growth (in days):

- The last time when all the replicate samples were negative
- The first time when one of the replicate samples was positive for toxin (or growth)

It should be noted that this approach leads to the omission of some negative data for the replicate samples (e.g. if two of the replicates are negative and one is positive, then the sample is recorded as positive).

References

The data have been collected from industry and the literature. Industry data are indicated by a code, as these data were provided in confidence. Literature data was obtained from the following publications:

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