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Growth of spoilage bacteria during storage and transport of meat

EFSA Panel on Biological Hazards (BIOHAZ)

Abstract

Pseudomonads and lactic acid bacteria (LAB) are the most relevant organisms for assessing the effect of specific chilling time–temperature scenarios on the growth of spoilage bacteria under aerobic and anaerobic (vacuum packs) conditions, respectively. Pseudomonad growth was modelled on beef, pork and lamb carcasses, chilled to specific target surface temperatures and compared with the growth that would be achieved if the carcasses were chilled to a core temperature of 7°C (Regulation (EC) No 853/2004). Pseudomonad growth with the combination of chilling the carcass surface to a target temperature (1–10°C for beef and lamb, and 5–10°C for pork) and transportation at that temperature plus ± 1°C was also modelled for 1–48 h (assuming an initial count of 1 CFU/cm^2). Finally, the growth of pseudomonads and LAB was modelled on meat intended for use in minced meat/meat preparations, stored at temperatures of 1–7°C (inclusive) for 1–12 days. The effect of storage temperature and initial count on the time to reach 10^7 CFU/cm^2 was also investigated. The outputs suggest that chilling bovine or ovine carcasses to between 4 and 10°C surface temperature, inclusive, results in similar or lower predicted pseudomonad growth as compared to chilling to a core temperature of 7°C. The results for porcine carcasses depended on the target surface temperature and chilling curve applied. It was also predicted that pseudomonads and LAB grow steadily on meat stored at 1–7°C and LAB counts exceeded 10^7 CFU/cm^2 when stored for 11 days at 7°C. It was concluded that the time–temperature chilling profiles that may be used to obtain similar or less growth to that obtained when chilling to a core temperature of 7°C is dependent on the initial contamination level.

Keywords: carcass chilling, time–temperature integration, spoilage, pseudomonads, lactic acid bacteria

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Summary

Following a request from the European Commission, the EFSA Panel on Biological Hazards (BIOHAZ) was asked to deliver a scientific opinion on the growth of spoilage bacteria, including *Clostridium*, on: (1) carcasses using similar time–temperature combinations as applied for pathogens in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates)', and to revise these chilling profiles if bacterial growth in excess of that which would be achieved if the carcasses were chilled to a core temperature of 7°C, as required by Regulation (EC) No 853/2004, was predicted and (2) raw meat materials intended for the production of minced meat or meat preparations using the time–temperature combinations applied for pathogens in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2 (minced meat from all species)', and to revise these chilling conditions if bacterial growth in excess of that which would be achieved using the current recommendations was predicted.

To fulfil this mandate, and based on a review of the scientific literature, pseudomonads were considered to be the main spoilage bacteria under aerobic conditions, i.e. on the carcasses and meat cuts stored in air (aerobically), and lactic acid bacteria (LAB) as the primary spoilage agents on meat cuts stored under anaerobic conditions (vacuum-packed), as is the norm in the red meat industry. *Brochothrix thermosphacta* may also cause meat spoilage under anaerobic conditions as can psychrophilic *Clostridium* spp. The latter were specifically requested for consideration in the Terms of Reference. Their growth was not modelled directly as it was considered that LAB, given their higher prevalence and similar growth rate, were a more appropriate target for modelling bacterial growth on meat stored under anaerobic conditions and it was assumed that the predictions for LAB would also apply to *Clostridium* spp.

While it was generally accepted that the vast majority of spoilage bacteria occur on the surface of the carcass, the term 'within' was included to cover *Clostridium* spp. that cause deep tissue spoilage, such as bone taint. Furthermore, at the specific request of the European Commission, this was expanded to include target carcass surface temperatures in combination with transport at ± 1°C of this target.

The growth of pseudomonads on beef, pork and lamb carcasses under similar time–temperature scenarios to those used in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates)' was calculated using available predictive models and compared with the two baseline scenarios used in that opinion; a mean chilling profile and a worst case (slowest decrease in carcass surface temperature) for each animal species, with the exception of lamb for which a mean chilling profile was not available.

The growth of both pseudomonads and LAB on meat cuts intended for use in minced meat and meat preparations and stored under aerobic and anaerobic conditions, respectively, was also predicted at alternative scenarios similar to those used in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2 (minced meat from all species)' using available predictive models. Moreover, the time (h) required by pseudomonads and LAB to reach a spoilage level of 10^7 CFU/cm^2 was estimated for various temperature conditions taking initial contamination levels into account.

The models used predicted that bovine or ovine carcasses could be chilled to between 1 and 10°C surface temperature, inclusive, without obtaining pseudomonad growth in excess of that, which would be achieved if the carcasses were chilled to a core temperature of 7°C as required by Regulation (EC) No 853/2004. Pseudomonad growth on porcine carcasses was modelled for target surface temperatures of between 5 and 10°C as the chilling curves used did not go below these temperatures. The results for porcine carcasses were different to those for beef and lamb as not all time–temperature combinations resulted in lower pseudomonad growth on porcine carcasses as compared with that which would be achieved if the carcasses were chilled to a core temperature of 7°C. Moreover, it is difficult to model the relationship between core and surface temperature of the carcasses. To ensure heat dissipating from the core does not heat the surface, sufficient heat must be removed from the carcass before transportation.

For meat cuts intended for minced meat or meat preparations, the growth of pseudomonads on poultry was estimated using the parameters pH 6.5 and water activity (a_w) 0.993, and on red meat, a pH of 5.7 and an a_w of 0.98. The results of this modelling analysis were inconclusive. Whether or not the predicted growth of pseudomonads and LAB (anaerobic conditions) obtained using the time–temperature combinations applied for pathogens in the previous opinion (EFSA BIOHAZ Panel, 2014b) was higher or lower than that obtained using current recommendations was dependent on the initial levels of contamination.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Current requirements

The maintenance of the cold chain is one of the main principles and basic requirements of European Union (EU) legislation on food hygiene.[1] Raw materials, ingredients, intermediate products and finished products that are likely to support the growth of pathogenic microorganisms and/or spoilage bacteria, are to be kept at temperatures that do not result in a risk to health. The cold chain must not be interrupted.

In the case of meat (including fresh meat, meat products, minced meat and meat preparations), EU legislation (Regulation (EC) No 853/2004) lays down specific requirements for its storage and transport regarding temperatures and maximum times of storage. Such requirements include:

- Fresh meat from animals other than poultry:
  - *Post-mortem* inspection must be followed immediately by chilling in the slaughterhouse to ensure a temperature throughout the meat of not more than 3°C for offal and 7°C for other meat, along a chilling curve that ensures a continuous decrease in the temperature. However, meat may be cut and boned during chilling in establishments attached to the slaughterhouse.
  - Apart from few exceptions, meat must reach these temperatures specified above before transport, and remain at that temperature during transport.
  - The maximum storage time between slaughter and production of minced meat and meat preparations is no more than 6 days and no more than 15 days from the slaughter of the animals in the case of boned, vacuum-packed beef and veal.
- Poultry meat:
  - After *post-mortem* inspection, slaughtered animals must be chilled to not more than 4°C as soon as possible, unless the meat is cut while warm in establishments attached to the slaughterhouse.
  - The maximum storage time between slaughter and production of minced meat and meat preparations is no more than 3 days.
- Minced meat, meat preparations:
  - Immediately after production, minced meat and meat preparations must be wrapped or packaged and be chilled to an internal temperature of no more than 2°C for minced meat and 4°C for meat preparations, or frozen to an internal temperature of not more than −18°C.
  - Minced meat and meat preparations must comply with the microbiological criteria laid down in Regulation (EC) No 2073/2005[2] as regards *Salmonella*, aerobic colony counts and *Escherichia coli*. These criteria remain applicable.

1.1.2. EFSA Opinions on public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 and Part 2

The European Food Safety Authority (EFSA) adopted the Scientific Opinion on 'the public health risks related to the maintenance of the cold chain during storage and transport of meat', Part 1 and Part 2, in 2014 (EFSA BIOHAZ Panel, 2014a,b).

Part 1 of the Opinion (EFSA BIOHAZ Panel, 2014a) concluded that it is possible to commence carcass transport before a target temperature is reached, without significantly increasing the risk linked to the growth of potentially harmful microorganisms, as long as the carcass temperature continues to decrease towards the target one during transportation. It also concluded that it is possible to develop different combinations of carcass surface target temperature at loading time, with transport time–air temperature combinations that ensure pathogen growth is no greater than that

[1] Article 4(3)(d) of Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. OJ L 139, 30.4.2004, p. 1–54.

[2] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338, 22.12.2005, p. 1–26.

achieved using the current chilling requirements. The opinion did not address the possible growth of spoilage bacteria.

Part 2 of the Opinion (EFSA BIOHAZ Panel, 2014b) recommended a number of time–temperature combinations for the storage of fresh meat prior to mincing, all of which gave the same or less bacterial growth than is possible under current legislative requirements. The opinion did not address the possible growth of spoilage bacteria.

1.1.3. Additional scientific advice

After discussions with Member States' experts and stakeholders, the Commission considered that further scientific advice is needed as regards the growth of spoilage bacteria in meat (carcases, cuts) during transport after slaughter, and in raw materials for minced meat and meat preparations.

The purpose of the request is to ensure compliance with Article 14(5) of Regulation (EC) No 178/2002[3] laying down that, 'in determining whether any food is unfit for human consumption, regard shall be had to whether the food is unacceptable for human consumption according to its intended use, for reasons of contamination, whether by extraneous matter or otherwise, or through putrefaction, deterioration or decay'.

The current requirement to chill meat immediately after *post-mortem* inspection remains applicable. In addition, the core temperature currently applicable will have to be reached as soon as possible after the transport stage.

Moreover, a need for a transport regime extending for up to 2 or 3 h for minimally chilled meat has been identified. Stakeholders have indicated that a time–temperature combination resulting in a transport regime of 1 h (combination 'e' in the recommendations of Part I of the Opinion) is somewhat short for practical purposes, as loading of the lorry can take up to 2 h. Therefore, it would be helpful if other options could be explored that might result in a combination of time and temperature which would result in transport of 2 or 3 h with minimal chilling. This could facilitate the short-term transport of minimally chilled meat to cutting/boning plants relatively close to the slaughterhouse.

1.1.4. Terms of Reference

EFSA is asked to provide a Scientific Opinion on the growth of spoilage bacteria, including *Clostridium*, in meat and in raw materials stored for minced meat and meat preparations as a consequence of applying flexibility in the maintenance of the cold chain during storage and transport of meat. In particular, EFSA is requested:

In relation to transport of meat of domestic ungulates:

1) to investigate the growth of spoilage bacteria both within and on the surface of meat carcasses or parts thereof during storage and transport using the same combinations of temperature and time conditions applied for pathogens adopted in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part I';
2) to revise, if needed, based on the outcome of Term of Reference (ToR) 1, the recommendations for maximum surface temperature and maximum transport time that would give equivalent growth to current requirements to ensure that the recommendations address at the same time both pathogens (as concluded in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1') and spoilage bacteria. Recommendations on short distance transport (2–3 h) should be included.

In relation to the production of minced meat from all species:

3) to investigate the growth of spoilage bacteria during storage of meat raw materials intended for the production of minced meat or meat preparations using the same combinations of temperature and time conditions for pathogens adopted in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2';

[3] Article 14(5) of 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. OJ L 31, 1.2.2002, p. 1–24.

4) to revise, if needed, based on the outcome of the ToR 3, the recommendations for maximum storage time that would give equivalent growth to current requirements to ensure that the recommendations address at the same time both pathogens (as concluded in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2') and spoilage bacteria.

In addition, at a later stage, the European Commission requested additional aspects to be addressed in this opinion, specifically:

5) to model the growth of spoilage bacteria at lower temperature transport regimes;
6) to investigate the growth of spoilage bacteria at higher temperature (7, 8, 9 and 10°C) transport regimes.

1.2. Additional information

1.2.1. Introduction to the assessment

In June 2014, EFSA published the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates)' (EFSA BIOHAZ Panel, 2014a). This opinion concluded that (1) *Salmonella* spp., verocytotoxigenic *E. coli* (VTEC), *Listeria monocytogenes* and *Yersinia enterocolitica* are the most relevant microbial pathogens when assessing the effects of beef, pork and lamb carcass chilling regimes on the potential risk to public health, and (2) most bacterial contamination occurs on the surface of the carcass. Moreover, it provided combinations of maximum surface temperatures at carcass loading and maximum chilling and transport times, resulting in pathogen growth equivalent to or less than that obtained when carcasses are chilled to a core temperature of 7°C in the slaughterhouse.

Part 2 of this opinion (EFSA BIOHAZ Panel, 2014b) covered minced meat and investigated the impact of storage time between slaughter and mincing on bacterial pathogen growth using predictive modelling. It concluded that time–temperature combinations, other than that legally required by Regulation (EC) No 853/2004 (red meat carcasses must be immediately chilled after *post-mortem* inspection to not more than 7°C throughout, and that this temperature be maintained until mincing, which must take place not more than 6 or 15 (vacuum-packed meat) days after slaughter), for the storage of fresh meat between slaughter and mincing are possible without increasing bacterial pathogen growth. Moreover, maximum times for the storage of fresh meat intended for minced meat preparation were provided for different storage temperatures.

Neither Part 1 nor Part 2 of the previous opinions (EFSA BIOHAZ Panel, 2014a,b) considered the impact of bacterial spoilage on the alternative storage time–temperature combinations.

This follow-up opinion investigates the growth of spoilage bacteria on the surface of the carcass (aerobic conditions) during chilling and subsequent transportation, using an extended range of time–temperature combinations, including those applied to pathogens in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates)' (EFSA BIOHAZ Panel, 2014a) and provides recommendations for the maximum surface temperature and maximum transport time that would give equivalent growth to current requirements (Regulation (EC) No 853/2004).

This opinion also investigates the growth of spoilage bacteria on raw meat materials used for minced meat (red meat and poultry) preparation during chilling and subsequent transportation using the same time–temperature combinations applied to pathogens in the opinion mentioned above (EFSA BIOHAZ Panel, 2014b), with a view to providing recommendations for maximum storage time, that would give equivalent growth to that which would be achieved based on the requirements in Regulation (EC) No 853/2004.

1.2.2. Meat spoilage

Beef, pork and lamb carcasses are chilled immediately after *post-mortem* inspection in slaughterhouse chilling rooms (Figure 1). As the carcasses are exposed to air this process is aerobic. After 24–96 h of chilling, the carcasses are typically moved to a boning hall where they are cut into primary cuts called primals. These are typically stored for up to 6 weeks in vacuum packs under

anaerobic conditions. Ground meat products may be prepared from trimmings from deboning or trimmings from primals after 6 weeks anaerobic storage. These may then be stored aerobically or anaerobically.

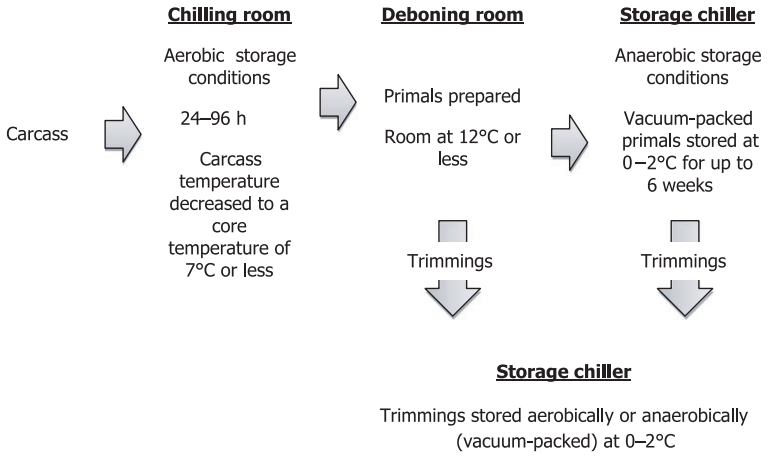

Figure 1: The chilling and chilled storage conditions used for beef, pork and lamb carcasses and associated primals and trimmings

Chilling red meat and poultry carcasses is essential to retard bacterial growth. Chilling is also required for appearance and eating quality. Most carcasses are refrigerated using a system based on forced convection air chilling (James and James, 2004), although spray chilling may also be used. Spray chilling is faster than air chilling and operates on the same principle as air chilling except potable water (instead of air) is chilled before being applied to the carcasses as a fine spray. It is primarily used in poultry, but may also be used in beef, pork and lamb processing plants (Brown and James, 1992; Brown et al., 1993; James and James, 2004). A more thorough review of meat chilling methods is provided in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates)' (EFSA BIOHAZ Panel, 2014a).

Regulation (EC) No 853/2004 requires that carcasses are immediately chilled after *post-mortem* inspection to ensure that the temperature throughout the meat is not higher than 7°C in the case of meat, and not higher than 3°C for offal. However, there is no provision on the time limit by when this temperature must be achieved. Moreover, beef and lamb carcasses are usually not chilled to below 10°C (core temperature) within the first 10 h to avoid cold shortening and toughening of the meat. Thus, bacteria will grow on the surface of the carcass until the temperature is reduced sufficiently to retard bacterial activity.

Meat is considered to be spoiled when discolouration, off-odour and/or slime develop and is usually caused by bacteria (Tsigarida and Nychas, 2001). Pseudomonads, *Lactobacillus* and *Enterococcus*, for example, produce slime on meat. *Enterococcus* may also produce hydrogen peroxide greening similar to hydrogen sulfide greening caused by *Clostridium* spp. The growth of bacteria on meat is influenced by temperature, pH, water activity, nutrient availability, storage atmosphere and competition from other organisms and small changes in these factors can greatly influence spoilage (Sumner and Jenson, 2011).

Although indigenous enzymes may also be involved, their contribution is considered to be negligible compared with bacterial action (Tsigarida and Nychas, 2001). The bacteria commonly found on fresh meat belong to a range of different genera, including *Achromobacter*, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Alteromonas*, *Arthrobacter*, *Bacillus*, *Campylobacter*, *Carnobacterium*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Escherichia*, *Flavobacterium*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Kocuria*, *Kurthia*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Listeria*, *Microbacterium*, *Micrococcus*, *Moraxella*, *Paenibacillus*, *Pantoea*, *Proteus*, *Providencia*, *Pseudomonas*, *Shewanella*, *Staphylococcus*, *Streptococcus*, *Vibrio*, *Weissella* and *Yersinia* (Nychas et al., 2007). These organisms originate from the animal (hide, fleece or skin and intestines) and the abattoir environment. The main spoilage defects and causal bacteria are shown in Table 1.

Table 1: The main spoilage defects and causal bacteria (adapted from Nychas et al., 2008)

Defect	Meat product	Causal bacteria
Slime	Fresh meat	Pseudomonads, *Lactobacillus*, *Enterococcus*, *Weissella* and *Brochothrix*
Hydrogen peroxide greening	Fresh meat	*Weissella*, *Leuconostoc*, *Enterococcus* and *Lactobacillus*
Hydrogen sulfide greening	Vacuum-packed fresh meat	*Shewanella* and *Clostridium*
Hydrogen sulfide production	Cured meats	*Vibrio* and Enterobacteriaceae
Sulfide odour	Vacuum-packed fresh meat	*Clostridium* and *Hafnia*
Cabbage odour	Bacon	*Providencia*
Cheesy or dairy odour	Vacuum-packed fresh meat	*Brochothrix thermosphacta*
Putrefaction	Ham	Enterobacteriaceae and *Proteus*
Bone taint	Whole meats	*Clostridium* and *Enterococcus*
Souring	Vacuum-packed meats	Lactic acid bacteria, *Enterococcus*, *Micrococcus*, *Bacillus* and *Clostridium*

The dominant spoilage genera (and species within a given genus) are determined by the storage conditions. Chilled storage selects for psychrophilic and psychrotrophic bacteria. Under aerobic conditions, the spoilage consortium of bacteria is usually dominated by pseudomonads (Stanbridge and Davis, 1998; Koutsoumanis et al., 2006). Three species of *Pseudomonas*, *Pseudomonas fragi*, *Pseudomonas fluorescens* and *Pseudomonas lundensis*, are primarily responsible for the formation of slime and off-odour, usually when their population reaches 10^7–10^8 colony forming units (CFU)/cm^2 (Nychas et al., 2008).

Enterobacteriaceae, especially cold-tolerant species, such as *Hafnia alvei*, *Serratia liquefaciens* and *Pantoea agglomerans*, are also commonly found on fresh meat but rarely contribute to spoilage unless there is temperature abuse (Nychas et al., 1998). Lactic acid bacteria (LAB) and *Brochothrix thermosphacta* are oxygen-tolerant anaerobes and are commonly detected on aerobically stored chilled meat, but are not considered as major spoilage contributors, with the possible exception of lamb (Holzapfel, 1998). These bacteria are, however, usually the predominant spoilage organisms of meat stored under anaerobic conditions (e.g. vacuum-packed or modified atmosphere). This type of spoilage, which typically occurs when maximum numbers (10^8 CFU/cm^2) are achieved (Jones, 2004), is characterised by souring rather than putrefaction.

Chilled meat stored under anaerobic conditions may also be spoiled by a range of psychrotolerant/psychrophilic *Clostridium* spp. These *Clostridium* spp. grow relatively slowly and spoilage typically occurs in correctly chilled batches (0–2°C) after 4–6 weeks, characterised by a putrid smell (H_2S) with a metallic sheen on the meat, with or without gas production. *Clostridium algidicarnis*, *Clostridium frigoris*, *Clostridium bowmanii*, *Clostridium frigidicarmis* and *Clostridium ruminantium* have been associated with spoilage without gas production (Broda et al., 1999, 2000; Adam et al., 2010; Cavill et al., 2011), while other species, such as *Clostridium estertheticum* and *Clostridium gasigenes*, produce large volumes of gas, primarily carbon dioxide (Moschonas et al., 2010; Yang et al., 2011). The packs inflate and eventually burst; thus, this type of spoilage is often referred to as 'blown pack spoilage' (Bolton et al., 2015). *Clostridium* spp. are also the primary causative agents of deep tissue spoilage of meat, including bone taint.

1.2.2.1. Factors affecting meat spoilage

Factors affecting meat spoilage include temperature, pH, water activity and storage atmosphere. During chilling, the temperature of the surface of the carcass changes (EFSA BIOHAZ Panel, 2014a). Meat is usually stored at temperatures of around 2°C (James and James, 2004), with the exception of meat being transported long distances where a temperature of −1.5°C is recommended (Jeremiah and Gibson, 2001). Small changes in temperature can significantly affect the shelf-life. Thus, increasing the temperature from −1.5 to 0, 2 or 5°C, will decrease the time to spoilage by approximately 30%, 50% and 70%, respectively (Sumner and Jenson, 2011).

The pH of the muscle is approximately 7.0 at slaughter and decreases to pH from 5.3 to 5.8 over 18–40 h in beef, and 6–12 h in pork. In lamb carcasses, this usually occurs in approximately 24 h

(McGeehin et al., 1999). Dark firm dry (DFD) meat can occur in all species but is more common in beef. DFD is the result of pre-slaughter stress and the depletion of glycogen reserves to below 0.6%, and DFD meat has a pH of 5.9–6.8. This higher pH promotes the growth of spoilage bacteria. Fresh meat has a water activity (a_w) of approximately 0.99 (ICMSF, 1998), which decreases during chilling to approximately 0.96–0.97 (Reid et al., 2015). Thus, a wide range of bacteria are able to survive and grow on meat and carcass surfaces.

1.2.2.2. Pseudomonad counts on beef, pork, lamb and poultry carcasses

The levels of bacterial contamination, including pseudomonads on beef, pork, lamb and poultry carcasses will depend on a variety of factors including season, animal/bird cleanliness, abattoir prerequisite activities especially good hygiene practices (GHP), sampling stage, etc. Thus, pseudomonad carcass counts may vary considerably not only within a given abattoir over time but also between abattoirs. The currently available data are presented in Table 2. Relatively few studies have reported pseudomonad counts on beef carcasses. A recent Irish study investigated pseudomonad levels on beef carcasses immediately pre-chill and in the first 96 h in the chillers. In this study, 10 carcasses were tested as per Commission Decision 2001/471/EC[4]; the neck, brisket, flank and rump (100 cm^2 of each) were sampled on each carcass using a sterile cellulose acetate sponge. The experiment was repeated on three separate occasions in the same commercial beef export abattoir. The mean pseudomonad count immediately before chilling was 1.14 log_{10} CFU/cm^2. Over the first 48 h, this count decreased by 0.11 log_{10} CFU/cm^2 and then increased by 0.83 log_{10} CFU/cm^2 to a final count of 1.86 log_{10} CFU/cm^2 between 48 and 96 h (Reid et al., 2015). Lasta et al. (1995) reported an initial pseudomonad count of 3.2 log_{10} CFU/cm^2 on beef briskets, which increased to 8.9 log_{10} CFU/cm^2 after 14 days storage at 5°C. The pseudomonad count on Romanian beef carcasses ranged from 1.04 to 5.48 log_{10} CFU/cm^2 in a study of 18 carcasses (Dan et al., 2003). In contrast, Gustavsson and Borch (1993) found very low counts (0.4 log_{10} CFU/cm^2) on the brisket, lateral forerib and foreleg during chilling.

Table 2: Pseudomonad counts on beef, pork and lamb carcasses reported in the scientific literature

Carcass type	Data provided	Reference
Beef	Mean count of 1.14 (range 0.23–2.35) log_{10} CFU/cm^2 (4 sites sampled as per Commission Decision 2001/471/EC) immediately before chilling increasing to 1.86 log_{10} CFU/cm^2 after 96 h chilling	Reid et al. (2015)
	3.2 log_{10} CFU/cm^2 (briskets) immediately before chilling increasing to 8.9 log_{10} CFU/cm^2 after 14 days storage at 5°C	Lasta et al. (1995)
	1.04–5.48 log_{10} CFU/cm^2	Dan et al. (2003)
	0.4 log_{10} CFU/cm^2	Gustavsson and Borch (1993)
Pork	2.74–6.57 log_{10} CFU/cm^2	Dan et al. (2005)
	4.37 (neck/chest), 4.49 (thigh), 5.45 (lateral abdominal) and 4.55 (coccygeal region) log_{10} CFU/cm^2	Sala et al. (2010)
Lamb	3.11 (after fleece removal), 3.09 (after evisceration) and 3.08 (after washing) log_{10} CFU/cm^2	Bhandare et al. (2007)
	3.32–3.51 log_{10} CFU/cm^2 (leg/flank regions)	Sauter et al. (1980)
Poultry	2.45–3.15 log_{10} CFU/cm^2, post-chill	Vareltzis et al. (1997)
	1.8 (before scalding), 1.7 (after scalding) and 3.1 after de-feathering) log_{10} CFU/g of neck skin	Geornaras et al. (1997)
	Mean count of 3.96 log_{10} CFU/cm^2 (ranging from 0.44 to 4.45 0.4 log_{10} CFU/cm^2	Holder et al. (1997)

CFU: colony forming unit.

Pseudomonad counts have also been reported for pork, and to a lesser extent lamb carcasses. In two different Romanian studies, the pseudomonad count on pork carcasses ranged from 2.74 to

[4] Commission Decision 2001/471/EC of 8 June 2001 laying down rules for the regular checks on the general hygiene carried out by the operators in establishments according to Directive 64/433/EEC on health conditions for the production and marketing of fresh meat and Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat. OJ L 165, 21.6.2001, p. 48–53.

6.57 $\log_{10}$ CFU/cm^2 (Dan et al., 2005), while Sala et al. (2010) obtained counts of 4.37, 4.49, 5.45 and 4.55 $\log_{10}$ CFU/cm^2 on the neck/chest, thigh, lateral abdominal region and the coccygeal region of pork carcasses. Bhandare et al. (2007) reported mean *Pseudomonas aeruginosa* counts on Indian sheep/goat carcasses of 3.11 $\log_{10}$ CFU/cm^2 after flaying (hide/fleece removal), 3.09 $\log_{10}$ CFU/cm^2 after evisceration and 3.08 $\log_{10}$ CFU/cm^2 after washing in the abattoir. Interestingly, Sauter et al. (1980) had previously obtained similar counts, 3.32 and 3.51 $\log_{10}$ CFU/cm^2, respectively, on the leg and flank regions of lamb carcasses in the USA.

There is also very limited data available for poultry carcasses. Vareltzis et al. (1997) obtained pseudomonad counts of 2.54–3.15 $\log_{10}$ CFU/cm^2 on poultry carcasses post-chilling. Geornaras et al. (1997) reported pseudomonad counts of 1.8, 1.7 and 3.1 $\log_{10}$ CFU/g on neck skin before scalding, after scalding and after defeathering, respectively. Holder et al. (1997) obtained a mean pseudomonad count on whole carcasses of 3.96 $\log_{10}$ CFU/cm^2. These ranged from 0.44 $\log_{10}$ CFU/cm^2 on the medial breast to 4.45 $\log_{10}$ CFU/cm^2 on the lateral drumstick during portioning of the carcass.

1.3. Approach to answering the Terms of Reference

The objective of the ToRs was to assess the impact of the time–temperature chilling profiles proposed as alternatives to the current legislation (Regulation (EC) No 853/2004) in the 'Scientific Opinion on public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1' on the growth of spoilage bacteria. Pseudomonads were selected for modelling bacterial spoilage of beef, pork and lamb carcasses stored aerobically because they are considered to be the main spoilage organisms under aerobic chilling conditions, and also because there were suitable models available. LAB were selected as the target organism for modelling meat stored under anaerobic conditions (e.g. vacuum-packed meat primals) as under anaerobic chilled conditions they are assumed to be the major spoilage organism, and again because suitable models were available to predict growth. Although psychrophilic *Clostridium* spp. and *Br. thermosphacta* may also cause spoilage of anaerobically stored meat cuts and the former were mentioned in the ToRs, these were not included in the analysis as it was assumed that their growth would be similar or slower than that of LAB (Mejlholm and Dalgaard, 2013).

The growth of pseudomonads at various alternative time–temperature scenarios during chilling and transportation was calculated, based on available predictive models, and compared with the two baseline scenarios (mean chilling profile and worst-case chilling profile, as per the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates)') for each animal species. For comparison, growth was expressed as $\log_{10}$ CFU/cm^2. The time (h) required by pseudomonads and LAB to reach a spoilage level of 10^7 CFU/cm^2 was estimated for the same combinations of temperature and time conditions applied for pathogens adopted in the aforementioned opinion (EFSA BIOHAZ Panel, 2014a).

2. Data and methodologies

The general approach proposed is based on the change in the temperature kinetics in terms of the bacterial growth potential using predictive microbiology models. The approach is also called 'time–temperature integration' or 'temperature function integration' (McMeekin, 2007). This approach is widely used and has been applied in previous EFSA opinions (EFSA BIOHAZ Panel, 2014a,b). Other examples illustrating the use of time–temperature integration in the area of meat refrigeration include Gill and Jones (1997), Dickson et al. (1993), Jericho et al. (1998) and Lovatt et al. (2006), all of which have used records of the temperatures at the surface and/or core of beef, pork and/or lamb carcasses. These temperature records, coupled with growth models made it possible to estimate microbial growth during chilling. The associated studies facilitate validation of the consequences of specific refrigeration methods (e.g. spraying during cooling, effect of passing through a refrigeration tunnel, etc.). Time–temperature integration has previously been applied in meat industry hazard analysis and critical control point (HACCP) plans. In Australia, for example, on the basis of a model predicting the rate of growth of *E. coli* as a function of the temperature, water activity and the lactate concentration (Mellefont et al., 2003), the Australian Quarantine Inspection Service (AQIS) has established the rules for the chilling of carcasses for export.[5] Using recorders placed in the carcasses during chilling, the growth of *E. coli* can be calculated and expressed in the form of a refrigeration index. The values

[5] Available online: http://www.daff.gov.au/SiteCollectionDocuments/aqis/exporting/meat/market-access-advice/maa-2010/approved-arrangements-guidelines-meat.pdf

calculated for each slaughter site must comply with values that may not be exceeded, to verify that the required refrigeration was applied to the carcasses.

More specifically, the output of time–temperature integration can be used in two different ways. In the first approach, the initial level of contamination is not considered, i.e. growth potential rather than final levels are calculated. The growth potential associated with a time–temperature is then compared with a growth potential obtained in a 'reference' situation (e.g. EFSA BIOHAZ Panel, 2014a,b) or to a target growth potential (e.g. 'less than a doubling', AFSCA, 2008).

In the second approach, the initial level of the bacteria of interest is taken into account. Time–temperature integration is then used to calculate the time to reach a target level given an initial level. ANSES (2010) used this second approach for defining the maximum delay time for entry into the cold rooms related to failure of the slaughter chain. Both the initial level and growth of pseudomonads were considered. The maximum times proposed were directly linked to the initial level of contamination that is specific to the general hygiene of each slaughterhouse; the lower the initial contamination, the higher the delay time. This approach is thus particularly appropriate for bacteria for which a high variability of initial contamination occurs.

As contamination levels on carcasses or meat cuts are highly variable for pseudomonads or LAB (see e.g. Augustin and Minvielle, 2008; Ghafir et al., 2008), both approaches were used.

Pseudomonas and LAB are considered the specific spoilage organisms (SSO) of meat during storage under aerobic conditions and in vacuum packs, respectively. The growth of pseudomonads at various alternative scenarios during chilling and transportation was calculated based on available predictive models (Figure 2).

To address ToRs 1 and 2, the alternative scenarios are compared with two baseline scenarios (mean and worst case, described below) for beef and pork and the worst-case scenario for lamb. Predicted growth is defined as the difference between the final concentration and a starting point equal to 1 CFU/cm^2. The time (h) required by pseudomonads to reach a spoilage level of 10^7 CFU/cm^2 was estimated for the same combinations of temperature and time conditions applied for pathogens adopted in the 'Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1 (meat of domestic ungulates) (EFSA BIOHAZ Panel, 2014a).

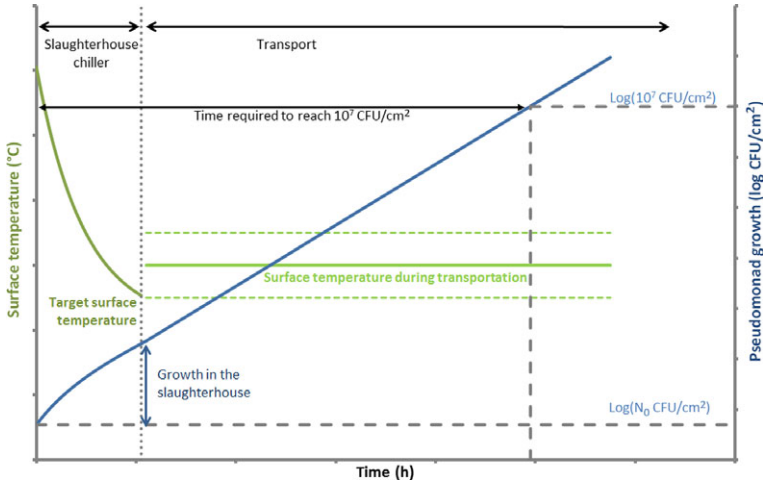

Figure 2: Pseudomonad growth was estimated for three different combinations of surface temperature (°C) and time (h)

In order to model the growth of pseudomonads, the same surface temperature–time baseline scenarios representing the current situation as those in the previous opinion (EFSA BIOHAZ Panel, 2014a) were applied. Using beef and pork carcass surface temperature data obtained in commercial slaughterhouse chillers, two chilling profiles were developed; a 'mean' profile calculated from the mean surface temperatures at various time points during carcass chilling and a 'worst-case' profile calculated using the highest temperatures recorded at each time point. As there was insufficient data available on the changes in surface temperature of lamb carcasses during chilling, it was not possible to obtain a 'mean profile' and, based on expert opinion, the chilling profile obtained was considered to be a 'worst-case' profile. The commercial chilling data also included data on the core temperature during carcass chilling, thus the time to reach 7°C, which marks the end of the chilling process, was obtained.

When modelling pseudomonad growth on carcasses during chilling in the slaughterhouse, the 'mean' and 'worst-case' temperature profiles were used for beef and pork, and the 'worst-case' profile for lamb. In order to model pseudomonad growth during the combination of chilling in the slaughterhouse and chilled storage during transport, two scenarios were developed;

1) The 'mean baseline scenario' (beef and pork) was the calculated mean surface temperature profile during chilling in the slaughterhouse when the carcasses were chilled to a core temperature of 7°C and transportation at a constant surface temperature of 4°C for 48 h.
2) The 'worst-case baseline scenario' (beef, pork and lamb) was the surface temperature profile composed of the highest temperature obtained at each time point during chilling in the slaughterhouse when the carcasses were chilled to a core temperature of 7°C and transportation at a constant surface temperature of 4°C for 48 h (Appendix A).

To address ToRs 3 and 4, the time (h) required by pseudomonads and LAB to reach a spoilage level of 10^7 CFU/cm^2 during storage was estimated for various temperature conditions and initial pseudomonads or LAB levels.

2.1. Models for growth of pseudomonads and lactic acid bacteria

Several secondary models for predicting growth rate of pseudomonads are available (e.g. Gill and Jones, 1992; Neumeyer et al., 1997; Pin and Baranyi, 1998; Dominguez and Schaffner, 2007). These models were established using growth rate data obtained in broth medium and/or meat. All of these models consider the effect of temperature. Growth of pseudomonads during carcass chilling and transportation was estimated using the ComBase model (available at www.combase.com). This model was selected because differences of predicted growth rates between models are limited (Baranyi et al., 1999; ANSES, 2010) and because the ComBase model includes the effect of pH and water activity.

Several secondary models for predicting growth rate of LAB are available (e.g. Devlieghere et al., 2000; Wijtzes et al., 2001; Mejlholm and Dalgaard, 2013). Growth of LAB was estimated using the Food Spoilage and Safety Predictor (FSSP™) v. 4.0 growth model (available at http://fssp.food.dtu.dk/). The FSSP™ is based on the Mejlholm and Dalgaard (2013) model for psychrotrophic LAB. The selection of this model was based on the fact that the most extensive validation has been carried out for this model.

The models and the assumed environmental conditions used in the growth predictions for pseudomonads and LAB are shown in Tables 3 and 4. The assumption of the lag phase absence, together with the assumed high a_w and pH, as well as no competition from other meat bacterial flora, represents conditions that are favourable for the growth of pseudomonads and LAB and most likely results in an overestimation of growth. However, as the approach used is based on the comparison of temperature scenarios, this is not expected to affect the results and conclusions.

Table 3: Characteristics of ComBase[(a)] growth model for pseudomonads

Model	Pseudomonads
Primary growth model	Baranyi and Roberts (1994)
Secondary growth model	Polynomial
Environmental parameters in model	Temperature, salt, a_w
Product validation studies	Unknown
Range of applicability	Temperature (0–20°C), a_w (0.961–1), pH (5.0–7.4)

a_w: water activity.
(a): www.combase.com

Table 4: Characteristics of FSSP™ v. 4.0[(a)] growth model for LAB

Model	LAB growth and growth boundary models
Primary growth model	Logistic model with delay
Secondary growth model	Simplified cardinal parameter type model
Environmental parameters in model	Temperature, atmosphere (CO_2), water phase salt/a_w, pH, smoke components/phenol, nitrite and organic acids in water phase of product (acetic acids, benzoic acid, citric acid, diacetate, lactic acid and sorbic acid)

Model	LAB growth and growth boundary models
Product validation studies	The model has been extensively validated using data from fresh and processed seafood and meat products (Mejlholm and Dalgaard, 2007, 2013, 2015)
Range of applicability	Temperature (0–25°C), atmosphere (0–100% CO_2), water phase salt (0.1–6.4%), pH (5.3–7.7), smoke components/phenol (0–21.2 ppm), nitrite (0–209 ppm in product), acetic acid (0–12,600 ppm in water phase), benzoic acid (0–1,800 ppm in water phase), citric acid (0–7,300 ppm in water phase), diacetate (0–3,000 ppm in water phase), lactic acid (0–67,000 ppm in water phase) and sorbic acid (0–1,600 ppm in water phase)

FSSP: Food Spoilage and Safety Predictor; LAB: lactic acid bacteria; a_w: water activity.
(a): http://fssp.food.dtu.dk/

The above models were used to predict growth of pseudomonads and LAB assuming a worst-case scenario based on growth without lag phase and optimum conditions for growth for meat characteristics, i.e. pH = 6.5, a_w = 0.993 or water phase salt (WPS) = 1. The shelf-life of meat (time to spoilage) was estimated as the time required by pseudomonads or LAB to reach a spoilage level of 10^7 CFU/g. For ToRs 3 and 4, the growth of pseudomonads on poultry was estimated using the parameters pH 6.5 and a_w 0.993, and on red meat, a pH of 5.7 and an a_w of 0.98.

The outputs of these modelling exercises are presented as summarised in Table 5. Tables 6–10 present the predicted growth of pseudomonads on beef, pork and lamb carcasses during chilling in the abattoir immediately after slaughter and dressing. Tables 11–37 present the predicted growth of pseudomonads on beef, pork and lamb carcasses during the combination of chilling in the abattoir and chilled transportation. Tables 38–43 model the growth of pseudomonads or LAB on red meat and/or poultry stored at a range of temperatures for up to 12 days.

The format for the tables predicting the effect of the combination of time during chilling (to a specific target carcass surface temperature) and transport on bacterial count has been changed to that used in the opinion covering the growth of pathogenic bacteria. Instead of predicting the time required to reach a given bacterial concentration equivalent to that which would be achieved if the carcass was chilled to a core temperature of 7°C and transported at that temperature, the format has been changed to predict the time required to reach 10^7 pseudomonads/cm^2 (end of shelf-life). This was done to directly address the ToRs in each opinion. Thus, the revised format, with shelf-life as the key metric, was considered to be more appropriate for addressing the ToRs in this opinion.

Table 5: An overview of the predictive modelling performed

Table	Assumed initial count	Carcass/ meat	Chilling profile	Temperature		Objective
				Target surface temperature during chilling	During transportation	
Predicted growth of pseudomonads on carcasses during chilling in the abattoir						
6	1 CFU/cm^2	Beef	M	1–10°C	NA	To compare the predicted growth with that which would be achieved if the carcass was chilled to a core of 7°C (as per Reg. (EC) No 853/2004)
7	1 CFU/cm^2	Beef	WC	1–10°C	NA	
8	1 CFU/cm^2	Pork	M	1–10°C	NA	
9	1 CFU/cm^2	Pork	WC	1–10°C	NA	
10	1 CFU/cm^2	Lamb	WC	1–10°C	NA	
Predicted growth of pseudomonads during transportation in a refrigerated vehicle						
11	1 CFU/cm^2	Beef, pork and lamb	NA	NA	5–10°C	To predict the growth of pseudomonads during transportation
12	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	1°C	0–2°C	To predict the time (h) until the pseudomonad count reaches 10^7 CFU/cm^2 (end of shelf-life)
13	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	2°C	1–3°C	
14	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	3°C	2–4°C	
15	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	4°C	3–5°C	
16	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	5°C	4–6°C	
17	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	6°C	5–7°C	

Table	Assumed initial count	Carcass/ meat	Chilling profile	Temperature		Objective
				Target surface temperature during chilling	During transportation	
18	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	7°C	6–8°C	
19	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	8°C	7–9°C	
20	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	9°C	8–10°C	
21	1–5 $\log_{10}$ CFU/cm^2	Beef	M & WC	10°C	9–11°C	
22–27	1–5 $\log_{10}$ CFU/cm^2	Pork	M & WC	5–10°C[(a)] (done separately as for beef carcasses, above)	4–11°C (the target carcass surface temperature ± 1°C as for beef, above)	
28–37	1–5 $\log_{10}$ CFU/cm^2	Lamb	WC	1–10°C (done separately as for beef carcasses, above)	0–11°C (the target carcass surface temperature ± 1°C as for beef, above)	
Predicted growth of pseudomonads and LAB on red meat and poultry stored under chilled conditions (and intended for used in ground meat/meat preparations)						
38	1 CFU/cm^2	Red meat (beef, pork or lamb)	NA	Stored at 1–7°C		To estimate pseudomonad growth during storage of 1–12 days
39	1 CFU/cm^2	Poultry	NA	Stored at 1–7°C		To estimate pseudomonad growth during storage of 1–12 days
40	1 CFU/cm^2	Red meat (beef, pork or lamb)	NA	Stored at 1–7°C		To estimate LAB growth during storage of 1–12 days
41	1–5 $\log_{10}$ CFU/cm^2	Red meat (beef, pork or lamb)	NA	Stored at 1–7°C		To predict the effect of storage temperature and initial count on the time (h) until the pseudomonad count reaches 10^7 CFU/cm^2 (end of shelf-life)
42	1–5 $\log_{10}$ CFU/cm^2	Poultry	NA	Stored at 1–7°C		
43	1–5 $\log_{10}$ CFU/cm^2	Red meat (beef, pork or lamb)	NA	Stored at 1–7°C		To predict the effect of storage temperature and initial count on the time (h) until the LAB count reaches 10^7 CFU/cm^2 (end of shelf-life)

CFU: colony forming unit; LAB: lactic acid bacteria; M: mean chilling profile; WC: worst-case chilling profile; NA: not applicable.
(a): Lower temperatures were not modelled as the surface temperature did not reach these values during chilling (see Appendix A, Figures A.1–A.3 for details).

3. Assessment

3.1. Answers to Terms of Reference 1 and 2

3.1.1. Growth of pseudomonads during carcass chilling in the slaughterhouse

The growth (expressed as the difference between the final concentration and a starting point of 1 CFU/cm^2) of pseudomonads on beef, pork and lamb carcasses was predicted using the mean and the worst-case temperature chilling profiles for final target surface temperatures of 1–10°C and

compared with that which would be achieved if the carcasses were chilled to a core temperature target of 7°C, as required by the Regulation (EC) No 854/2004. The results are shown in Table 6 (beef carcasses and mean chilling profile), Table 7 (beef carcasses and worst-case chilling profile), Table 8 (pork carcasses and mean chilling profile), Table 9 (pork carcasses and worst-case chilling profile) and Table 10 (lamb carcasses and worst-case chilling profile).

The predicted pseudomonad counts using the mean chilling temperature profile on beef carcasses, chilled to a target surface temperature of 1–10°C, were less than that which would be achieved if the carcasses were chilled to a core temperature of 7°C (Table 6). Substituting the worst-case chilling profiles for the mean chilling profile suggested that equivalent or less growth would only be achieved when the carcasses were chilled to a target surface temperature of 4–10°C (Table 7). Modelling the growth of pseudomonads on pork carcasses could only be done for surface target temperatures of 4–10°C (mean chilling profile) and 7–10°C (worst-case chilling profile) as the surface temperatures on the respective chilling curves never reached the lower temperatures. The predicted growth obtained suggests that target surface temperatures of 6–10°C (mean chilling profile, Table 8) and 7–10°C (worst-case chilling profile, Table 9) would yield lower pseudomonad counts as compared to chilling to a core temperature of 7°C. The predicted pseudomonad counts using the worst-case chilling temperature profile on lamb carcasses, chilled to a target surface temperature of 4–10°C, were less than that which would be achieved if the carcasses were chilled to a core temperature of 7°C (Table 10).

3.1.1.1. Results for beef carcasses

Table 6: Predicted growth (log CFU/cm^2) of pseudomonads during beef carcass chilling based on the calculated mean temperature chilling profile. Assuming an initial pseudomonad count of 1 CFU/cm^2, the growth of pseudomonads on the surface of a beef carcass chilled to a core temperature of 7°C is compared with that which would be achieved if the carcass was chilled to target surface temperatures of 1–10°C. The times to achieve each of these target temperatures are also given

Chilling temperature limit		**Growth (log CFU/cm^2)**
Bacterial growth on the carcass when chilled to a core temperature of 7°C		**2.18**
Surface temperature (°C)	**Time in chiller to reach target temperature (h)**	
1	18.8	1.93
2	14.8	1.78
3	12.5	1.69
4	10.9	1.61
5	9.7	1.55
6	8.5	1.47
7	7.7	1.41
8	6.8	1.35
9	6.2	1.29
10	5.6	1.22

CFU: colony forming unit.

Table 7: Predicted growth (log CFU/cm^2) of pseudomonads during beef carcass chilling based on the worst-case temperature chilling profile. Assuming an initial pseudomonad count of 1 CFU/cm^2, the growth of pseudomonads on the surface of a beef carcass chilled to a core temperature of 7°C is compared with that which would be achieved if the carcass was chilled to target surface temperatures of 1–10°C. The times to achieve each of these target temperatures are also given

Chilling temperature limit		**Growth (log CFU/cm^2)**
Bacterial growth on the carcass when chilled to a core temperature of 7°C		**3.99**
Surface temperature (°C)	**Time in chiller to reach target temperature (h)**	
1	46.5	4.77
2	37.0	4.42
3	31.0	4.17
4	27	3.98
5	23.8	3.80
6	21.2	3.64
7	19.0	3.48
8	17.0	3.32
9	15.2	3.17
10	13.8	3.02

CFU: colony forming unit.

3.1.1.2. Results for pork carcasses

Table 8: Predicted growth (log CFU/cm^2) of pseudomonads during pork carcass chilling based on the calculated mean temperature chilling profile. Assuming an initial pseudomonad count of 1 CFU/cm^2, the growth of pseudomonads on the surface of a pork carcass chilled to a core temperature of 7°C is compared with that which would be achieved if the carcass was chilled to target surface temperatures of 1–10°C. The times to achieve each of these target temperatures are also given

Chilling temperature limit		**Growth (log CFU/cm^2)**
Bacterial growth on the carcass when chilled to a core temperature of 7°C		**1.59**
Surface temperature (°C)	**Time in chiller to reach target temperature (h)**	
1	–[a]	–
2	–	–
3	–	–
4	22.5	1.81
5	21.2	1.71
6	14.2	1.27
7	9.8	0.95
8	8.0	0.73
9	4.8	0.53
10	3.0	0.36

CFU: colony forming unit.
(a): Surface temperature never reached this value, therefore no growth estimate is provided.

It was noted that the time required to chill the pork carcasses to a surface target temperature of 5°C (21.2 h) was longer than the time required to achieve a core temperature of 7°C (19.2 h) as required by Regulation (EC) No 853/2004. Furthermore, the predicted growth exceeded that which would have been achieved by chilling of a core temperature of 7°C.

Table 9: Predicted growth (log CFU/cm^2) of pseudomonads during pork carcass chilling based on the calculated worst-case temperature chilling profile. Assuming an initial pseudomonad count of 1 CFU/cm^2, the growth of pseudomonads on the surface of a pork carcass chilled to a core temperature of 7°C is compared with that which would be achieved if the carcass was chilled to target surface temperatures of 7–10°C. The times to achieve each of these target temperatures are also given. It was not possible to model the growth for target carcass surface temperatures of 1–6°C (inclusive) as the chilling profiles used (worst case) never reached these temperatures

Chilling temperature limit		**Growth (log CFU/cm^2)**
Bacterial growth on the carcass when chilled to a core temperature of 7°C		**3.11**
Surface temperature (°C)	**Time in chiller to reach target temperature (h)**	
1	–[(a)]	–
2	–	–
3	–	–
4	–	–
5	–	–
6	–	–
7	26.2	3.00
8	18.0	2.37
9	14.0	2.01
10	11.0	1.71

CFU: colony forming unit.
(a): Surface temperature never reached this value, therefore no growth estimate is provided.

3.1.1.3. Results for lamb carcasses

Table 10: Predicted growth (log CFU/cm^2) of pseudomonads during lamb carcass chilling based on the calculated worst-case temperature chilling profile. Assuming an initial pseudomonad count of 1 CFU/cm^2, the growth of pseudomonads on the surface of a lamb carcass chilled to a core temperature of 7°C is compared with that which would be achieved if the carcass was chilled to target surface temperatures of 1–10°C. The times to achieve each of these target temperatures are also given

Chilling temperature limit		**Growth (log CFU/cm^2)**
Bacterial growth on the carcass when chilled to a core temperature of 7°C		**3.11**
Surface temperature (°C)	**Time in chiller to reach target temperature (h)**	
1	35.5	3.68
2	28.5	3.42
3	23.8	3.23
4	20.8	3.08
5	18.2	2.95
6	16.2	2.82
7	14.5	2.70
8	13.0	2.58
9	11.8	2.47
10	10.5	2.34

CFU: colony forming unit.

3.1.2. Growth of pseudomonads on the carcasses during chilling in the slaughterhouse and transportation

The chill chain for beef, pork and lamb carcasses starts with carcass chilling in the slaughterhouse and continues through to the boning hall and into the chilled storage of meat primals (cuts). Transportation of the chilled carcasses may occur between carcass chilling in the slaughterhouse and the boning operations. The growth of pseudomonads on the carcasses during chilling was modelled in

the previous section (Section 3.1.1). In this section, the growth of pseudomonads on the carcasses during chilling was extended to include growth during transportation. At the specific request of the European Commission, the range of target surface temperatures was extended to include 1–10°C (inclusive), where possible, with transport temperatures of ± 1°C either side of the target temperature (e.g. chilling to a target surface temperature of 4°C and transport at 3, 4 and 5°C).

The predicted growth of pseudomonads on carcasses during transportation 1–10°C (inclusive) for 1–48 h is shown in Table 11, assuming the initial count was 1 CFU/cm^2. However, in the real world, the initial pseudomonad count on carcasses is likely to be higher than 1 CFU/cm^2. In order to model pseudomonad growth from entry into the chiller in the slaughterhouse from *post-mortem* inspection to the end of transportation (i.e. during the combination of chilling in the slaughter plant and during transportation) growth was predicted for a range of different scenarios. These included chilling the carcasses to a surface temperature of 1–10°C (inclusive) using both the mean and/or worst-case temperature profiles and combining these with transport of 1°C either side of the target surface temperature, assuming the initial carcass count was 1–5 $\log_{10}$ CFU/cm^2 (inclusive). The results, expressed as the time required to reach 10^7 CFU/cm^2, are shown for beef using the 'mean chilling' and 'worst-case baseline' as follows: Table 12, chilled to a surface temperature of 1°C and transported at 0, 1 and 2°C; Table 13, chilled to a surface temperature of 2°C and transported at 1, 2 and 3°C; Table 14, chilled to a surface temperature of 3°C and transported at 2, 3 and 4°C; Table 15, chilled to a surface temperature of 4°C and transported at 3, 4 and 5°C; Table 16, chilled to a surface temperature of 5°C and transported at 4, 5 and 6°C; Table 17, chilled to a surface temperature of 6°C and transported at 5, 6 and 7°C; Table 18, chilled to a surface temperature of 7°C and transported at 6, 7 and 8°C; Table 19, chilled to a surface temperature of 8°C and transported at 7, 8 and 9°C; Table 20, chilled to a surface temperature of 9°C and transported at 8, 9 and 10°C and Table 21, chilled to a surface temperature of 10°C and transported at 9, 10 and 11°C. Tables 22–27 are as for beef (above) but for pork while Tables 28–37 present the predicted growth for pseudomonads on lamb using the 'worst-case chilling profile' and the same temperature combination as presented above for beef.

While it is not practical to describe every result a few examples will be provided. Predicted pseudomonad growth ranged from 0.04 $\log_{10}$ CFU/cm^2 during transportation at 1°C for 1 h to 5.15 $\log_{10}$ CFU/cm^2 when transported at 10°C for 48 h (Table 11). The predicted time for pseudomonads to reach 10^7 CFU/cm^2 on beef carcasses was 135.7 h using the mean chilling profile and 41.7 h using the worst-case chilling profile, when the initial count was 1 $\log_{10}$ CFU/cm^2, the target surface temperature during chilling was 1°C and the carcasses were transported at 0°C (Table 12). Increasing the carcass chilling target surface temperature to 2°C and transporting at 1°C changed the predicted times to achieved 10^7 CFU/cm^2 to 120.6 and 45.1 h, respectively (Table 13). While it might have been expected that chilling to and transporting at higher temperatures would result in higher growth and a reduced time to achieve 10^7 CFU/cm^2, this is not always the case as, especially with the worst-case chilling profile, the longer times required in the slaughterhouse to achieve the lower target temperature results in higher predicted growth and thus shorter shelf-life.

The predicted time for pseudomonads to reach 10^7 CFU/cm^2 on pork carcasses was 75.4 h using the mean chilling profile and 44.8 h using the worst-case chilling profile, when the initial count was 1 $\log_{10}$ CFU/cm^2, the target surface temperature during chilling was 7°C and the carcasses were transported at 6°C (Table 24).

The predicted time for pseudomonads to reach 10^7 CFU/cm^2 on lamb carcasses (worst-case chilling profile only) when the initial count was 1 $\log_{10}$ CFU/cm^2 was 77.3 h when the target surface temperature during chilling was 1°C and the carcasses were transported at 0°C (Table 28), 73.7 h when the target surface temperature during chilling was 2°C and the carcasses were transported at 1°C (Table 29) and 69.3 h when the target surface temperature during chilling was 2°C and the carcasses were transported at 2°C (Table 30).

Table 11: Predicted growth (increase in the concentration) of pseudomonads during transportation only for up to 48 h, assuming the initial count was 1 CFU/cm^2 upon entry into the chilled lorry

Time (h)	Temperature (°C)									
	1	2	3	4	5	6	7	8	9	10
	log CFU/cm^2									
1	0.04	0.04	0.05	0.05	0.06	0.07	0.08	0.09	0.10	0.11
2	0.07	0.08	0.09	0.10	0.12	0.13	0.15	0.17	0.19	0.21
3	0.11	0.12	0.14	0.16	0.18	0.20	0.23	0.26	0.29	0.32
6	0.21	0.24	0.28	0.31	0.35	0.40	0.45	0.51	0.57	0.64
12	0.42	0.48	0.55	0.62	0.71	0.80	0.91	1.02	1.15	1.29
24	0.84	0.96	1.10	1.25	1.42	1.61	1.82	2.05	2.30	2.58
48	1.68	1.92	2.21	2.50	2.84	3.21	3.63	4.09	4.60	5.15

CFU: colony forming unit.

3.1.2.1. Time required by pseudomonads to reach 10^7 CFU/g (h) at various alternative scenarios

Results for beef carcasses

Table 12: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcasses chilled to a target surface temperature of 1°C and transported at 0, 1 and 2°C using different initial counts (1–5 log$_{10}$ CFU/cm^2) and the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level (log$_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	0	135.7	102.3	69.0	35.7	2.3
	1	116.3	87.7	59.1	30.6	2.0
	2	101.8	76.8	51.8	26.8	1.8
Worst baseline scenario	0	41.7	8.3	NA[(a)]	NA	NA
	1	35.7	7.1	NA	NA	NA
	2	31.3	6.3	NA	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 log$_{10}$ CFU/cm^2 or higher.

Table 13: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 2°C and transported at 1, 2 and 3°C using different initial counts (1–5 log$_{10}$ CFU/cm^2) and the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level (log$_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	1	120.6	92.0	63.4	34.9	6.3
	2	105.5	80.5	55.5	30.5	5.5
	3	91.7	70.0	48.3	26.5	4.8
Worst baseline scenario	1	45.1	16.6	NA[(a)]	NA	NA
	2	39.5	14.5	NA	NA	NA
	3	34.3	12.6	NA	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 log$_{10}$ CFU/cm^2 or higher.

Table 14: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 3°C and transported at 2, 3 and 4°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	2	107.8	82.8	57.8	32.8	7.8
	3	93.7	72.0	50.2	28.5	6.7
	4	82.9	63.7	44.4	25.2	6.0
Worst baseline scenario	2	45.8	20.8	NA(a)	NA	NA
	3	39.8	18.0	NA	NA	NA
	4	35.2	16.0	NA	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Table 15: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 4°C and transported at 3, 4 and 5°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	3	95.7	73.9	52.2	30.4	8.7
	4	84.6	65.4	46.2	26.9	7.7
	5	74.6	57.6	40.7	23.7	6.8
Worst baseline scenario	3	43.9	22.2	0.4	NA(a)	NA
	4	38.8	19.6	0.4	NA	NA
	5	34.2	17.3	0.3	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Table 16: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 5°C and transported at 4, 5 and 6°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	4	85.8	66.5	47.3	28.1	8.8
	5	75.4	58.5	41.5	24.6	7.6
	6	66.4	51.5	36.6	21.6	6.7
Worst baseline scenario	4	42.3	23.1	3.8	NA(a)	NA
	5	37.3	20.3	3.4	NA	NA
	6	32.8	17.9	3.0	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Table 17: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 6°C and transported at 5, 6 and 7°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	5	76.8	59.8	42.9	25.9	9.0
	6	67.6	52.7	37.8	22.8	7.9
	7	59.6	46.4	33.3	20.1	7.0
Worst baseline scenario	5	40.0	23.1	6.1	NA[(a)]	NA
	6	35.2	20.3	5.4	NA	NA
	7	31.1	17.9	4.7	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Table 18: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 7°C and transported at 6, 7 and 8°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	6	68.5	53.6	38.7	23.7	8.8
	7	60.4	47.2	34.1	20.9	7.8
	8	53.8	42.1	30.4	18.6	6.9
Worst baseline scenario	6	37.6	22.7	7.8	NA[(a)]	NA
	7	33.2	20.0	6.8	NA	NA
	8	29.5	17.8	6.1	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Table 19: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 8°C and transported at 7, 8 and 9°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	7	61.2	48.0	34.9	21.7	8.6
	8	54.5	42.8	31.1	19.3	7.6
	9	48.5	38.1	27.7	17.2	6.8
Worst baseline scenario	7	35.3	22.1	8.9	NA[(a)]	NA
	8	31.4	19.7	8.0	NA	NA
	9	28.0	17.5	7.1	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Table 20: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 9°C and transported at 8, 9 and 10°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	8	55.2	43.5	31.8	20.0	8.3
	9	49.2	38.7	28.3	17.8	7.4
	10	43.9	34.6	25.3	15.9	6.6
Worst baseline scenario	8	33.2	21.5	9.7	NA(a)	NA
	9	29.5	19.1	8.7	NA	NA
	10	26.4	17.1	7.7	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Table 21: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on beef carcass chilled to a target surface temperature of 10°C and transported at 9, 10 and 11°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	9	49.8	39.4	28.9	18.5	8.0
	10	44.5	35.1	25.8	16.5	7.2
	11	39.7	31.4	23.0	14.7	6.3
Worst baseline scenario	9	31.1	20.7	10.2	NA(a)	NA
	10	27.8	18.5	9.1	NA	NA
	11	24.9	16.5	8.2	NA	NA

CFU: colony forming unit; NA: not applicable.
(a): It was noted that using the worst-case chilling profile the pseudomonad concentration reached or exceeded 10^7 CFU/cm^2 during chilling in the abattoir (before transportation) when the initial contamination levels were 3 $\log_{10}$ CFU/cm^2 or higher.

Results for pork carcasses

Table 22: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on pork carcass chilled to a target surface temperature of 5°C and transported at 4, 5 and 6°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	4(a)	–	–	–	–	–
	5	72.7	55.8	38.8	21.9	4.9
	6	64.0	49.1	34.2	19.3	4.3

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level (log_{10} CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	4[(a)]	–	–	–	–	–
	5[(a)]	–	–	–	–	–
	6[(a)]	–	–	–	–	–

CFU: colony forming unit.
(a): Surface temperature does not reach this value during chilling.

Table 23: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on pork carcass chilled to a target surface temperature of 6°C and transported at 5, 6 and 7°C using different initial counts (1–5 log_{10} CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level (log_{10} CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	5	80.2	63.2	46.3	29.3	12.4
	6	70.6	55.7	40.7	25.8	10.9
	7	62.2	49.1	35.9	22.8	9.6
Worst baseline scenario	5[(a)]	–	–	–	–	–
	6[(a)]	–	–	–	–	–
	7[(a)]	–	–	–	–	–

CFU: colony forming unit.
(a): Surface temperature does not reach this value during chilling.

Table 24: Predicted time (h) required by pseudomonads to reach 10^7 CFU cm^{-2} on pork carcass chilled to a target surface temperature of 7°C and transported at 6, 7 and 8°C using different initial counts (1–5 log_{10} CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level (log_{10} CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	6	75.4	60.4	45.5	30.6	15.7
	7	66.4	53.3	40.1	27.0	13.8
	8	59.2	47.5	35.8	24.0	12.3
Worst baseline scenario	6	44.8	29.9	14.9	0[(a)]	0[(a)]
	7	39.5	26.3	13.2	0[(a)]	0[(a)]
	8	35.5	23.8	12.1	0.4	0[(a)]

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 25: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on pork carcass chilled to a target surface temperature of 8°C and transported at 7, 8 and 9°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	7	69.3	56.2	43.0	29.9	16.7
	8	61.8	50.1	38.3	26.6	14.9
	9	55.0	44.6	34.1	23.7	13.3
Worst baseline scenario	7	47.8	34.6	21.4	8.3	0 (a)
	8	42.6	30.8	19.1	7.4	0
	9	37.9	27.5	17.0	6.6	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 26: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on pork carcass chilled to a target surface temperature of 9°C and transported at 8, 9 and 10°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	8	64.1	52.4	40.7	29.0	17.2
	9	57.1	46.7	36.2	25.8	15.3
	10	51.0	41.7	32.3	23.0	13.7
Worst baseline scenario	8	46.8	35.1	23.3	11.6	0(a)
	9	41.6	31.2	20.8	10.3	0
	10	37.2	27.9	18.5	9.2	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 27: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on pork carcass chilled to a target surface temperature of 10°C and transported at 9, 10 and 11°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Mean baseline scenario	9	58.9	48.4	38.0	27.6	17.1
	10	52.6	43.2	33.9	24.6	15.3
	11	46.8	38.5	30.1	21.8	13.4
Worst baseline scenario	9	44.8	34.3	23.9	13.5	0(a)
	10	40.0	30.7	21.3	12.0	2.7
	11	35.8	27.5	19.1	10.8	2.4

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Results for lamb carcasses

Table 28: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 1°C and transported at 0, 1 and 2°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	0	77.3	44.0	10.7	0[(a)]	0
	1	66.3	37.7	9.1	0	0
	2	58.0	33.0	8.0	0	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 29: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 2°C and transported at 1, 2 and 3°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	1	73.7	45.1	16.6	0[(a)]	0
	2	64.5	39.5	14.5	0	0
	3	56.1	34.3	12.6	0	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 30: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 3°C and transported at 2, 3 and 4°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	2	69.3	44.3	19.3	0[(a)]	0
	3	60.2	38.5	16.7	0	0
	4	53.3	34.0	14.8	0	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 31: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 4°C and transported at 3, 4 and 5°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	3	63.5	41.7	20.0	0[(a)]	0
	4	56.2	36.9	17.7	0	0

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
	5	49.5	32.5	15.6	0	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 32: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 5°C and transported at 4, 5 and 6°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	4	58.7	39.4	20.2	1.0	0[(a)]
	5	51.7	34.7	17.8	0.8	0
	6	45.5	30.6	15.7	0.7	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 33: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 6°C and transported at 5, 6 and 7°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	5	53.9	36.9	20.0	3.1	0[(a)]
	6	47.5	32.5	17.6	2.7	0
	7	41.8	28.7	15.5	2.4	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 34: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 7°C and transported at 6, 7 and 8°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	6	49.3	34.3	19.4	4.5	0[(a)]
	7	43.4	30.3	17.1	3.9	0
	8	38.7	27.0	15.2	3.5	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 35: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 8°C and transported at 7, 8 and 9°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	7	45.0	31.8	18.7	5.5	0[(a)]
	8	40.1	28.4	16.6	4.9	0
	9	35.7	25.3	14.8	4.4	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 36: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 9°C and transported at 8, 9 and 10°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	8	41.4	29.7	17.9	6.2	0[(a)]
	9	36.8	26.4	16.0	5.5	0
	10	32.9	23.6	14.3	4.9	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

Table 37: Predicted time (h) required by pseudomonads to reach 10^7 CFU/cm^2 on lamb carcass chilled to a target surface temperature of 10°C and transported at 9, 10 and 11°C using different initial counts (1–5 $\log_{10}$ CFU/cm^2) and using the mean and worst-case chilling profiles

Scenario	Carcass transport temperature (°C)	Time required by pseudomonads to reach 10^7 CFU/cm^2 (h) during transportation				
		Initial pseudomonad level ($\log_{10}$ CFU/cm^2)				
		1	2	3	4	5
Worst baseline scenario	9	38.2	27.8	17.3	6.9	0[(a)]
	10	34.1	24.8	15.5	6.2	0
	11	30.5	22.1	13.8	5.4	0

CFU: colony forming unit.
(a): Pseudomonad concentration reached 10^7 CFU/cm^2 before transportation.

3.2. Answers to Terms of Reference 3 and 4

In order to investigate the growth of spoilage bacteria during storage of meat intended for the production of minced meat or meat preparations, the growth of pseudomonads (aerobic storage) was modelled at temperatures of 1–7°C using pH 6.0 and an a_w of 0.993 for poultry, and a pH of 5.7 and an a_w of 0.975–0.98 for red meat. The results are presented as the predicted growth ($\log_{10}$ CFU/cm^2) of pseudomonads on red meat (Table 38) and poultry (Table 39) stored aerobically at 1–7°C for 1–12 days assuming the initial concentration was 1 CFU/cm^2; the predicted growth ($\log_{10}$ CFU/cm^2) of LAB on red meat stored anaerobically at 1–7°C for 1–12 days assuming the initial concentration was 1 CFU/cm^2 (Table 40); the effect of storage temperature and initial concentration on the time (days) required to reach 10^7 CFU/cm^2 pseudomonads on red meat (Table 41) and poultry (Table 42) stored aerobically for a range of initial concentrations (1–5 $\log_{10}$ CFU/cm^2) and finally the effect of storage temperature and initial concentration on the time (days) required to reach 10^7 CFU/cm^2 LAB on red meat stored anaerobically, for a range of initial concentrations (1–5 $\log_{10}$ CFU/cm^2) (Table 42).

The results suggest, for example, that pseudomonad growth on red meat ranges from 0.4 log_{10} CFU/cm^2 (stored aerobically at 1°C for 1 day) to 9.5 log_{10} CFU/cm^2 (stored aerobically at 7°C for 12 days, assuming the initial concentration was 1 CFU/cm^2 (Table 38). The equivalent values on poultry were 0.8 log_{10} CFU/cm^2 and 9.5 log_{10} CFU/cm^2, respectively (Table 39). Predicted LAB growth (under anaerobic conditions) was lower, with the equivalent values of 0.2 log_{10} CFU/cm^2 and 7.8 log_{10} CFU/cm^2, respectively (Table 40). The time required for pseudomonads to reach 10^7 CFU/cm^2 on red meat was 15 days when stored at 1°C and the initial count was 1 log_{10} CFU/cm^2 (Table 41) and 7.1 days on poultry (Table 42). The equivalent time for LAB on red meat stored anaerobically was 35.5 days (Table 43).

Table 38: The predicted growth (log_{10} CFU/cm^2) of pseudomonads in red meat stored aerobically at different temperatures and times (days), assuming the initial concentration was 1 CFU/cm^2

Growth potential (log_{10} CFU/cm^2)							
Time (days)	Storage temperature (°C)						
	1	2	3	4	5	6	7
1	0.4	0.5	0.5	0.6	0.7	0.8	0.9
2	0.8	0.9	1.1	1.2	1.4	1.5	1.7
3	1.2	1.4	1.6	1.8	2.0	2.3	2.6
4	1.6	1.8	2.1	2.4	2.7	3.1	3.5
5	2.0	2.3	2.6	3.0	3.4	3.9	4.4
6	2.4	2.8	3.2	3.6	4.1	4.6	5.2
7	2.8	3.2	3.7	4.2	4.8	5.4	6.1
8	3.2	3.7	4.2	4.8	5.5	6.2	7.0
9	3.6	4.1	4.7	5.4	6.1	7.0	7.9
10	4.0	4.6	5.3	6.0	6.8	7.7	8.7
11	4.4	5.1	5.8	6.6	7.5	8.5	9.5
12	4.8	5.5	6.3	7.2	8.2	9.3	9.5

CFU: colony forming unit.

Table 39: The predicted growth (log_{10} CFU/cm^2) of pseudomonads in poultry stored aerobically at different temperatures and times (days), assuming the initial concentration was 1 CFU/cm^2

Growth potential (log_{10} CFU/cm^2)							
Time (days)	Storage temperature (°C)						
	1	2	3	4	5	6	7
1	0.8	1.0	1.1	1.2	1.4	1.6	1.8
2	1.7	1.9	2.2	2.5	2.8	3.2	3.6
3	2.5	2.9	3.3	3.7	4.2	4.8	5.5
4	3.4	3.8	4.4	5.0	5.7	6.4	7.3
5	4.2	4.8	5.5	6.2	7.1	8.0	9.1
6	5.0	5.8	6.6	7.5	8.5	9.5	9.5
7	5.9	6.7	7.7	8.7	9.5	9.5	9.5
8	6.7	7.7	8.8	9.5	9.5	9.5	9.5
9	7.6	8.6	9.5	9.5	9.5	9.5	9.5
10	8.4	9.5	9.5	9.5	9.5	9.5	9.5
11	9.2	9.5	9.5	9.5	9.5	9.5	9.5
12	9.5	9.5	9.5	9.5	9.5	9.5	9.5

CFU: colony forming unit.

Table 40: The predicted growth (log_{10} CFU/cm^2) of LAB in red meat stored anaerobically at different temperatures and times (days), assuming the initial concentration was 1 CFU/cm^2

Growth potential (log_{10} CFU/cm^2)							
Time (days)	Storage temperature (°C)						
	1	2	3	4	5	6	7
1	0.2	0.2	0.3	0.4	0.5	0.5	0.7
2	0.3	0.5	0.6	0.7	0.9	1.1	1.3
3	0.5	0.7	0.9	1.1	1.4	1.6	2.0
4	0.7	0.9	1.2	1.5	1.8	2.2	2.6
5	0.8	1.1	1.5	1.9	2.3	2.7	3.3
6	1.0	1.4	1.8	2.2	2.7	3.3	3.9
7	1.2	1.6	2.1	2.6	3.2	3.8	4.6
8	1.4	1.8	2.4	3.0	3.6	4.4	5.2
9	1.5	2.1	2.7	3.3	4.1	4.9	5.9
10	1.7	2.3	2.9	3.7	4.6	5.5	6.5
11	1.9	2.5	3.2	4.1	5.0	6.0	7.2
12	2.0	2.7	3.5	4.5	5.5	6.6	7.8

CFU: colony forming unit; LAB: lactic acid bacteria.

Table 41: Effect of storage temperature and initial microbial load on the time (days) required by pseudomonads to reach a level of 10^7 CFU/cm^2 on red meat stored aerobically

Time required by pseudomonads to reach 10^7 CFU/cm^2 (days)					
Storage temperature (°C)	Initial pseudomonad level (log_{10} CFU/cm^2)				
	1	2	3	4	5
1	15.0	12.5	10.0	7.5	5.0
2	13.1	10.9	8.7	6.5	4.4
3	11.4	9.5	7.6	5.7	3.8
4	10.0	8.3	6.7	5.0	3.3
5	8.8	7.3	5.9	4.4	2.9
6	7.8	6.5	5.2	3.9	2.6
7	6.9	5.7	4.6	3.4	2.3

CFU: colony forming unit.

Table 42: The effect of storage temperature and initial microbial load on the time (days) required by pseudomonads to reach a level of 10^7 CFU/cm^2 on poultry stored aerobically

Time required by pseudomonads to reach 10^7 CFU/cm^2 (days)					
Storage temperature (°C)	Initial pseudomonad level (log_{10} CFU/cm^2)				
	1	2	3	4	5
1	7.1	6.0	4.8	3.6	2.4
2	6.3	5.2	4.2	3.1	2.1
3	5.4	4.5	3.6	2.7	1.8
4	4.8	4.0	3.2	2.4	1.6
5	4.2	3.5	2.8	2.1	1.4
6	3.7	3.1	2.5	1.9	1.2
7	3.3	2.7	2.2	1.6	1.1

CFU: colony forming unit.

Table 43: The effect of storage temperature and initial microbial load on the time (days) required by LAB to reach a level of 10^7 CFU/cm^2 on red meat stored anaerobically

Time required by LAB to reach 10^7 CFU/cm^2 (days)					
Storage temperature (°C)	Initial LAB level ($\log_{10}$ CFU/cm^2)				
	1	2	3	4	5
1	35.5	29.6	23.7	17.8	11.8
2	26.3	21.9	17.5	13.1	8.8
3	20.3	17.0	13.6	10.2	6.8
4	16.2	13.5	10.8	8.1	5.4
5	13.2	11.0	8.8	6.6	4.4
6	10.9	9.1	7.3	5.5	3.6
7	9.2	7.7	6.2	4.6	3.1

CFU: colony forming unit; LAB: lactic acid bacteria.

4. Conclusions

- Based on current knowledge, pseudomonads were considered to be the main spoilage organisms on meat stored under aerobic conditions, while LAB are primarily responsible for spoilage under the anaerobic conditions encountered in vacuum-packed primals. In both cases, a population of 10^7 CFU/cm^2 was considered as the level at which spoilage occurred (end of shelf-life).
- Modelling pseudomonad growth on beef, pork and lamb carcasses suggested that equivalent or lower growth, as compared to that achieved if the carcass was chilled to a core temperature of 7°C, would be obtained on: beef carcasses chilled to a surface temperature of 1–10°C (mean chilling profile) and 4–10°C (worst-case chilling profile); pork carcasses with a target surface temperatures of 6–10°C (mean chilling profile) and 7–10°C (worst-case chilling profile) and lamb carcasses chilled to a target surface temperature of 4–10°C.
- Pseudomonad growth was predicted during the combination of chilling the carcass surface to a specific target temperature (1–10°C for beef and lamb and 5–10° for pork) and transportation at that temperature plus ± 1°C. For example, for a target surface temperature during chilling of 1°C and carcass transportation temperature of 2°C, the predicted time for pseudomonads to reach a spoilage level of 10^7 CFU/cm^2 on beef carcasses, assuming no lag phase, was 101.8 h using the mean chilling profile and 31.3 h using the worst-case chilling profile, when the initial count was 1 $\log_{10}$ CFU/cm^2 (Table 12)
- The predicted time for pseudomonads to reach 10^7 CFU/cm^2 on beef, pork and lamb carcasses depends on the chilling profile used, the initial count, the target surface temperature on the carcass during chilling in the slaughterhouse and the temperature during transportation. While it might have been expected that the time to reach 10^7 CFU/cm^2 would be reduced as the target surface and/or transport temperatures decreased, this is not always the case as achieving a relatively higher carcass surface temperature requires less time which, especially when using the worst-case chilling profile, may result in lower predicted growth and thus a longer time to achieved 10^7 CFU/cm^2.
- The predicted time required for spoilage bacteria to reach 10^7 CFU/cm^2 on red meat and poultry depended on their initial contamination levels and storage temperature. At a high initial contamination level (5 $\log_{10}$ CFU/cm^2) and storage at 7°C, the predicted time for pseudomonads to reach 10^7 CFU/cm^2 was 2.3 days on red meat cuts (Table 41) and 1.1 days on poultry (Table 42), both stored aerobically, and 3.1 days for LAB on red meat cuts stored anaerobically (Table 43).
- While this opinion investigated the growth of spoilage bacteria on the surface of meat carcasses or parts thereof during storage and transport, the scientific opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat (Part 1) investigated the growth of pathogenic bacteria using similar combinations of temperature and time conditions. A comparison of the predicted growth of the spoilage bacteria (pseudomonads) with the predicted growth for pathogens (*L. monocytogenes* and *Y. enterocolitica*), suggests that the spoilage bacteria grow more rapidly and will limit the target carcass surface temperature and transport time–temperature combinations that may be applied.

- The time–temperature chilling profiles that may be used to achieve similar or less growth of pseudomonads, as compared to that obtained when chilling to a core temperature of 7°C, depended on the initial contamination levels. Thus, the conclusions regarding the application of different chilling profiles in the 'Scientific Opinion on public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 1' may or may not apply to spoilage bacteria (pseudomonads) depending on the initial concentration of these bacteria on the carcass.
- Depending on the animal contamination and hygiene condition during processing, the initial contamination level of spoilage bacteria on meat carcasses and cuts can vary significantly. A slaughterhouse with effective prerequisite programmes (good manufacturing practice (GMP)/GHP) can significantly reduce the contamination with spoilage bacteria and thus carcasses can be transported for longer times, at a given temperature, before spoilage occurs. In contrast, lack of hygiene control usually leads to high levels of spoilage bacteria limiting the transportation time before spoilage.

Recommendation

- It is recommended that FBOs are fully informed of the relationship between the effectiveness of the prerequisite programme (GHP/GMP) in the abattoir/boning halls and contamination levels of spoilage bacteria on beef, pork, lamb and poultry carcasses. As lower initial carcass counts facilitate greater flexibility in the time–temperature combinations that may be used in the abattoir chilling rooms and during transportation (with all the associated benefits in terms of reduced costs, etc.) such information should prove strong motivation for better hygiene in the meat sector.

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Abbreviations

AQIS	Australian Quarantine Inspection Service
a_w	water activity
BIOHAZ	EFSA Panel on Biological Hazards
CFU	colony forming unit
DFD	dark firm dry
FBO	food business operator
FSSP™	Food Spoilage and Safety Predictor
GHP	good hygiene practice
GMP	good manufacturing practice
HACCP	Hazard Analysis and Critical Control Point
LAB	lactic acid bacteria
SSO	specific spoilage organisms
ToR	Term of Reference
WPS	water phase salt

Appendix A – Baseline scenarios for chilling of beef, lamb and pork

The approaches taken to develop the baseline scenarios are slightly different for the different species because of the type and amount of input data that were available.

A.1. Beef

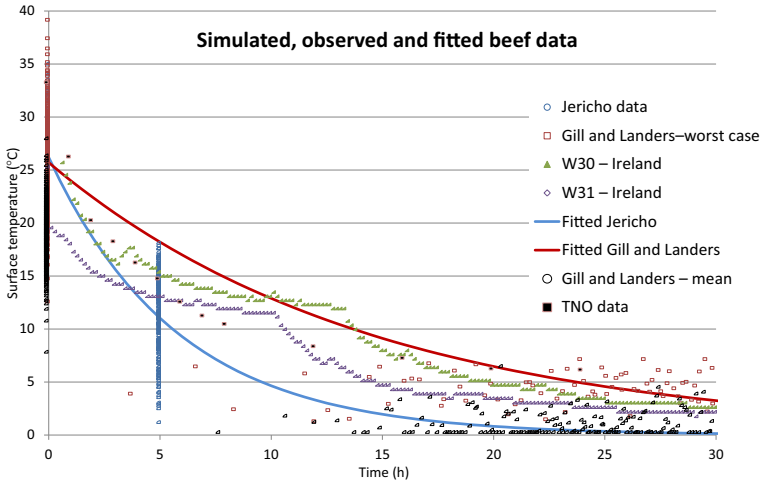
Data describing the distribution of initial and final carcass surface temperatures and chilling times (Gill and Landers, 2003), or the frequency of temperatures at the start and after 5 h of chilling (Jericho et al., 1998), were used to simulate chilling of beef. Data reflecting maximum time and temperatures during chilling in four slaughterhouses (Gill and Landers, 2003) were also used to develop a 'worst case' but still compliant baseline scenario. As described in the approach (Section 2 of EFSA BIOHAZ Panel, 2014a), simulated data were fitted to an exponential model to estimate the chilling rate and the initial surface temperature.

The best fit of the exponential equation to simulated data are shown in Figure A.1 and fitted parameters and goodness of fit estimates are shown in Table A.1. For a comparison with observed data, surface temperatures during the chilling of beef carcasses (using data obtained from a commercial beef slaughterhouse and reviewed by the BIOHAZ Panel) were fitted to the same equation (Figure A.1). In addition, observed beef carcass surface temperatures during chilling from a recent Dutch study are included for comparison (TNO, 2013). Comparisons of all temperature profiles based on graphs and fitted parameters show that the observed data are between the worst-case temperature profile based on Gill and Landers (2003) and that based on data from Jericho et al. (1998). Thus, these temperature profiles may be used as mean and worst-case scenarios (Figure A.1). The fitted parameters in Table A.1 were used to develop the chilling temperature profiles for beef baseline scenarios.

Table A.1: Parameter and goodness of fit estimates when the exponential decay function was fitted to simulated or observed data

K (SE) (/h)	5th, 95th percentile	T_0 (SE)	5th, 95th percentile	R^2	Dataset/comment
0.173 (0.005)	0.181, 0.165	26.3 (0.3)	25.8, 26.7	0.823	Jericho et al. average scenario (Jericho et al., 1998)
0.069 (0.003)	0.076, 0.063	25.8 (0.3)	25.3, 26.2	0.872	Worst case – based on maximum time and temperatures (Gill and Landers, 2003)
0.173 (0.018)	0.233, 0.147	18.7 (0.2)	18.4, 19.0	0.931	Mean times and temperatures (Gill and Landers, 2003)
0.066 (0.001)	0.068, 0.064	19.8 (0.2)	19.4, 20.2	0.900	Beef carcass chilling data (Appendix B) w30/31 – for comparison

The fitted parameters were used to develop baseline scenarios for chilling of beef. The scenario is defined in terms of k, the rate of chilling (SE, standard error), and T_0 (SE), the initial carcass surface temperature. Fifth and 95th percentiles and the R^2 of the fit are also shown.



Lines show best fit to the exponential decay function; $T = T_0 \times e^{-k*t}$, where T, T_0 are temperatures at time t and time zero, and k is the rate coefficient.

Figure A.1: Simulated beef carcass surface temperature data based on Jericho et al. (1998) and Gill and Landers (2003), using mean or maximum temperatures and times, and a comparison with observed Irish (data supplied by the beef industry and reviewed by the BIOHAZ Panel) and Dutch (TNO, 2013) data for carcass chilling

Beef baseline scenarios

Based on the comparison in Figure A.1 two scenarios were defined, 'average' and a 'worst-case' scenarios defined by the following equations:

- Average: $T = 26.3 \times e^{-0.173*t}$
- Worst case: $T = 25.8 \times e^{-0.069*t}$

Time to 7°C in the core (based on Irish beef industry data after review by the BIOHAZ Panel):

- Mean: 26.6 h
- Median: 27.3 h
- 95th percentile: 30.6 h.

A.2. Lamb

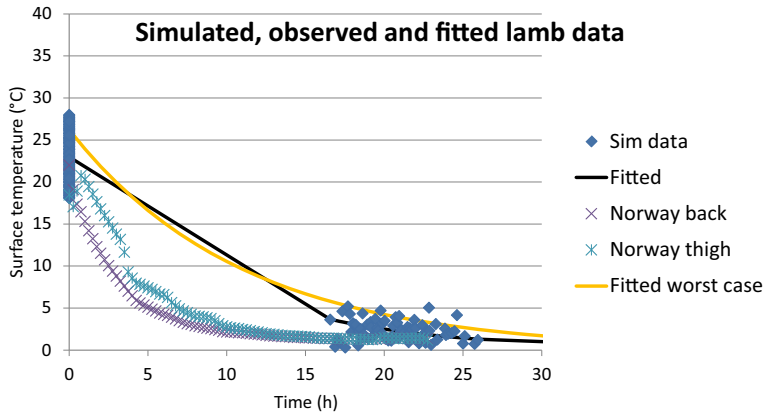
Data describing the distribution of initial and final carcass surface temperatures, as well as distribution of chilling times (Gill and Jones, 1997), were used to simulate chilling of lamb. As described in the approach (Section 2 of EFSA BIOHAZ Panel, 2014a), simulated data were then fitted to an exponential model to estimate the chilling rate and the initial surface temperature.

Table A.2: Parameter and goodness of fit estimates, when the exponential decay function was fitted to simulated or observed data, were developed

K (SE) (/h)	5th, 95th percentile	T_0	5th, 95th percentile	R^2	Dataset/comment
0.111 (0.006)	0.122, 0.102	23.0 (0.2)	22.6, 23.4	0.962	Simulated data based on Gill and Jones (1997)
0.091 (0.003)	0.097, 0.086	26.2 (0.2)	25.9, 26.5	0.982	Simulated worst case (upper quartile); data based on Gill and Jones (1997)
0.192 (0.006)	0.201, 0.182	22.0 (0.4)	21.2, 22.7	0.964	Lamb – thigh (Appendix B, EFSA BIOHAZ Panel, 2014a)
0.238 (0.007)	0.253, 0.224	19.3 (0.4)	18.6, 20.1	0.962	Lamb – back (Appendix B, EFSA BIOHAZ Panel, 2014a)

The fitted parameters were used to develop baseline scenarios for the chilling of lamb. The scenario is defined in terms of k, the rate of chilling (SE, standard error), and T_0 (SE), the initial carcass surface temperature. Fifth and 95th percentiles and the R^2 of the fit are also shown.

Data reflecting the upper quartiles of simulated temperatures based on the data in Gill and Jones (1997) were used to develop a 'worst case' but still compliant baseline scenario. This scenario/model was compared with the fitted model based on all data. There was only a very small difference in the rate of temperature decrease (Table A.2). A comparison of chilling based on simulated data with observed data from Norway (EFSA BIOHAZ Panel, 2014a Appendix B) also indicates that the rates based on simulated data may be used to represent a worst case (Figure A.2). However, the rate equations do not fit very well during extended chilling (> 24 h) and therefore the mean temperature during the period between 24 and 67 h was used for times greater than 24 h.



Lines show best fit to the exponential decay function; $T = T_0 \times e^{-k*t}$, where T, T_0 are temperatures at time t and time zero, and k is the rate coefficient.

Figure A.2: Simulated lamb carcass surface temperature data based on Gill and Jones (1997, using all data or the upper quartiles of temperatures = worst case), and a comparison with observed Norwegian data for pork carcass chilling (reviewed by the BIOHAZ Panel before application)

Lamb baseline scenarios

Based on the comparison in Figure A.2 one scenario was defined.
Baseline:

- 0–24 h: $T = 26.2 \times e^{-0.091*t}$
- >24 h: $T = 2.3$
- Time to 7°C in the core (assumption based in data in Gill and Jones, 1997): 21.5 h.

A.3. Pork

A total of 42 surface chilling curves from five French slaughterhouses were obtained from ANSES (2014). Observed surface temperatures showed a rapid decline followed by a small increase and then, after about 5 h, a gradual decline again with a long tail (Figure A.3). Consequently, the simple exponential equation could not be used to describe this chilling. The following equation was therefore used to describe the pig baseline scenarios:

$$T = T_a + (T_0 - T_a) \times e^{-k*t}$$

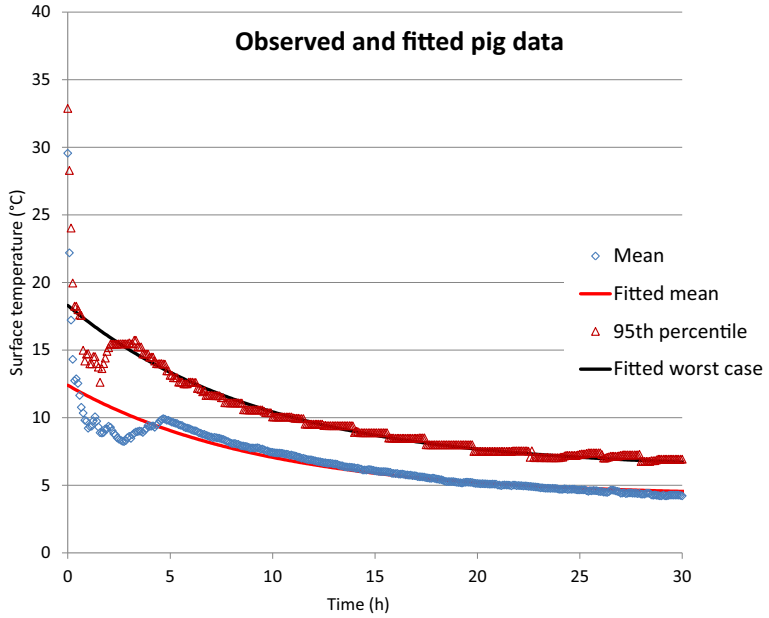
where T_a is the asymptotic final temperature and the other parameters are as described above.

From the 42 chilling curves, the mean and the 95th percentile surface temperature was estimated for each measured time interval. The modified exponential equation was fitted to these curves (Figure A.3 and Table A.3).

Table A.3: Parameter and goodness of fit estimates when the modified exponential decay function was fitted to the mean or the 95th percentile of the observed data

K (SE) (/h)	K (5th, 95th percentile)	T_0	T_0 (5th, 95th percentile)	T_a	T_a (5th, 95th percentile)	R^2	Dataset/ comment
0.105 (0.004)	0.112, 0.099	12.4 (0.2)	12.1, 12.7	4.2 (0.1)	4.2, 4.3	0.820	Mean temperatures
0.105 (0.003)	0.110, 0.100	18.3 (0.2)	18.0, 18.6	6.2 (0.1)	6.1, 6.3	0.910	95th percentile temperatures

The fitted parameters were used to develop baseline scenarios for chilling of pigs. The scenario is defined in terms of k, the rate of chilling (SE, standard error), T_0 (SE), the initial carcass surface temperature, and T_a (SE) the asymptotic final temperature. Fifth and 95th percentiles and the R^2 of the fit are also shown.



Lines show best fit to the modified exponential decay function: $T = T_a + (T_0 - T_a) \times e^{-k*t}$, where T, T_0, T_a are temperatures at time t, time zero, final asymptotic temperature and k is the rate coefficient.

Figure A.3: Observed and fitted mean and 95th percentiles (worst case) pig carcass surface temperature based on data from five slaughterhouses

Pig baseline scenarios

Based on the results shown in Figure A.3 and Table A.3 two scenarios were defined; an 'average' and a 'worst case' case based on the 95th percentile temperatures but still compliant case scenario defined by the following equations:

- Average: $T = 4.2 + (12.4 - 4.2) \times e^{-0.105*t} = 4.2 + 8.2 \times e^{-0.105*t}$
- Worst case: $T = 6.2 + (18.3 - 6.2) \times e^{-0.105*t} = 6.2 + 12.1 \times e^{-0.105*t}$
- Time to 7°C in the core (based on ANSES, 2014):
  - Mean: 19.3 h
  - Median: 17.8 h
  - 95th percentile: 27.5 h.