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SCIENTIFIC OPINION

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)[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

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ABSTRACT

Fresh meat intended for the production of minced meat may be contaminated by a range of pathogens including *Salmonella* spp. and verocytotoxigenic *Escherichia coli* (VTEC). These may grow if the temperatures are not maintained below 5 °C along the continuum from carcass chilling to mincing. Moreover *Listeria monocytogenes* and *Yersinia enterocolitica* will grow at chill temperatures, albeit slowly, but significant growth may occur during prolonged storage. Current legislation (Regulation (EC) 853/2004) requires that red meat carcasses are 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. The corresponding figures for poultry are 4 °C and 3 days. The impact of storage time between slaughter and mincing on bacterial pathogen growth was investigated using predictive modelling. Storage time-temperature combinations that allow growth of *Salmonella*, VTEC, *L. monocytogenes* and *Y. enterocolitica* equivalent to those obtained under the conditions defined by Regulation (EC) 853/2004 were identified. As the modelling assumed favourable pH and a_w for bacterial growth, no microbial competition and no lag phase, the equivalent times reported are based on worst-case scenarios. This analysis suggested, for example, that red meat, vacuum packed beef and poultry could be stored at 2 °C for up to 14, 39 and 5 days, respectively, without more bacterial pathogen growth occurring than that which would be achieved under current legislative conditions. It was therefore concluded that alternative time-temperature combinations for the storage of fresh meat between slaughter and mincing are possible without increasing bacterial pathogen growth, and maximum times for the storage of fresh meat intended for minced meat preparation are provided for different storage temperatures. The impact of spoilage on maximum storage times was not considered.

KEY WORDS

red meat, poultry, chilled storage, time-temperature, minced meat

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Biological Hazards was asked to deliver a scientific opinion on the transportation of carcasses and the production of minced meat. Transportation was dealt with in part 1 of this opinion. This document (part 2) deals with minced meat and has two objectives: (1) to assess the impact of the storage time of fresh meat intended for the production of minced meat on the risk linked to the microbiological growth of potentially harmful microorganisms; and (2) to recommend, if appropriate, in relation to such risk, maximum times of storage of fresh meat intended for the production of minced meat

Regulation (EC) 853/2004 requires that carcasses are immediately chilled after *post-mortem* inspection to ensure that the temperature throughout the meat is not more than 7 °C in the case of meat and not more than 3 °C for offal. Minced meat must be prepared from animals other than poultry within no more than 6 days after slaughter with the exception of boned, vacuum-packed beef and veal, for which minced meat may be prepared up to 15 days post slaughter. Poultry meat must be chilled to not more than 4 °C as soon as possible after *post-mortem* inspection and the maximum storage time between slaughter and the production of minced meat must be no more than 3 days.

The requirement for maximum storage times between slaughter and the production of minced meat is creating problems for the meat industry. For example, beef carcasses may be matured in slaughterhouse chillers for periods in excess of those currently permitted under Regulation (EC) 853/2004. This opinion investigates the possibility of extending the duration between slaughter and minced meat preparation without increasing the growth of potentially harmful bacteria; more specifically the impact of the time and temperature of storage (between slaughter and the preparation of minced meat) of fresh beef, pork, lamb and poultry on the growth of potentially harmful microorganisms. Target pathogens were selected based on their occurrence on red meat or poultry, and/or their ability to grow at chilled temperatures and included *Salmonella* spp., verocytotoxigenic *Escherichia coli* (VTEC), *Listeria monocytogenes* and *Yersinia enterocolitica*. Parasitic and viral pathogens do not grow on fresh meat and were therefore excluded. *Campylobacter* spp. pathogenic for humans, although prevalent on poultry carcasses, were also excluded as these bacteria do not usually grow outside of their host and never at temperatures below 30 °C.

The available data on growth of the relevant pathogens in the different meats during storage at different temperatures are limited and could not be used for a systematic approach for addressing the TORs. Instead, microbial growth models were used to predict pathogen growth potential on the meat surface during the storage period between slaughter and minced meat preparation using the most favourable conditions of pH and a_w (water activity). Moreover, a lag phase before growth commenced was assumed to be absent and inactivation during storage and competition from other microorganisms were not considered. Thus, the predicted growth potential related to ideal conditions and represents a worst case scenario. To assess the impact of the time of storage of fresh red meat intended for the production of minced meat on the risk linked to microbiological growth of potentially harmful microorganisms, the growth potential of *Salmonella* spp., VTEC, *L. monocytogenes* and *Y. enterocolitica* was estimated at 7 °C for 5 days (baseline scenario 1) and 14 days (baseline scenario 2) and for an extended period using predictive models. These parameters were selected based on current legislation which states that a maximum temperature of 7 °C should be maintained and the maximum time between slaughter and minced meat preparation should be 6 or 15 days in the case of boned vacuum-packed meat. Allowing for carcass chilling, which requires on average 24 hours, this leaves 5 and 14 days, respectively, before the production of minced meat. To assess the impact of storage time of poultry meat on the growth potential of pathogenic microorganisms, the growth of *L. monocytogenes* and *Y. enterocolitica* was predicted during storage at 4 °C for 3 days (baseline scenario 3). Neither *Salmonella* spp. nor VTEC will grow at this temperature. This was based on current legislation which mandates a maximum storage temperature at 4 °C and a maximum storage time of 3 days between slaughter and mincing. As poultry carcass chilling requires only approximately 2 hours this did not significantly reduce the 3 days storage time. In order to recommend maximum times of storage of fresh meat intended for the production of minced meat, pathogen growth potential

was predicted using different time-temperature scenarios and compared with that obtained using baseline scenario 1 and 2 (red meat) and baseline scenario 3 (poultry meat). Combinations of extra days at temperatures of 1 °C to 6 °C were evaluated and those that resulted in equivalent growth potential to that obtained with the relevant baseline scenarios were considered to represent equivalent risk.

As an example, a cautionary worst-case approach was applied based on the pathogen and the lactic acid model giving the shortest maximum storage times that resulted in equivalent growth potential. The predicted shortest equivalent time for storage of red meat at each temperature, was 12, 11, 9, 8, 7 and 6 days at 1, 2, 3, 4, 5 and 6 °C, respectively before growth equivalent to that obtained at 7 °C after 6 days (baseline scenario 1) would occur. In vacuum-packed red meat, growth equivalent to that obtained at 7 °C after 15 days (baseline scenario 2) was predicted after 48, 39, 31, 25, 20 and 17 days at 1, 2, 3, 4, 5 and 6 °C, respectively. For poultry, growth equivalent to that obtained at 4 °C after 3 days (baseline scenario 3) was obtained after 5, 4 and 3 days at 1, 2 and 3 °C, respectively. It was concluded that the storage times can be extended while maintaining equivalent risk by decreasing the storage temperature. The impact of spoilage on maximum storage times was not considered.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Current requirements

The maintenance of the cold chain is one of the main principles and basic requirements of EU legislation on food hygiene[4]. Raw materials, ingredients, intermediate products and finished products likely to support the growth of pathogenic micro-organisms are not to be kept at temperatures that might result in a risk to health. The cold chain must not to be interrupted.

In the case of meat (including fresh meat, meat products, minced meat and meat preparations), EU legislation lays down specific requirements for the storage and transport of meat regarding temperatures and maximum times of storage. Such requirements are:

- In the case of meat from animals other than poultry:

    a. 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 of the temperature. However, meat may be cut and boned during chilling in establishments attached to slaughterhouses.

    b. Meat must reach the temperature specified before transport, and remain at that temperature during transport. However, transport may also take place, if the competent authority so authorises, to enable the production of specific products, provided that it takes place in accordance with the requirements that the competent authority specifies in respect of transport from one given establishment to another, and that the meat leaves the slaughterhouse, or a cutting room on the same site as the slaughter premises, immediately and transport takes no more than two hours.

    c. The maximum storage time between slaughter and production of minced meat is no more than six days and no more than fifteen days from the slaughter of the animals in the case of boned, vacuum-packed beef and veal.

- In the case of poultry meat:

    a. 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.

    b. Meat must reach a temperature of not more than 4 °C before transport, and be maintained at that temperature during transport. However, if the competent authority so authorises, livers for the production of foie-gras may be transported at a temperature of more than 4 °C, provided that such transport takes place in accordance with the requirements that the competent authority specifies in respect of transport from one given establishment to another, and that the meat leaves the slaughterhouse, or a cutting room, immediately and transport takes no more than two hours.

    c. The maximum storage time between slaughter and production of minced meat is no more than three days.

[4] 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 the foodstuffs

Available scientific advice and recent studies

The Belgian (AFSCA) and French (ANSES) food safety agencies have issued in 2004, 2008 and 2009 opinions regarding the transport of meat that has not reached the required temperature upon leaving the slaughterhouse:

- Avis 2004/01-"Problématique du transport de viande non complètement refroidie ('transport à chaud')":

http://www.afsca.be/home/com-sci/doc/avis04/Avis_2004-01.pdf

- Avis 31-2008-"Transport à chaud de carcasses de porcs (dossier Sci Com 2008/23)".

http://www.afsca.be/comitescientifique/avis/_documents/AVIS31-2008_FR_DOSSIER2008-23.pdf

- Avis 19-2009 Projet d'arrêté royal modifiant l'arrêté royal du 30/12/1992 relatif au transport des viandes fraîches, des produits à base de viande et des préparations de viandes (dossier Sci Com 2009/17)

http://www.afsca.be/comitescientifique/avis/_documents/AVIS19-2009_FR_DOSSIER2009-17_000.pdf

- Opinion (2008-SA-0283) of the French Food Safety Agency (AFSSA) on the transport of pig carcasses that have not reached the required temperature upon leaving the slaughterhouse.

http://www.anses.fr/sites/default/files/documents/MIC2008sa0283.pdf

In addition:

- A scientific study, enclosed with this request, carried out in France by IFIP (Institut du Porc), was submitted for the opinion of the French Food Safety Agency (ANSES). The study evaluates the difference in bacterial growth induced by refrigerated transport of carcasses loaded at more than 7 °C, compared to the same carcasses remaining in cold storage. The study proposes combinations of time/temperature for the transport of such carcasses. The advice of ANSES is expected by end of 2013.

- A scientific research project was carried out in the UK on the public health risks of different time and temperature regimes for the period between slaughter and production of minced meat. That study (enclosed) concludes that, provided effective HACCP-based procedures are in place, the age of meat at mincing does not require a prescribed limit in days as a control for food safety and quality.

Before considering any derogations from the requirements described in 1.1, EFSA is requested to provide an opinion in relation to the public health risks as a consequence of applying flexibility in the maintenance of the cold chain during storage and transport of meat.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is asked to issue a scientific opinion on the public health risks as a consequence of applying flexibility in the maintenance of the cold chain during storage and transport of meat, taking into account the above mentioned studies and any other relevant scientific data. In particular, EFSA is requested:

In relation to transport of meat of domestic ungulates:

1. To assess if it is possible to apply alternative core temperatures, higher than 7 °C, in combination with specific transport durations for the transport of meat (carcasses) after the slaughter, without increasing significantly the risk linked to the microbiological growth of potentially harmful microorganisms, and

2. To recommend, if appropriate, in relation to such risk, combinations of a maximum core temperature for the loading of meat (carcasses) and a maximum time for transportation.

EFSA delivered an opinion addressing the terms of reference 1 and 2 (EFSA BIOHAZ Panel, 2014).

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

3. To assess the impact of the time of storage of fresh meat intended for the production of minced meat on the risk linked to the microbiological growth of potentially harmful microorganisms, and

4. To recommend, if appropriate, in relation to such risk, maximum times of storage of fresh meat intended for the production of minced meat.

EFSA is requested to deliver an opinion addressing the terms of reference 3 and 4 not later than 15 July 2014.

ASSESSMENT

1. Introduction

Current legislation, Regulation (EC) 853/2004[5], requires that carcasses are immediately chilled after *post-mortem* inspection to ensure that the temperature throughout the meat is not more than 7 °C in the case of meat and not more than 3 °C for offal. The same regulation defines minced meat as 'boned meat that has been minced into fragments and contains less than 1 % salt.' The raw material for minced meat must be derived from skeletal muscle including adherent fatty tissues and not from scrap cuttings or scrap trimmings (other than whole muscle cuttings), mechanically separated meat (MSM), meat containing bone fragments, meat containing skin or head meat with the exception of the masseters, the non-muscular part of the *linea alba*, the region of the carpus and the tarsus, bone scrapings or the muscles of the diaphragm (unless the serosa has been removed).

Minced meat must be prepared from animals other than poultry within no more than 6 days after slaughter with the exception of boned, vacuum-packed beef and veal, for which minced meat may be prepared up to 15 days post slaughter. Poultry meat must be immediately chilled to not more than 4 ˚C as soon as possible after *post-mortem* inspection, unless the meat is cut while warm and the maximum storage time between slaughter and the production of minced meat must be no more than 3 days. The regulations regarding transportation of livers for use in the production of foie-gras are as for those for red meat.

In the mincing process, fresh or semi-frozen meat pieces are pressed in a rotating spiral shaft or pump-type system against a rotating knife and through a static end plate with holes of 1.5 mm to 10mm in diameter. This process disrupts the meat cellular structure and the ordered fibrillar structures including myofibres and connective tissue, releasing tissue fluids. As a result, minced beef, pork, lamb and poultry meat is a highly nutritious medium that readily supports bacterial growth. Moreover, intact carcasses and meat cuts are primarily contaminated on their surfaces but mincing redistributes surface bacteria throughout the product. Minced meat is therefore a highly perishable product that must be chilled immediately. Regulation (EC) 853/2004 requires that minced meat must be wrapped or packaged and chilled to an internal temperature of not more than 2 °C or frozen to an internal temperature of not more than -18 °C. These conditions must be maintained during storage and transport.

The requirement regarding the maximum storage time between slaughter and the production of minced meat is creating a problem for the meat industry. For example, it may be desirable to mature beef carcasses in the slaughterhouse chillers for periods in excess of those currently permitted under Regulation (EC) 853/2004 to improve meat quality. However, it may be possible to extend the duration between slaughter and minced meat preparation without increasing the growth of potentially harmful bacteria. Most microbiological pathogens will not grow at chill temperatures, and those that are capable of growth, such as *Listeria monocytogenes* and *Yersinia enterocolitica*, will multiply slowly, if at all. Thus, if the initial microbiological load on carcasses and cross-contamination during subsequent processing are controlled and the integrity of the chill chain is maintained from carcass to minced meat, the impact of time of storage on public health risk should be minimal. The former is dependent on the development and application of effective hazard analysis and critical control point (HACCP) and prerequisite actions including those covered by good hygiene practices (GHP), as required under Regulation (EC) 852/2004[6].

This opinion, Transport of Meat (Part 2), deals with the terms of reference (TOR) 3 and 4. Carcass chilling and transportation (TOR 1 and 2) were covered in Transport of Meat (Part 1) (EFSA BIOHAZ Panel, 2014). It therefore investigates the impact of the time of storage (between slaughter and the

[5] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin OJ L 139, 30/04/2004, p. 55–205.

[6] Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuff OJ L 139, 30.4.2004, p. 1.

preparation of minced meat) of fresh beef, pork, lamb and poultry meat on the growth of potentially harmful microorganisms and recommends storage time-temperature combinations that would result in microbial growth equivalent to that obtained under the conditions defined by Regulation (EC) 853/2004. Other factors that may affect shelf life and risk, such as microbial load and contamination by specific pathogens are not covered in this opinion.

2. Approach to addressing the terms of reference (TOR)

The available data on growth of the relevant pathogens in the different meats during storage at different temperatures are limited and could not be used for a systematic approach to address the TORs. Thus, to assess pathogen growth and evaluate different time and temperature storage scenarios, the growth potential of relevant bacterial pathogens on the meat surface during the storage period between slaughter and minced meat preparation was estimated using published predictive microbial growth models. Values for model variables, e.g. pH and a_w, favouring growth were used, and a lag phase before growth commenced was assumed to be absent. Moreover, inactivation during storage and competition from other microorganisms was not considered, and the effect of storage time-temperature conditions on the growth of meat spoilage bacteria and the organoleptic rejection of the products was not taken into account. Thus the predicted growth is potential growth that would be achieved under ideal conditions and may be considered to represent a worst-case scenario. However, as a comparative approach between baseline and alternative scenarios was applied, the above assumptions are not expected to significantly affect the outputs. Details of the modelling are described in Section 6.

2.1. Approach to addressing TOR 3

To assess the impact of the time of storage of fresh red meat intended for the production of minced meat on the risk linked to microbiological growth of potentially harmful microorganisms (TOR 3), the growth potential of *Salmonella* spp., VTEC, *L. monocytogenes* and *Y. enterocolitica* was estimated at 7 °C for 5 days (baseline scenario 1) and 14 days (baseline scenario 2) and for an extended period using predictive models. These parameters were selected based on current legislation, which states that a maximum temperature of 7 °C should be maintained and the maximum time between slaughter and minced meat preparation should be 6 days or 15 days in the case of boned vacuum-packed meat. Allowing for carcass chilling (24 hours), that leaves 5 and 14 days before the production of minced meat.

To assess the impact of storage time of poultry on the growth potential of pathogenic microorganisms, *L. monocytogenes* and *Y. enterocolitica* growth was predicted at 4 °C for 3 days (baseline scenario 3). This was based on current legislation, which mandates a maximum storage temperature of 4 °C and a maximum storage time of 3 days between slaughter and mincing. As poultry carcass chilling requires only approximately 2 hours, this did not significantly reduce the 3 days' storage time.

2.2. Approach to addressing TOR 4

To recommend maximum times of storage of fresh meat intended for the production of minced meat (TOR4), the pathogen growth potential achieved using different time-temperature scenarios was compared with that which would be obtained using baseline scenarios 1 and 2 (red meat) and baseline scenario 3 (poultry meat). Combinations of extra days at temperatures of 1° to 6 °C were evaluated, and those that gave equivalent growth to that obtained in the relevant baseline scenarios were considered to represent equivalent risk.

3. Hazard identification

3.1. Bacterial hazards that may be influenced by chilling time-temperature combinations

The first step in assessing the impact of the time of storage of fresh meat intended for the production of minced meat on the risk linked to the microbial growth of potentially harmful organisms is to identify the relevant pathogenic organisms that may contaminate fresh beef, pork, lamb and/or poultry meat and are capable of multiplication at the temperatures encountered during minced meat preparation and storage. Parasitic and viral pathogens do not grow on fresh meat and should therefore

be excluded. In the earlier 'Scientific Opinion on public health hazards to be covered by inspection of meat (poultry)', *Campylobacter* spp. were identified as a priority hazard in poultry (EFSA Panels on Biological Hazards (BIOHAZ), on Contaminants in the Food Chain (CONTAM), and on Animal Health and Welfare (AHAW), 2012). However, in red meats *Campylobacter* spp. are infrequently reported in minced beef and pork in Europe, probably because these organisms are particularly sensitive to drying during carcass chilling (EFSA and ECDC, 2013) and die off in vacuum-packed meat at the chilling temperatures used in the European red meat sector (Gill and Harris, 1982; Hanninen et al., 1984; Vanlaack et al., 1993). Regardless of meat type, *Campylobacter* spp. do not usually grow outside of their host and never at temperatures below 30 °C (Hazeleger et al., 1998). Moreover, inoculation studies suggest that *Campylobacter* spp. decreases on chicken meat during chilled storage (Meredith et al., 2013). For these reasons *Campylobacter* spp. was not considered for inclusion in answering the terms of this mandate. Pathogenic bacteria such *Salmonella* spp. and pathogenic *E. coli* (VTEC) are found on red meat and/or poultry meat and will grow slowly at temperatures as low as 5-7 °C. *Y. enterocolitica* is found on fresh pork and will grow at -2 °C. *L. monocytogenes* is an environmental contaminant that may also contaminate fresh meat and can grow at temperatures as low as -1 °C. These four bacterial hazards will be discussed in this section.

3.2. *Salmonella* spp.

Contaminated foodstuffs serving as a source of *Salmonella* infection for humans include table eggs closely followed by pig meat, whereas the risks associated with broiler and turkey meat are similar and approximately two-fold lower (EFSA BIOHAZ Panel, 2012). In the European Union (EU), *S.* Enteritidis and *S.* Typhimurium are the serovars most frequently associated with human illness. Human *S.* Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat, whereas *S.* Typhimurium cases are mostly associated with the consumption of contaminated pig meat or bovine meat (EFSA and ECDC, 2014). It is estimated that around 10.6 %, 17 %, 56.8 % and 2.6 % of the human salmonellosis cases in the EU are attributable to broilers, laying hens (eggs), pigs and turkeys, respectively (EFSA BIOHAZ Panel, 2012). Of the broiler-associated human salmonellosis cases, around 82 % and 6.5 % are estimated to be due to the serovars *S.* Enteritidis and *S.* Infantis, respectively (Hald et al., 2012). In the Netherlands serovars of *Salmonella* spp. from humans and animals were studied from 1984 to 2001. The human strains (n = 59 168) were clinical isolates, and the animal strains (n = 65 567) were from clinical and non-clinical infections. The most prevalent serovars were as follows: in humans, serovars Typhimurium and Enteritidis; in cattle, serovars Typhimurium and Dublin; in pigs, serovar Typhimurium; and in chickens, serovars Enteritidis, Infantis, and Typhimurium (van Duijkeren et al., 2002). In the EU, approximately 9 % of turkey carcasses are *Salmonella*-positive and the top six serovars that contribute to human cases are *S.* Enteritidis, *S.* Kentucky, *S.* Typhimurium, *S.* Newport, *S.* Virchow and *S.* Saintpaul (EFSA BIOHAZ Panel, 2012). While there are few data on the prevalence of pathogens on trimmings and meat cuts used for minced meat products, Scanga et al. (2000) detected *Salmonella* spp. on up to 5.3 % of beef trimmings. Data from Belgium suggest that 3.5 % to 4.2 % of minced beef samples are contaminated with *Salmonella* spp. (Ghafir et al., 2005). Prendergast et al. (2009) reported that 2.35 % of minced pork samples in Ireland were *Salmonella* positive. Regulation (EC) 2073/2005[7] sets down microbiological criteria for foodstuffs. In 2012, as in 2011 and in previous years, the highest levels of non-compliance with *Salmonella* criteria generally occurred in foods of meat origin that are intended to be cooked before consumption. Minced meat and meat preparations from poultry intended to be eaten cooked had the highest level of non-compliance (category 1.5; 8.7 % of single samples and 5.7 % of batches) (EFSA and ECDC, 2014). *Salmonella* spp. have a reported minimum growth temperature of 5 °C and an optimum temperature of 35 °C to 43 °C (James and James, 2014), a pH growth range of 4.5 to 9.0 and a minimum a_w for growth of 0.94 (Oliveira de Almeida Møller, 2012) and based on these figures, growth should be absent or very slow in correctly chilled meat intended for preparation of mince.

[7] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs OJ L 338, 22/12/2005, p. 1–26.

3.3. Verocytotoxigenic *Escherichia coli* (VTEC)[8]

The pathogenic *E. coli* found on fresh meat are mainly VTEC that may contaminate beef and/or lamb (EFSA BIOHAZ Panel, 2013a, b). Contaminated bovine meat is considered to be a major source of food-borne VTEC infections in humans. In 2012, 9 Member States reported data on VTEC in fresh bovine meat from 10 investigations with 25 or more samples. VTEC was detected in 7 of these 10 investigations. A total of 4 603 bovine meat units (single or batch) were tested for VTEC and 58 units (1.3 %) were found to be VTEC-positive and 6 units (0.1 %) were VTEC O157-positive (EFSA and ECDC, 2014). Data from several European countries showed that the VTEC prevalence in minced beef ranged from 0 % to 3 % (EFSA and ECDC, 2013). The reported prevalence of VTEC in minced beef in various European studies is 0.76 % in the UK (Chapman et al., 2000; 2001), 2.8 % in Ireland (Cagney et al., 2004), 0.12 % in France (Vernozy-Rozand et al., 2002), 0.18 % in Belgium (Tutenel et al., 2003), 0.43 % to 13 % in Italy (Conedera et al., 2004; Parisi et al., 2010), 11.5 % in Spain (Mora et al., 2007), 1.1 % in the Netherlands (Heuvelink et al., 1999) and 2.3 % in Switzerland (Fantelli and Stephan, 2001). Pathogenic *E. coli*, such as VTEC, have a reported minimum growth temperature of 6 ºC to 7 ºC, an optimum temperature of 35 °C to 42 °C (James and James, 2014) and will grow between pH 4.4 and 10.0 and a minimum a_w of 0.95 (Desmarchelier and Fegan, 2003).

3.4. *Listeria monocytogenes*

L. monocytogenes has been reported on beef, pork and lamb carcasses (Sheridan et al., 1994; Nicholas, 1995; McEvoy et al., 1998) and on up to 5.4 % of beef trimmings (Scanga et al., 2000), while the reported prevalence in minced beef was 10.9 % (Fantelli and Stephan, 2001) and 4.7 % to 16 % (Sheridan et al, 1994; Sheridan et al., 1997). Skovgaard and Nørrung, (1989) reported that 12 % of minced pork and 36.1 % of minced poultry contained *L. monocytogenes*. Other poultry studies found *L. monocytogenes* prevalence ranging from 12 to 60 % (Farber and Peterkin, 1991). *L. monocytogenes* grow optimally at 30 °C to 37 °C (James and James, 2014) but are also capable of growing at -1 °C, although inoculation studies in ground beef suggest survival but no growth at 4 °C (Johnson et al., 1988).

Glass and Doyle (1989) found that growth of *L. monocytogenes* on meat was highly dependent on product type and pH. The organism tended to grow well on meat products with a pH value near or above 6.0, whereas it grew poorly or not at all on meats near or below pH 5.0. Poultry supported the growth of *L. monocytogenes* better than other meats, and roast beef, summer sausage and hot dogs supported the least growth.

3.5. *Yersinia enterocolitica*

Further evidence of the link between pigs, pork carcasses and associated products is presented in the earlier 'Scientific Opinion on the public health hazards to be covered by inspection of meat (swine) (EFSA Panels on Biological Hazards (BIOHAZ), on Contaminants in the Food Chain (CONTAM), and on Animal Health and Welfare (AHAW), 2011). These bacteria have an optimum growth temperature of 28 °C to 29 °C, but they are also capable of growth at -2 ºC (James and James, 2014). Investigative studies on the growth of *Y. enterocolitica* on meat are inconclusive. Several studies observed growth under chilled storage (Stern et al., 1980; Lee et al., 1981; Gill and Reichel, 1989; Lindberg and Borch, 1994; Nissen et al., 2000; Nissen et al., 2001). In contrast, other studies suggest these bacteria compete poorly with other micro-organisms on the meat (Fukushima and Gomyoda, 1986; Schiemann, 1989; Kleinlein and Untermann, 1990). Fukushima and Gomyoda (1986) reported good survival but no growth in ground pork stored at 6 °C and 25 °C.

The occurrence of *Y. enterocolitica* in poultry meat is described, but generally the recovered isolates belong to apathogenic biotypes and no data on the occurrence of *Y. enterocolitica* in poultry flocks or carcasses is included in the EU monitoring data according to 'Scientific Opinion on the public health hazards to be covered by inspection of meat (poultry)' (EFSA Panels on Biological Hazards (BIOHAZ), on Contaminants in the Food Chain (CONTAM), and on Animal Health and Welfare

[8] VTEC and STEC are used synonymously in this opinion

(AHAW), 2012). However in Germany, Stengel (1985) isolated *Y. enterocolitica* biotype 4/ serotype O:3 (n=3) and biotype 2/ serotype O:9 (n=3) from 130 samples of poultry. This is probably the first and only time that these virulent serotypes have been isolated from poultry, and there was no obvious opportunity for cross-contamination from pigs or pork.

4. Carcass chilling and further processing

Red meat primary chilling has been described in Transport of Meat (Part 1) (EFSA BIOHAZ Panel, 2014). As with other meat species, poultry carcasses are chilled to reduce the growth rate of spoilage and pathogenic organisms and preserve the quality of the meat. Carcasses are chilled in the poultry processing plant immediately after dressing using immersion, spray or air chilling methods (Figure 1). The whole carcasses are typically stored in the chillers for up to 24 hours during which ageing occurs. This short refrigerated storage period is in contrast to red meat carcasses and is designed to reduce water loss and facilitate high throughput. However, breast meat should not be removed from the carcass prior to the completion of *rigor mortis*, as this would result in muscle fibre contraction and shortening with toughening of the meat (Fletcher, 2002). The rate of chilling also influences the taste, texture and appearance of poultry meat (James et al., 2006).

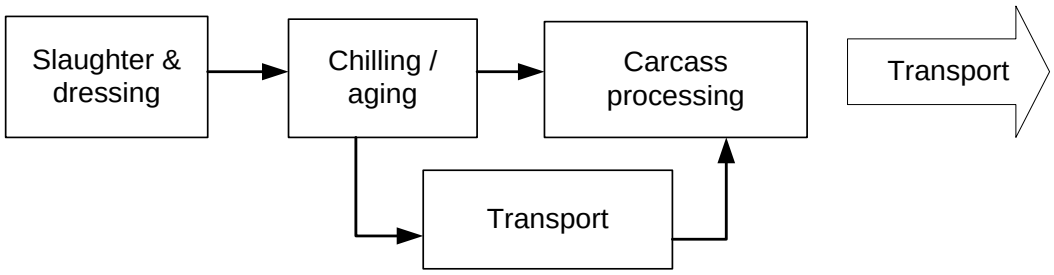

Figure 1: Summary flow diagram for chilling, further processing and transportation of poultry carcasses

4.1. Primary chilling methods for poultry

Primary chilling methods for red meat carcasses have been described in Transport of Meat (Part 1) (EFSA BIOHAZ Panel, 2014). In the poultry processing plant, dressed carcasses are conveyed continuously on rails through a room or tunnel that terminates in a chilling room. The part of a chicken that is slowest to cool is the internal deep breast, the temperature of which ranges from 29.6 °C to 42.4 °C, with a mean of 37.7 °C, before entering the chillers (May et al., 1961). Immersion and spray chilling may be used instead of air chilling as these are more efficient in terms of chilling times and reducing weight loss. Depending on the weight of the bird, rates of chilling of up to 1.12 °C min^{-1}, 0.9 °C min^{-1} and 0.28 °C min^{-1} can be achieved with immersion, spray and/or air chilling, respectively (James et al., 2006). The last may be substantially improved if blast chilling is used. With an air temperature as low as -40 °C, a carcass chilling rate of over 2 °C min^{-1} has been observed (James et al., 2006). Allen et al. (2000) describe air and water chilling systems used in UK poultry processing plants. The former run at 3 °C while the latter is comprised of a 3 unit counter-flow system that uses chlorinated water (mean total residual of 45mL/L) operating at a temperature that ranges from 15.9 °C at the carcass entry point to 5.1 °C at the chiller exit. The combination of air and spray chilling is common with the water sprays being applied in a pre-chill area or in the first section of the tunnel.

Some poultry plants operate a two-stage cycle; blast chilling at approximately -2 °C (to get the temperature of the bird down as quickly as possible) for 45 minutes followed by a maturation chilling process in which the chickens circulate at 0 °C for approximately 2 hours before entering the packing hall. The target temperature for birds entering this stage is < 4 °C but small birds are typically held at 2-3 °C and larger birds at 2.5 °C and 3.5 °C. Once packaged, the whole birds are stored in holding chills at approximately 2 °C ambient temperature. Minced poultry is typically produced in an area at 8 °C and the temperature of the raw materials or finished minced product should not go above 4 °C.

Deep or super chilling is used in the USA but not in Europe. Carcasses are chilled in water exposed to air at approximately -15 °C for 30 minutes, packaged and returned to the air freezer until the required temperature of the meat is achieved before storage and distribution at -1 °C to -2 °C. Poultry meat freezes between -1.5 °C and -2.8 °C but in the USA meat from carcasses kept above -3.3 °C can be marketed as fresh (Franatico, 2003).

5. Processing of red meat and poultry carcasses

After chilling and maturation, beef, pork and lamb carcasses are moved to the boning hall/cutting room. Cutting and boning must be carried out at ambient temperatures of 12 °C or less in accordance with European Food Hygiene regulations. Whereas many plants operate at 8 °C or less to inhibit the growth of spoilage organisms, there is considerable variability in operating temperatures between plants. Processing time will also affect pathogen growth and associated risk, and for this reason Mackey and Roberts (1991) suggested that boning operations should be completed within 2 hours, thus inhibiting the growth of all bacteria including psychrotrophic organisms. Trimmings destined for mincing are typically stored chilled or frozen. A recent UK study reported chilled trimming temperatures ranging from 0.7 °C to 2.9 °C for beef, from -0.7 °C to 3.8 °C for pork and from -1.2 °C to 4.6 °C for lamb trimmings immediately before mincing (James and James, 2012). Moreover, the same study reported a slight rise in temperature during mincing from 0.32 °C to 3.2 °C, from 0.18 °C to 5.7 °C and from 3.4 °C to 5.6 °C for lamb and from 0.6 °C to 1.2 °C and 1.98 to 3.6 °C for pork mince.

The conditions under which minced meat is packaged also vary and include aerobic, anaerobic and modified atmospheric packaging. This will also affect microbial growth. At 4 °C minced beef has a typical shelf-life of 1-2 days when stored aerobically, which is extended to 7-14 days under anaerobic conditions (James and James, 2012). In addition to carryover of bacterial contamination from the carcasses to trimmings to be minced, the public health risk associated with minced meat is also influenced by cross-contamination that occurs during deboning, and several studies have reported significantly increased bacterial loads as a result of inadequate GHP (Gill and McGinnis, 2000; Bouvet et al., 2002).

The most commercially valuable poultry cuts are the fillets, which are usually removed during further processing. The legs (drumsticks) and wings may also be sold commercially. The remaining meat is usually recovered using manual or mechanical processes, minced if required and used in a variety of poultry products. There is also a market for whole chickens. Carcass processing may take place on site or off site, this requiring transportation, possibly to another country.

The UK risk assessment (James and James, 2012) concluded that the initial bacterial load of carcasses is a key factor in the microbiological safety and quality of meat to be minced, and this should be controlled by the cleanliness of animals at slaughter, hygienic slaughter and dressing. The hygienic conditions under which meat is stored, cut and boned, and minced are also important factors that influence the microbiological quality of minced meat. The only part of the process that was identified as having a more important effect on the safety and quality of minced meat made from unwrapped chilled meat that had been stored longer than the current number of days allowed was the cleanliness of the storage rooms.

6. Modelling

6.1. Pathogen growth

The impact of time and storage temperature on microbial growth and risk was evaluated by estimating the growth potential under conditions favourable for growth. Growth potential is defined as the increase in the number of bacterial cells during storage expressed as $\log_{10}$ colony-forming units (CFU) per cm^2. Thus, to obtain the number of cells after a certain storage period the growth potential during that period is added to the initial contamination level ($\log_{10}$ CFU per cm^2).

Growth of pathogens during storage was estimated using available secondary models predicting the maximum specific growth rates of *Salmonella* spp., VTEC, *L. monocytogenes* and *Y. enterocolitica* at different storage temperatures. Parameter values reflecting favourable growth conditions in meat were assumed for the environmental factors included in the secondary models. The predicted maximum specific growth rates were then implemented in primary growth models to estimate pathogen growth over time (Table 1).

The assumption of the absence of a lag phase, together with the assumed high a_w and near-neutral pH of meat, as well as the assumed absence of competition from other meat bacterial flora, represents conditions that are favourable for extended growth of the target pathogens and results in an over-estimation of growth. Thus, growth estimated with predictive models developed in broth media under these conditions represent the maximum growth potential and is not expected to occur in meat during most storage conditions but represents a worst-case scenario. However, since the approach used in TOR 4 is based on the comparison of time and temperature scenarios in terms of growth potential and is estimated under the same conditions and with the same model, this is not expected to affect the results and conclusions.

For *Salmonella* spp. (no lactic acid) and *Y. enterocolitica* (with lactic acid) only aerobic models were available (Table 1). For VTEC and *L. monocytogenes* anaerobic models were used to predict growth during storage of vacuum-packed meat but these did not include the effect of lactic acid. Corresponding aerobic models were included to evaluate the effect of the absence of oxygen in vacuum-packed meat.

In addition, models including lactic acid as a parameter were used to evaluate the effect of lactic acid in meat on the growth of *L. monocytogenes* and VTEC (Table 1). For VTEC, the model of Ross et al. (2003), although developed for *E. coli* growth, was used, assuming that the kinetic behaviour of VTEC is similar to that of other *E. coli*. The performance of this model in foods has been successfully evaluated (Mellefont et al., 2003). For *Listeria* the ComBase model and the model of Mejlholm and Dalgaard (2010) were used. The latter model has been validated for meat products, sea-food, poultry products, and non-fermented dairy products (Mejlholm et al., 2010) and is included in the freely available SSSP (Seafood Spoilage and Safety Predictor) program[9]. Furthermore, to evaluate the impact of model selection on estimated growth potential, and the effect of the assumption of a high pH of 6.5, estimated growth was compared between alternative *L. monocytogenes* growth models and for poultry between pH of 6.0 and 6.5, respectively.

Details on the models are described in Appendix A.

[9] Seafood Spoilage and Safety Predictor, version 3.1, free software distributed from http://sssp.dtuaqua.dk. Secondary Cardinal parameter model and primary logistic model .

Table 1: Models and assumptions used to predict growth potential of the selected pathogens. The initial level of pathogens, N_0, was set to N_0=0 $\log_{10}$ CFU per cm^2 (i.e. one bacterial cell), and a lag before growth was assumed to be absent

Organism	Secondary Model	Primary model	Temperature range[b] (°C)	pH (meat)	a_w (meat)	Oxygen	Total lactic acid mM[c]
Salmonella spp.	ComBase[a]	ComBase[a]	7.0-40.0	6.5	0.993	Aerobic	NI
Escherichia coli	Ross et al., 2003	Baranyi and Roberts, 1994[d]	7.6-47.4	6.5	0.993	Aerobic	51.7
E.coli O157:H7	PMP[e]	PMP[e]	5.0-42.0	6.5	0.993	Aerobic	NI
E.coli O157:H7	PMP[e]	PMP[e]	5.0-42.0	6.5	0.993	Anaerobic	NI
Listeria monocytogenes	ComBase[a]	ComBase[a]	1.0-40.0	6.5 or 6.0	0.993	Aerobic	51.7
L. monocytogenes	PMP[e]	PMP[e]	4.0-37.0	6.5	0.993	Aerobic	NI
L. monocytogenes	PMP[e]	PMP[e]	4.0-37.0	6.5	0.993	Anaerobic	NI
L. monocytogenes	SSSP[f]	SSSP[f]	2.0-25.0	6.5	0.993	Aerobic	51.7
Yersinia enterocolitica	ComBase[a]	ComBase[a]	-1.0-37.0	6.5	0.993	Aerobic	51.7

a: Used ComBase predictive models and interface at the website; www.combase.cc (last accessed: 26 March 2014). Polynomial secondary models and Baranyi and Roberts (1994) primary model.
b: Temperature range used for the development of the model
c: Naturally occurring
d: The same approach as described in Transport of meat (Part 1) (EFSA BIOHAZ Panel, 2014).
e: Used Pathogen Modeling Program predictive models at the website; http://pmp.errc.ars.usda.gov/PMPOnline.aspx (last accessed: 26 March 2014). Polynomial secondary model. The Baranyi and Roberts (1994) primary model was implemented in R software in the assessment.
f: Seafood Spoilage and Safety Predictor, version 3.1, free software distributed from http://sssp.dtuaqua.dk. Secondary cardinal parameter model and primary logistic model.
NI: parameter not included in the model.

6.2. Development of baseline scenarios

The impact of storage time on the microbiological growth potential was estimated based on the predictive models and the assumed intrinsic and extrinsic conditions of the meat during storage. Storage times from 1 to 21 days at temperatures of 7 °C or 4 °C were evaluated. The estimated growth potential at the different storage times was compared with a baseline time and temperature storage scenario compliant with current legislation. In a deterministic approach, growth potential was evaluated at a constant storage temperature.

Storage of meat prior to mincing is prescribed to be at a maximum temperature throughout the meat of 7 °C (red meat) or 4 °C (poultry meat), for up to 6 (red meat) or 3 (poultry meat) days. In the case of vacuum-packed red meat, storage times up to 15 days are allowed. Taking into consideration that chilling of beef, pork and sheep carcasses to a core temperature of 7 °C according to the mean baseline takes around 1 day in the slaughterhouse (EFSA BIOHAZ Panel, 2014), and chilling of poultry can be completed within hours, the following storage baselines were assumed in the deterministic approach:

Red meat:

- Baseline scenario 1 Carcass or aerobically stored/unpacked meat: Storage at 7 °C for 5 days.
- Baseline scenario 2 Vacuum-packed meat: Storage at 7 °C for 14 days.

Poultry meat:

- Baseline scenario 3 Poultry meat: Storage at 4 °C for 3 days.

6.3. Development of alternative scenarios

The predicted pathogen growth potential under conditions favouring growth was used to interpret different storage time and temperature scenarios in terms of microbial growth and, thus, potential risk. To address TOR 4, a similar approach to that used in Transport of Meat (Part 1) (EFSA BIOHAZ Panel, 2014) was applied to find combinations of storage temperatures and storage times that would result in the same growth potential as storage baselines that are consistent with current legislation. The rationale is that equivalent growth potential equates to equivalent risks. The combinations of storage times at different temperatures were defined by estimating growth at temperatures below baseline temperatures, 1 to 6 °C (red meat) and 1 to 3 °C (poultry meat), respectively, and finding the times corresponding to the same potential growth as baseline storage at 7 °C for 5 days (red meat), 7 °C for 14 days (vacuum-packed red meat) or 4 °C for 3 days (poultry meat).

6.4. Results for addressing TOR 3

Growth was predicted for the four target pathogens at the currently mandated maximum temperature for storage of red meat of 7 °C. For *Salmonella* spp. and VTEC a growth potential of up to 1.92 and 3.10 $\log_{10}$ CFU per cm^2, respectively, was estimated after 5 days while a growth potential of up to 5.81 and 6.18 $\log_{10}$ CFU per cm^2, respectively, were predicted for *L. monocytogenes* and *Y. enterocolitica* after the same time period (Table 2).

For red meat stored at 7 °C the estimated growth potential of all pathogens was high. After 5 days of storage (red meat baseline scenario 1), levels had increased, depending on the pathogen and the model, up to between 0.9 $\log_{10}$ CFU per cm^2 (*E. coli* – Ross model) and 6.2 $\log_{10}$ CFU per cm^2 (*Y. enterocolitica*-ComBase lactic acid model) (Table 2).

For poultry meat stored at 4 °C, growth was estimated only for *L. monocytogenes* and *Y. enterocolitica* as this temperature is below the minimum growth temperature for *Salmonella* spp. and VTEC (Table 3). Although it is very unlikely that poultry meat would be contaminated with *Y. enterocolitica*, this scenario has been included for completeness. Most models indicated that the growth potential was

greater for *Y. enterocolitica* than *L. monocytogenes*. After 3 days of storage a growth potential of up to 2.1 $\log_{10}$ CFU per cm^2 and 2.4 $\log_{10}$ CFU per cm^2 was predicted for *L. monocytogenes* and *Y. enterocolitica*, respectively (Table 3).

Based on the comparison between models for VTEC and *L. monocytogenes*, models including the effect of lactic acid predicted less growth than models not including this factor (Table 2).

The effect on growth of vacuum-packed storage of meat, i.e. based on comparisons between models for aerobic and anaerobic growth, is less clear. The PMP (Pathogen Modelling Program) model predicted a lower growth potential of VTEC in anaerobic conditions than in aerobic conditions (Table 2). In contrast, at both 7 °C and 4 °C, PMP models predicted more rapid growth of *L. monocytogenes* in anaerobic conditions than in aerobic conditions. In the PMP model the maximum population density of *L. monocytogenes* was greater in aerobic conditions than in anaerobic conditions at 7 °C but not at 4 °C (Tables 2 and 3).

The effect of using a pH of 6.0 instead of 6.5 resulted in about a 1 log difference in the estimate of the growth potential of *L. monocytogenes* in poultry (Table 3). In comparison, differences between predictions from different models including the same factors were greater than the estimated effect of pH. Two models describing the effect of lactic acid on growth of *L. monocytogenes* were compared. The ComBase lactic acid model predicted at most a growth potential over 2 $\log_{10}$ units greater than the SSSP lactic acid model at both 7 and 4 °C (Tables 2 and 3).

For the maximum population density of pathogen growth, default values for the different models were used. These levels represent a worst-case scenario. The maximum population density of the pathogens in meat is expected to be significantly lower, mainly as a result of the growth of the natural microflora present in fresh meat.

Table 2: Estimated worst-case growth potential (log_{10} CFU per cm^2) of selected bacteria in red meat stored at 7 °C for different times (days), pH=6.5, a_w=0.993 (=1.29 % w/w), lactic acid=0 or 51.7 mM (4 654 ppm).

	Growth potential in red meat (log_{10} CFU per cm^2)								
Time (days)	***Salmonella* spp. (ComBase model)**	***E. coli* for VTEC (Ross lactic acid model)**	***E. coli* O157:H7 (Aerobic PMP model)**	***E. coli* O157:H7 (Anaerobic PMP model)**	***Listeria monocytogenes* (Aerobic PMP model)**	***Listeria monocytogenes* (Anaerobic PMP model)**	***Listeria monocytogenes*, (ComBase lactic acid model)**	***Listeria monocytogenes*, (SSSP lactic acid model)**	***Yersinia enterocolitica*, (ComBase lactic acid model)**
1	0.38	0.18	0.62	0.45	1.04	1.16	0.74	0.46	1.25
2	0.77	0.36	1.24	0.90	2.09	2.32	1.50	0.92	2.50
3	1.16	0.54	1.86	1.35	3.14	3.48	2.23	1.39	3.74
4	1.54	0.72	2.48	1.81	4.18	4.65	2.98	1.85	4.98
5	**1.92**	**0.90**	**3.10**	**2.26**	**5.23**	**5.81**	**3.71**	**2.32**	**6.18**
6	2.31	1.08	3.72	2.71	6.27	6.97	4.47	2.78	7.22
7	2.69	1.26	4.34	3.16	7.32	8.11	5.20	3.24	7.92
8	3.08	1.44	4.96	3.61	8.34	9.01	5.92	3.70	8.20
9	3.46	1.62	5.58	4.06	9.18	9.31	6.61	4.16	8.28
10	3.84	1.80	6.20	4.52	9.52	9.34	7.24	4.63	8.30
11	4.22	1.98	6.82	4.97	9.56	9.34	7.75	5.09	8.30
12	4.60	2.16	7.44	5.42	9.57	9.34	8.11	5.55	8.30
13	4.98	2.34	8.05	5.87	9.57	9.34	8.33	6.02	8.30
14	**5.36**	**2.52**	**8.61**	**6.32**	**9.57**	**9.34**	**8.43**	**6.47**	**8.30**
15	5.73	2.70	9.05	6.77	9.57	9.34	8.48	6.92	8.30
16	6.10	2.88	9.29	7.21	9.57	9.34	8.51	7.34	8.30
17	6.45	3.06	9.37	7.65	9.57	9.34	8.52	7.71	8.30
18	6.79	3.24	9.39	8.05	9.57	9.34	8.52	7.98	8.30
19	7.12	3.42	9.40	8.38	9.57	9.34	8.52	8.16	8.30
20	7.41	3.60	9.40	8.61	9.57	9.34	8.52	8.27	8.30
21	7.67	3.78	9.40	8.72	9.57	9.34	8.52	8.33	8.30

Table 3: Estimated worst-case growth potential (log_{10} CFU per cm^2) of selected bacteria in poultry meat stored at 4 °C for different times (days). pH 6.5 or 6.0, a_w=0.993 (=1.29 % w/w), lactic acid=0 or 51.7 mM (4 654 ppm).

	Growth potential in poultry meat (log_{10} CFU per cm^2)				
Time (days)	***Listeria monocytogenes*, (Aerobic PMP model)**	***Listeria monocytogenes*, (Anaerobic PMP model)**	***Listeria monocytogenes*, (ComBase lactic acid model pH 6.5 / 6.0)**	***Listeria monocytogenes*, (SSSP lactic acid model)**	***Yersinia enterocolitica*, (ComBase lactic acid model)**
1	0.59	0.70	0.41 / 0.34	0.22	0.79
2	1.18	1.41	0.82 / 0.67	0.45	1.59
3	**1.78**	**2.12**	**1.23 / 1.01**	**0.67**	**2.38**
4	2.37	2.82	1.64 / 1.35	0.89	3.18
5	2.96	3.53	2.04 / 1.68	1.12	3.96
6	3.55	4.23	2.45 / 2.02	1.34	4.75
7	4.15	4.94	2.86 / 2.35	1.56	5.52
8	4.74	5.64	3.27 / 2.69	1.79	6.27
9	5.33	6.35	3.67 / 3.03	2.01	6.95
10	5.92	7.06	4.08 / 3.36	2.24	7.52
11	6.52	7.75	4.48 / 3.69	2.46	7.91
12	7.11	8.41	4.89 / 4.03	2.68	8.13
13	7.70	8.95	5.29 / 4.36	2.91	8.23
14	8.27	9.23	5.68 / 4.70	3.13	8.28
15	8.80	9.32	6.07 / 5.03	3.35	8.29

6.5. Results for addressing TOR 4

Since the minimum growth temperatures of *Salmonella* spp. and VTEC are higher than those for *L. monocytogenes* and *Y. enterocolitica*, the growth potential of the last two bacteria during storage is greater. Based on the assumed minimum temperatures for growth of *Salmonella* and VTEC, growth will not occur below 7 °C and, thus, these pathogens cannot be used to define equivalent storage times at temperatures below 7 °C. In contrast, the impact of storage time and temperature is important for the estimated population densities of *L. monocytogenes* and *Y. enterocolitica* and these pathogens are more useful for defining storage times that would result in a growth potential equivalent to that of the baseline scenario.

At both temperatures, that is, for both red meat and poultry, maximum storage times for equivalent growth potential based on *Y. enterocolitica* are shorter than those based on *L. monocytogenes*. Storage of red meat at 1 °C for a maximum of 17 days (*L. monocytogenes*) or 12 days (*Y. enterocolitica*) results in a growth potential equivalent to that of the baseline scenario. Storage of red meat at 4 °C for a maximum time of 8.8 to 10.4 days, depending on the model (*L. monocytogenes*), or 8 days (*Y. enterocolitica*) results in a growth potential equivalent to baseline scenario 1 of 5 days at 7 °C.

The model used for defining maximum storage time does have an impact on the result even when the same environmental factors are included in the model, and this impact appears to be greater at the lower storage temperatures, i.e. for longer storage times. The maximum difference in the assessment of maximum storage times between models for red meat was around 7 days. Maximum storage times at 2 °C based on the SSSP *Listeria* lactic acid model was 21 days and based on the ComBase *Listeria* lactic acid model maximum storage was 14 days (Table 4). For *L. monocytogenes* on vacuum-packed red meat stored at 2 °C, the ComBase lactic acid model predicted equivalent growth at 39.2 days while the corresponding figure with the SSSP lactic acid model was 58 days (Table 4). These models are based on different experimental data, as well as different primary and secondary models, all of which may contribute to the differences in predictions. The SSSP Listeria model has been successfully validated in meat and meat products, which supports the use of this model for predicting growth

potential (Mejlholm et al., 2010). Validation studies of the ComBase models used have not been published but should be possible to develop with data in ComBase. However, this was not possible to do within the current timeframe.

In conclusion, the assessment shows that the pathogen, the model and the assumptions used to evaluate and define maximum storage times that will result in growth potential equivalent to that of the baseline scenarios will have an impact on the results. The impact is greater at the lower temperatures and thus longer storage times. Based on the evaluation and in terms of growth potential, the model used had a greater impact than a change in pH, in some cases over 2 $\log_{10}$ units. More important for estimated maximum equivalent storage times, the choice of pathogen resulted in greater differences than the choice of predictive model for a given pathogen in maximum storage time differences of 7 days.

Table 4: Storage times (days) at different temperatures corresponding to growth potential of the selected bacteria equivalent to that of red meat stored for 5 or 15 days at 7 °C. pH 6.5, a_w=0.993, lactic acid= 0 or 51.7 mM (4 654 ppm).

	***Listeria monocytogenes*, (Aerobic PMP model[a])**	***Listeria monocytogenes*, (Anaerobic PMP model[a])**	***Listeria monocytogenes*, (ComBase lactic acid model)**	***Listeria monocytogenes*, (SSSP lactic acid model)**	***Yersinia enterocolitica*, (ComBase lactic acid model)**
	Estimated growth potential after 5 days of storage at 7 °C (log_{10} CFU per cm^2)				
	5.23	5.81	3.71	2.32	6.18
Storage temperature (°C)	Storage times corresponding to growth potential of the selected bacteria equivalent to that of baseline scenario 1 (days)				
1	NA	NA	17.2	NA	12.4
2	NA	NA	14.0	20.8	10.8
3	NA	NA	11.0	14.2	9.3
4	8.8	8.2	9.1	10.4	7.9
5	7.2	6.9	7.4	7.9	6.7
6	6.0	5.8	5.9	6.2	5.8
	Estimated growth potential after 14 days of storage at 7 °C (log_{10} CFU per cm^2)				
	9.57= max, reached after 12 days	9.34= max, reached after 10 days	8.43	6.47	8.30= max, reached after 10 days
Storage temperature (°C)	Storage times corresponding to growth potential of the selected bacteria equivalent to that of baseline scenario 2 (days)				
1	NA	NA	47.9	NA	23.9
2	NA	NA	39.2	58.0	20.9
3	NA	NA	30.7	39.8	17.9
4	19.6	16.1	25.4	29.0	15.2
5	16.1	13.5	20.5	22.1	12.8
6	13.3	11.5	16.6	17.3	11.1

NA: not applicable as the storage temperature is below the temperature range of the model.
a: The lower temperature range of the PMP model is 5 °C but a minimum growth temperature of 7 °C was assumed in the assessment.

Table 5: Storage times at different temperatures corresponding to equivalent growth potential of the selected bacteria as in poultry meat stored for 3 days at 4 °C. pH=6.5 or 6.0, a_w=0.993, lactic acid= 0 or 51.7 mM (4 654 ppm).

	***Listeria monocytogenes*, Aerobic PMP model**	***Listeria monocytogenes*, ComBase lactic acid model**	***Listeria monocytogenes*, SSSP lactic acid model**	***Yersinia enterocolitica*, ComBase lactic acid model**
	Estimated growth potential after 3 days of storage at 4 °C (log_{10} CFU per cm^2)			
	1.78	1.23	0.67	2.38
Storage temperature (°C)	Storage times corresponding to growth potential of the selected bacteria equivalent to that of baseline scenario 3 (days)			
1	NA	5.7	NA	4.7
2	NA	4.6	6.0	4.1
3	NA	3.7	4.1	3.5

NA: not applicable as the storage temperature is below the temperature range of the model.

6.5.1. Alternative equivalent storage scenarios

The results presented in tables 4 and 5 form the basis for recommendations for maximum storage times at alternative storage temperatures depending on the desired level of caution. The level of caution is reflected in the choice of pathogen and model used to estimate the alternative storage scenario based on the equivalent growth potential. It is expected that different pathogens will display different growth potentials and temperature dependence, but the reasons for differences between the models for a given pathogen, especially when they include the same environmental factors, are harder to interpret. However, it seems reasonable to use models that include the effect of lactic acid present in the meat on growth. Thus, different approaches can be taken when defining storage times and temperatures. A precautionary approach can be applied, by selecting the pathogen and model that gives the shortest maximum storage times. Alternatively, maximum storage times can be based on the most credible model, i.e. a model validated for meat, or a mixture of these approaches can be applied.

As an example, the results in Tables 4 and 5 were used to illustrate alternative storage temperature and time scenarios with the same growth potential as in the corresponding baseline scenario (Table 6). A cautionary worst-case approach was applied based on the pathogen and the lactic acid model giving the shortest maximum storage times that resulted in equivalent growth potential. For example, red meat could be stored for 12, 11, 9, 8, 7 and 6 days at 1, 2, 3, 4, 5 and 6 °C, respectively before growth equivalent to that obtained at 7 °C after 6 days (based on 853/2004) would be obtained (Table 6).

For vacuum-packed red meat it is possible to evaluate storage only at 4 °C to 6 °C based on *L. monocytogenes* and baseline scenario 2 because the PMP predictive model describing anaerobic conditions, is applicable only at temperatures of 4 °C or more. In vacuum-packed red meat equivalent growth (to that obtained at 7 °C after 15 days, starting immediately *post mortem*) at 1, 2, 3, 4, 5 and 6 °C was predicted after 48, 39, 31, 25, 20 and 17 days, respectively (Table 6).

For poultry, equivalent growth to that obtained at 4 °C after 3 days was obtained after 5, 4 and 3 days at 1, 2 and 3 °C, based on the model predicting the shortest equivalent time of storage (Table 6).

Table 6: Maximum storage times for meat prior to mincing at alternative storage temperatures with the same growth potential as baseline scenarios compliant with current legislation. Storage time scenarios are based on models including the effect of lactic acid for *Y. enterocolitica* and *L. monocytogenes* yielding the shortest storage time at each temperature

		Maximum storage time (days)	
	Storage temperature (°C)	***Y. enterocolitica***	***L. monocytogenes***
Red meat, baseline scenario 1	1	12	17
	2	11	14
	3	9	11
	4	8	9
	5	7	7
	6	6	6
Vacuum packed red meat baseline scenario 2	1	NA	48
	2	NA	39
	3	NA	31
	4	NA	25
	5	NA	20
	6	NA	17
Poultry baseline scenario 3	1	5	6
	2	4	5
	3	3	4

NA: not applicable as the maximum population density was reached prior to storage of meat for 15 days in the baseline scenario.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General conclusions

- The data available on the growth of the relevant pathogens in/on the different meats during storage at different temperatures are limited and could not be used for a systematic approach to addressing the TORs. Thus, to assess pathogen growth and evaluate different storage scenarios, the growth potential of relevant bacterial pathogens on meat, between slaughter and minced meat preparation, was estimated using published predictive microbiology growth models assuming favourable growth conditions.

- The predicted pathogen growth potential was used to interpret different storage time and temperature scenarios in terms of microbial growth and, thus, potential risk. Combinations of storage temperatures and storage times that would result in a growth potential equivalent to that of storage baseline scenarios that are consistent with current legislation are assumed to represent equivalent risk.

- Growth estimated using models developed in laboratory media and assuming favourable conditions represents the maximum growth potential and is not expected to occur in meat during most storage conditions but represents a worst-case scenario. However, since equivalent growth potential is estimated under the same conditions and with the same model this is not expected to affect the results and conclusions

- Pathogenic bacteria such as *Salmonella* and pathogenic *E. coli* (VTEC) are found on red meat and/or poultry and may grow at temperatures as low as 5-7 °C. *Y. enterocolitica* is found on fresh pork and may grow at -2 °C. *L. monocytogenes* is an environmental contaminant that may also contaminate fresh meat and can grow at temperatures as low as -1 °C. These four bacterial hazards are discussed in this opinion. *Campylobacter* spp. do not usually grow outside their host and never at temperatures below 30 °C. Moreover, inoculation studies suggest that *Campylobacter* spp. decrease on poultry meat during chilled storage. For these reasons *Campylobacter* spp. were not considered for inclusion in addressing the terms of this mandate.

- The growth of *Salmonella* spp., VTEC, *L. monocytogenes* and *Y. enterocolitica* on red meat and poultry was predicted using different models at 1 to 6 °C and compared with the following baseline scenarios; (1) red meat stored under normal aerobic conditions at 7 °C for 5 days; (2) red meat stored in vacuum packs at 7 °C for 14 days and (3) poultry meat stored under aerobic conditions at 4 °C for 3 days.

- Current legislation (Regulation (EC) 853/2004) allows for storage of red meat for 6 days at a maximum temperature of 7 °C, 15 days for vacuum-packed meat also stored at a maximum temperature of 7 °C and 3 days for poultry meat at 4 °C. As it takes on average 24 hours (12 to 48 hours depending on the animal species) to chill red meat carcasses, this leaves 5 and 14 days' storage under aerobic and anaerobic (vacuum-packed) conditions, respectively.

- Storing red meat at temperatures below 5 °C extends the time available before growth equivalent to that obtained at 7 °C is achieved and prevents the growth of pathogens such as *Salmonella* spp. and VTEC.

- The impact of spoilage on maximum storage times was not considered.

Answers to TOR 3

To assess the impact of the time of storage of fresh meat intended for the production of minced meat on the risk linked to the microbiological growth of potentially harmful microorganisms.

- Growth was predicted for the four target pathogens at the currently mandated maximum temperature for storage of red meat of 7 °C. For *Salmonella* spp. and VTEC, a growth potential of up to 1.92 and 3.10 $\log_{10}$ CFU per cm^2, respectively, was estimated after 5 days while a growth potential of up to 5.81 and 6.18 $\log_{10}$ CFU per cm^2, respectively, was predicted for *L. monocytogenes* and *Y. enterocolitica* over the same time period.

- For poultry meat stored at 4 °C, growth was estimated only for *L. monocytogenes* and *Y. enterocolitica*, as this temperature is below the minimum growth temperature for *Salmonella* and VTEC. After 3 days of storage a growth potential up to 2.1 $\log_{10}$ CFU per cm^2 and 2.4 $\log_{10}$ CFU per cm^2 was predicted for *L. monocytogenes* and *Y. enterocolitica*, respectively.

- The model used influenced the predicted equivalent growth potential. For example, for *L. monocytogenes* on red meat stored at 2 °C, the ComBase lactic acid model predicted equivalent growth at 39.2 days, while the corresponding figure with the SSSP lactic acid model was 58 days.

Answers to TOR 4

To recommend, if appropriate, in relation to such risk, maximum times of storage of fresh meat intended for the production of minced meat.

- A cautionary worst-case approach was applied based on the pathogen and the lactic acid model giving the shortest maximum storage times that resulted in equivalent growth potential. In this example, red meat could be stored for 12, 11, 9, 8, 7 and 6 days at 1, 2, 3, 4, 5 and 6 °C, respectively, before growth equivalent to that obtained at 7 °C after 6 days (based on Regulation (EC) 853/2004) would be obtained.

- In vacuum-packed red meat equivalent growth (to that obtained at 7 °C after 15 days, starting immediately *post mortem*) at 1, 2, 3, 4, 5 and 6 °C was predicted after 48, 39, 31, 25, 20 and 17 days, respectively.

- For poultry, growth equivalent to that obtained at 4 °C after 3 days was obtained after 5, 4 and 3 days at 1, 2 and 3 °C, based on the model predicting the shortest equivalent time of storage.

RECOMMENDATIONS

- To support a risk-based approach additional data on time and temperature and other parameters affecting pathogen growth in meat are required for describing the variability of these parameters. Such data could then be used in risk assessments that would relate bacterial growth to public health risk.

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APPENDIX

Appendix A. Implementation of predictive growth models

Maximum specific growth rates were calculated by setting the appropriate user-defined model parameters in the ComBase Predictive Models Tool (reference ComBase[10]), the Pathogen Modeling Program (PMP[11]) and the Seafood Spoilage and Safety Predictor (SSSP)[12]. To estimate the growth potential at different storage times and temperatures, the primary models included in the tools available on websites or downloaded programs described in Table 1 were used. However, for VTEC (Ross et al., 2003) the primary model of Baranyi and Roberts (1994) was implemented in Excel as described in this opinion (Part 1), and for PMP models the same primary model was implemented in R statistical and modelling software (R Core Team, 2013) using the nlstools[13] package. The reason for implementing PMP models in R and not using the PMP website model was to enable the use of an initial level of 0 $\log_{10}$ cfu per cm^2 instead of the minimum adjustable initial pathogen level of 3 $\log_{10}$ cfu per cm^2. If this high initial level had been used, the maximum population density would have been reached prematurely, leading to an underestimation of the growth potential. Model parameters used were: lag phase = 0 (i.e. physiological state = 1.0), $N_0 = 0$ $\log_{10}$ cfu/cm^2, $a_w = 0.993$, pH 6.5, lactic acid = 0 or 51.7 mM (4 654 ppm). The minimum temperatures for growth were assumed to be 7.0 °C for *Salmonella* spp. and VTEC, 1.0 °C for *L. monocytogenes* and –1.0 °C for *Y. enterocolitica*.

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