

Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness¹

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-064)

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SUMMARY

Following a request from the European Commission, the Panel on Biological Hazards was asked to deliver a scientific opinion on updating the scientific literature from the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and provide scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness.

The BIOHAZ Panel concludes that after a general decline in the 1990s, the number of cases of listeriosis has increased since 2000 in Europe. The disease is still associated with pregnancy, but it is now predominantly associated with immuno-compromised persons amongst the older section of the population (> 60 years). At present, no routine methods permit the differentiation between virulent and avirulent strains of *L. monocytogenes*.

The foods which could be associated with transmission of listeriosis were mostly ready-to-eat foods that support growth of *L. monocytogenes*. Surveys of foods have not only collected data on the prevalence and contamination levels of *L. monocytogenes* in different food types, but also revealed associations with other parameters including: food packaging type, preparation practices (e.g. the use of slicing machines for meat products), storage temperatures, the stage of sampling with respect to shelf life, the lack of an effective HACCP system, and lack of

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education and training of food handlers. Growth of *L. monocytogenes* is a function of the type of food, the storage time and the storage temperature. Storage temperature at retail and domestic refrigerators can vary significantly, especially for the domestic refrigerators.

Microbiological criteria have been implemented in Europe according to the categories of ready-to-eat foods (e.g. foods intended for sensitive consumers, foods supporting or not supporting growth of *L. monocytogenes*). Application of microbiological criteria is only one of several management activities to ensure that ready-to-eat foods are of low risk for human. Microbiological criteria will assist in controlling the levels of *L. monocytogenes* e.g. absence in 25 g or ≤ 100 cfu/g at the point of consumption. Recent risk assessment concluded that most listeriosis cases are due to foods markedly above the latter limit.

The most recent Codex document on microbiological criteria for *L. monocytogenes* in ready-to-eat foods suggests a zero tolerance throughout the shelf life of the product for ready-to-eat foods in which growth of this microorganism can occur. Applying this criterion close to the end of shelf life could classify products as unsatisfactory, although they are of low risk. An additional option proposed in this Codex document is therefore to tolerate 100 cfu/g throughout the shelf life provided that the manufacturer is able to demonstrate that the product will not exceed this limit throughout the shelf life. For ready-to-eat foods that support growth of *L. monocytogenes*, it is impossible to predict with high degree of certainty that the level will or will not exceed 100 cfu/g during the shelf life of these products. Thus, applying this option may result in accepting a probability that foods with more than 100 cfu/g will be consumed. The impact on public health would depend whether the levels markedly above 100 cfu/g are reached.

The BIOHAZ Panel recommends that more thorough investigation of sporadic and outbreak cases of listeriosis as well as consumption data of ready-to-eat foods that support growth of *L. monocytogenes* are needed to better assess the risk and improve our knowledge of the foods responsible of listeriosis. Comparison between studies (e.g. surveys) should be made only when similar sampling strategies are applied and studies should focus on ready-to-eat foods able to support growth of *L. monocytogenes*. As the application of microbiological criteria is only one of several management activities to ensure that ready-to-eat foods are of low risk for humans, application of GHP in combination with HACCP should be consistently applied to minimise the initial contamination at manufacturing level, and/or reducing the potential for growth of *L. monocytogenes*. The chill chain especially at the domestic level and dietary and food storage advice (particularly for the elderly) should be improved to reduce the risk of listeriosis.

Key words: ready-to-eat foods, *Listeria monocytogenes*, microbiological criteria

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BACKGROUND AS PROVIDED BY DG SANCO

EU scientific opinions and new EU criteria

The Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) in 1999 and the Scientific Committee on Food (SCF) in 2000 recommended that it should be an objective to keep the *Listeria monocytogenes* concentration in food below 100 cfu/g in order to ensure the safety of foodstuffs.

These scientific opinions form the basis of the Community *L. monocytogenes* criteria for ready-to-eat foods in Commission Regulation (EC) No 2073/2005² on microbiological criteria for foodstuffs. The new Community criteria cover all ready-to-eat foods during their entire shelf life. A distinction has been made between the following categories for ready-to-eat foods:

- Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes
- Ready-to-eat foods able to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes
- Ready-to-eat foods unable to support the growth of *L. monocytogenes*, other than those intended for infants and for special medical purposes

Codex Committee on Food Hygiene meeting in 2006

At the 38th Session of the Codex Committee on Food Hygiene (CCFH), Houston, USA, 4-9 December 2006, the Draft Guidelines on the Application of General Principles of Food Hygiene to the Control of *Listeria monocytogenes* in Ready-to-Eat Foods were considered.

In the meeting the European Community and its Member States (ECMS) were generally very pleased and supported the Draft Guidelines as well as Annex I: Recommendations for an environmental monitoring program for *Listeria monocytogenes* in processing areas. The Committee agreed to forward the Draft Guidelines including Annex I for final adoption at step 8.

However, the ECMS expressed the need for further development of microbiological criteria for *L. monocytogenes* in ready-to-eat foods. The Committee was reminded of the history of the development of the Draft Guidelines which arose out of the need for microbiological criteria for *L. monocytogenes*. The ECMS' view was that the proposed guidelines would ultimately benefit from an inclusion of microbiological criteria. While the ECMS consider that there is already enough scientific data available from international risk assessments and evaluations to allow Codex set criteria for ready-to-eat foods in international trade, the ECMS urged the Committee to take a firm commitment to engage in the elaboration of appropriate criteria for ready-to-eat foods in the coming year.

The Committee was informed that the European Community has recently established *L. monocytogenes* criteria covering all ready-to-eat foods during their shelf life in order to harmonise criteria within the EU and thus facilitating international trade of foods. Furthermore, scientific justification and broad consensus was sought for the establishment of the new EU criteria.

The Committee agreed to establish a physical working group to be led by Germany with the terms of reference to develop microbiological criteria on *L. monocytogenes* in ready-to-eat

² Regulation (EC) No 2073/2005, OJ L 338, 22.12.2005, p. 1, corrected by OJ L 278, 10.10.2006, p. 32, and OJ L 283, 14.10.2006, p. 62.

foods. The Committee was of the view that this work on microbiological criteria would be completed over two sessions of the Committee for adoption by the Codex Alimentarius Commission in 2009.

It is obvious that in the context of development of *L. monocytogenes* criteria, the link between the criteria, the outcome of internationally available risk assessments as well as the new concepts on microbiological metrics (e.g. FSOs, POs, PCs) will be considered although it was noted by the CCFH (Houston 2006) that the practical application of these new metrics is still at a very early stage of development.

From the EU perspective, the EFSA is particularly requested to give scientific elements to the Commission in order to identify the EU position on the establishment of *L. monocytogenes* criteria in the CCFH working group.

TERMS OF REFERENCE AS PROVIDED BY DG SANCO

The European Food Safety Authority is asked to:

- Update the scientific literature from the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods.
- Provide scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods (absence in 25 g, 100cfu/g and higher levels) and the related risk for human illness.

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ASSESSMENT

1. Introduction

In 1999, the Scientific Committee on Veterinary Measures relating to Public Health (SCVPH) issued an opinion on *Listeria monocytogenes* (SCVPH, 1999). The scope of the present scientific opinion is to update the scientific literature of the SCVPH opinion on *L. monocytogenes*. For this purpose, the elements of the microbiological risk assessment (*i.e.* hazard identification, hazard characterisation and risk characterisation) as described and presented in the SCVPH opinion are followed and updated taking into consideration the new available scientific information from 1999 to 2007. Moreover, for the purpose of this opinion, the following definition of ready-to-eat food is used:

Ready-to-eat food: means food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern³.

Finally, scientific advice on different levels of *L. monocytogenes* in ready-to-eat foods and the related risk for human illness will be provided.

2. Hazard identification (update of § 4.1. in the SCVPH 1999 opinion)

The hazard identification as described in the SCVPH opinion on *L. monocytogenes* has been updated and the previous conclusions remain valid: “*While listeriosis is an infrequently occurring disease, the high case fatality rate results in listeriosis being an infrequent but serious public health threat in particular for high risk groups such as elderly, other immunocompromised persons (i.e. cancer, HIV, rheumatic diseases) and pregnant women*”.

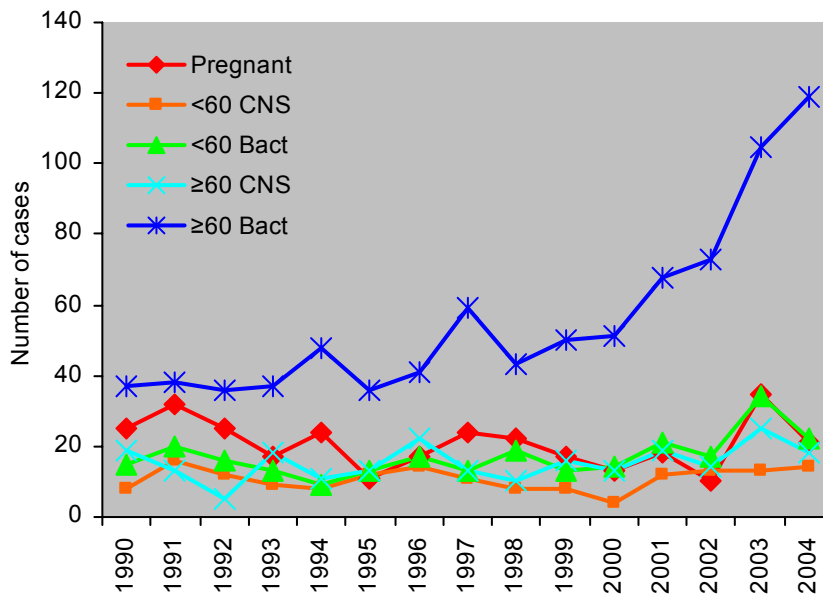
Human listeriosis showed a general decline in the numbers of cases reported during the 1990s within Europe from peaks in incidence during the 1980s, and this decline coincided with a substantial reduction in both the proportion of ready-to-eat food products contaminated by *L. monocytogenes*, and the levels of contamination detected (McLauchlin, 1996a; Goulet *et al.*, 2001; McLauchlin, 2006). However the epidemiological pattern of human listeriosis shows evidence of change after 2000 in some Member States.

Collated numbers of cases for statutory reporting of zoonoses from 26 Member States (EFSA, 2006; EFSA, 2007a) show an increase from 910 human listeriosis cases reported in 2001 to 1,427 cases in 2005, and 1,583 cases in 2006. Although this increase from 2001 to 2006 may reflect changes in surveillance systems and improved rates of reporting, at least for some Member States (see below) this appears to reflect true increases in the numbers of cases. Amongst the 26 Member States reporting cases of human listeriosis in 2005, the average incidence over all the EU was 0.3 cases per 100,000 of the population: 12 Member States reported rates of ≤ 1 cases per 100,000, and the highest rates were reported in Denmark (0.90 cases per 100,000), Belgium (0.8 cases per 100,000), Finland (0.7 cases per 100,000) and Germany (0.6 cases per 100,000). In 2006, the highest reported rates were in Denmark (1.0 cases per 100,000), Finland and Luxembourg (0.9 cases per 100,000), Czech Republic (0.8 cases per 100,000) and Germany (0.6 cases per 100,000).

³ According to the Regulation (EC) No 2073/2005, OJ L 338, 22.12.2005, p. 1, corrected by OJ L 278, 10.10.2006, p. 32, and OJ L 283, 14.10.2006, p. 62.

Surveillance methods between Member States have been previously compared (de Valk *et al.*, 2005) and there are considerable national differences. However these different rates probably reflect real differences in the numbers of cases in different Member States. 67% of all cases within the EU in 2005 were reported from Germany, France and the UK, and all three of these Member States reported increases since 2001: the increases in France were considerably smaller than those for Germany and the UK. The increase in Germany was described in greater detail by Koch and Stark, (2006) and despite changes in surveillance and raised diagnostic awareness (listeriosis became a notifiable disease in 2001) a more than doubling of the numbers of reported cases occurred between 2001 and 2005. The increase in cases in Germany occurred almost exclusively in patients ≥ 60 years of age and did not appear to be linked to any single common-source outbreak: the cases overall were predominantly sporadic in nature. The increase in the UK has also been described in some detail (Gillespie *et al.*, 2006), and showed similar characteristics to that in Germany, although there is no evidence on the relationship between these two national increases. The increase in the UK occurred predominantly in patients aged ≥ 60 years of age and who presented bacteraemia (the presence of bacteria in the blood) without central nervous system infection. The numbers of cases reported amongst patients < 60 years of age, those with central nervous system invasion, and those associated with pregnancy have remained similar since 1990 (Figure 1). Increases occurred in most regions of England, Wales, Scotland and Northern Ireland, amongst both genders, were due to multiple subtypes of *L. monocytogenes*, could not be explained by common source outbreaks and were predominantly sporadic in nature. The UK increase was independent of demographic changes and has resulted in an approximate tripling in the age specific rates of listeriosis in England and Wales between 1990 to 2006 (Figure 2). A doubling in the total numbers of cases in Denmark and a 25% increase has occurred in France between 2002 and 2006 (EFSA, 2007a).

Within Europe, 55.6 % of all human listeriosis cases were reported in patients above 65 years (EFSA, 2007a), therefore although this disease continues to occur in association with pregnancy, it is now predominantly an infection of immuno-compromised individuals amongst the older sections of the population. Control measures including dietary advice were previously predominantly targeted towards the pregnant woman. However because of the change in the age distribution of cases, together with a demographic shift in the population as a whole, control measures now will therefore be most effective when targeted towards older segments of the population.



CNS: Central Nervous System
Bact.: Bacteraemia

Figure 1. Numbers of reported listeriosis cases in England and Wales 1990-2006 (data from Gillespie *et al.*, 2006).

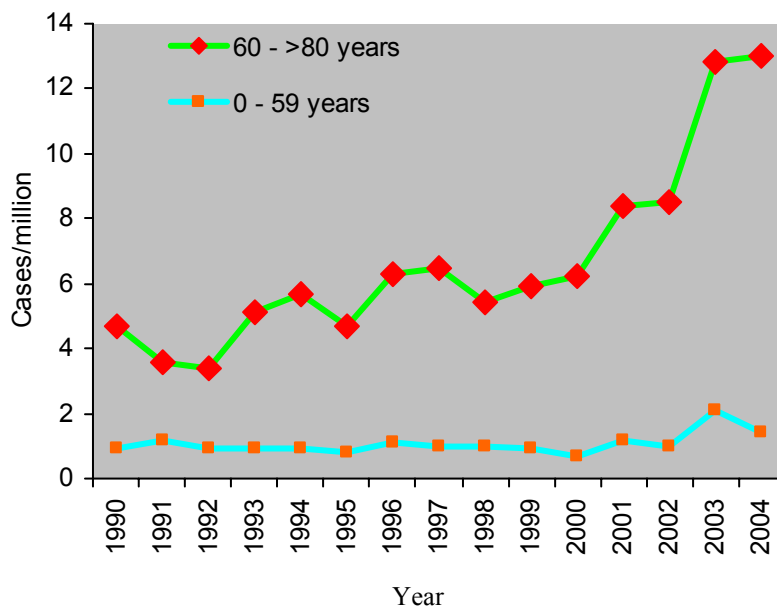


Figure 2. Age specific rates of listeriosis in England and Wales 1990-2006 (data from Gillespie *et al.*, 2006).

Data on listeriosis in USA, show a similar marked reduction to that in Europe which coincided with a decrease in contamination of ready-to-eat food products (Voetsch *et al.*, 2007). Similarly to Europe, human listeriosis in the USA is predominantly sporadic in nature (only 5% of cases could be identified as associated with outbreaks), 88% were not associated with pregnancy, 71% were aged >44, and 40% aged ≥ 70 (Varma *et al.*, 2007; Voetsch *et al.*, 2007).

Previous analysis of sporadic cases and outbreaks of human listeriosis have shown that the foods associated with transmission are predominantly ready-to-eat, capable of supporting the growth of *L. monocytogenes* (McLauchlin, 1996). Data on outbreaks and sporadic cases of human listeriosis from the world literature were previously presented (SCVPH, 1999), and a list of foodborne incidents which have been described since the SCVPH opinion is shown in Table 1. The foods associated with these incidents showed similar characteristics to those outlined above.

Table 1. Some foodborne listeriosis cases reported since 1999

Note: only outbreaks and sporadic cases for which the vehicle was identified are mentioned.

Year	Country	Numbers of cases ^(a)	Vehicle	Reference
1998	USA	108	Frankfurters	(Mead <i>et al.</i> , 2006)
1999	England	4	Hospital sandwiches	(Gillespie <i>et al.</i> , 2006)
1999-2000	France	10	Pork rillettes	(de Valk <i>et al.</i> , 2001)
1999-2000	France	32	Jellied pork tongue	(de Valk <i>et al.</i> , 2001)
2000	New Zealand	4*	Sliced cooked meat	(Sim <i>et al.</i> , 2002)
2000	USA	13	Mexican style soft cheese	(MacDonald <i>et al.</i> , 2005)
2000	USA	30	Cooked turkey	(Olsen <i>et al.</i> , 2005)
2001	USA	16*	Sliced cooked turkey	(Frye <i>et al.</i> , 2002)
2001	Sweden	50*	Cheese	(Carrique-Mas <i>et al.</i> , 2003)
2001	Japan	38*	Cheese	(Makino <i>et al.</i> , 2005)
2002	USA	54	Cooked turkey meat	(Gottlieb <i>et al.</i> , 2006)
2002	Canada	17	Cheese	(Gaulin <i>et al.</i> , 2003)
2003	England	17	Butter	(Gillespie <i>et al.</i> , 2006)
2003	England	18	None identified	(Gillespie <i>et al.</i> , 2006)
2003	Wales	2	Hospital sandwiches	(Gillespie <i>et al.</i> , 2006)
2003	England	5	Hospital sandwiches	(Gillespie <i>et al.</i> , 2006)
2004	England	2	Hospital sandwiches	(Gillespie <i>et al.</i> , 2006)
2005	Switzerland	10	Tomme cheese	(Bille <i>et al.</i> , 2006)
2006	Czech Republic	78	Soft cheese	(Vit <i>et al.</i> , 2007; EFSA, 2007a)
2006	Germany	6	Hard cheese	(EFSA, 2007a)

(a): All cases severe systemic disease except those marked with * where febrile gastroenteritis was the predominant case presentation.

3. Hazard characterisation (update of § 4.2. in the SCVPH 1999 opinion)

3.1. Virulence and pathogenicity (update of § 4.2.2 in the SCVPH 1999 opinion)

The surface protein InternalinA, encoded by the *inlA* gene, has a key role in the internalisation of *L. monocytogenes* in the intestinal epithelium by promotion of invasion of enterocytes, translocation across the intestinal barrier and mediation of access to deeper tissues (Lecuit *et al.*, 2001). A specific interaction between *inlA* and human E-cadherin has been reported (Lecuit *et al.*, 1999). It was found that while guinea pigs, chickens and rabbits carry a homologue to the human E-cadherin encoding a proline in position 16, rats and mice have a glutamic acid at this position, rendering the E-cadherin of these rodents unable to promote entry of *L. monocytogenes* into the intestinal epithelium.

During the last decade several attempts have been made to find an animal virulence model suitable for differentiation between *L. monocytogenes* isolates (Schlech *et al.*, 1993; O'Driscoll *et al.*, 1996; Larsen *et al.*, 2002; Smith *et al.*, 2003; Takeuchi *et al.*, 2006). Most of these models have been based on rats or mice. Examples of animal models carrying a homologue to the human E-cadherin include a nonhuman primate model (Smith *et al.*, 2003) and a genetically modified mouse model (Lecuit *et al.*, 1999). Some models bypassed the intestines by injecting the bacteria into the peritoneum (O'Driscoll *et al.*, 1996; Takeuchi *et al.*, 2006) others have used the oral gastric route (Schlech *et al.*, 1993; Larsen *et al.*, 2002; Smith *et al.*, 2003). Except for the primate model, these models do not include the complete foodborne route of exposure, and have not been applicable for investigating differences in virulence. Epidemiological evidence and behaviour of wild type strains using *in vivo* and *in vitro* models suggest that there are naturally occurring a-virulent or virulent attenuated strains (McLauchlin *et al.*, 2004; Liu *et al.*, 2007). For example, Jacquet *et al.* (2004) reported that isolates from food were more likely to express a truncated version of the internalin A protein than those causing disease in humans. However virulence is a product of the expression of multiple genes (Liu *et al.*, 2007) and phenotypic and genotypic tests are not routinely available to characterise the expression of all virulence genes. Hence routine methods which can differentiate between pathogenic, non-pathogenic or less virulent strains are not currently available. Therefore, the conclusion from the SCVPH opinion that “*all L. monocytogenes, including those present in food, should be regarded as potentially pathogenic*” is still valid.

3.2. Dose/Response (update of § 4.2.3 in the SCVPH 1999 opinion)

Since 1999, new attempts to characterise the hazard of *L. monocytogenes* with dose-response models have been published (Chen *et al.*, 2003; FDA/USDA/CDC, 2003; FAO/WHO, 2004; McLauchlin *et al.*, 2004; Chen *et al.*, 2006). Dose-response modelling is now usually based on the family of singly hit models. The basic assumption underlying these models are (i) single-hit (any viable microorganism has a non zero probability of causing infection and illness), (ii) independent action (the single-hit probability is independent of the total number ingested).

A FAO/WHO working group proposed an international consensus, with two models, one for the population with increased susceptibility (supposed to represent 15% to 20% of the total population but 90 to 98% of the cases) and the other for the rest of the population (FAO/WHO, 2004). For example, for a dose of 1×10^{10} cfu/serving (e.g. 100 g of a food contaminated at 1×10^8 cfu/g), the median listeriosis rate in the susceptible population is estimated at 1 in 100 servings, with lower (5%) and upper (95%) uncertainty bounds of 1 in 400 and 1 in 10. For the same dose, in the healthy population, the median listeriosis rate is estimated at 1 in 4,000 servings, with lower (5%) and upper (95%) uncertainty bounds of 1 in 30,000 and 1 in 400.

The FAO/WHO (2004) working group also further detailed the characterisation of the different susceptible population groups (elderly, infants, pregnant women, and immuno-compromised patients) relative to the general population. The relative susceptibility of populations at risk, based on epidemiological data from France and the US, was calculated. In the FAO/WHO model, the estimated *r*-value (*i.e.* the average probability that ingestion of cfu leads to illness) is constant for a given population. However, in the available data, the maximum levels of *L. monocytogenes* per individual serving are unspecified (reported as > x cfu/g) which introduces some additional uncertainty. Therefore the *r*-values were calculated in the FAO/WHO model for various assumptions of this highest dose level. For the most susceptible group (transplant patients), the estimated *r*-values varied from 5.8×10^{-10} (estimated highest log dose 7.5) to 2.3×10^{-11} (estimated highest log dose 10.5). In comparison, similar *r*-values estimates ranged from 2.23×10^{-13} to 7.45×10^{-15} for the non-immuno-compromised population. These *r* values are extremely low compared to other foodborne pathogenic bacteria.

Setting the susceptibility of non-immuno-compromised population to 1, those people having received organ transplants are 2584-fold more susceptible when they are challenged with an infective dose of log 7.5. Elderly people (above 60 years) may be 1.6-7.5 fold more susceptible than younger, non immuno-compromised people. The uncertainties in the resultant dose–response relationships are then considerable. However, with all these models, there is common agreement that the risk per serving would be roughly proportional to the exposure (number of cells ingested by the consumer); the uncertainty, how large it may be, concerning only the value of the proportionality parameter. For this reason, it is reasonable to assume (*i*) that any mitigation strategy able to reduce the exposure will reduce the risk, (*ii*) that, except at very high doses, 1,000 servings with a specified level of contamination has the same public health impact as 10,000 servings with ten-fold less organisms (FAO/WHO, 2004), (*iii*) that most predicted cases result from the exposure of the susceptible population to the very few highest contamination levels.

4. Exposure Assessment (update of §4.3. in the 1999 opinion)

4.1. Occurrence in foods

The 2004-2005 data support the previous conclusions from the SCVPH that *L. monocytogenes* is ubiquitous and that almost all food categories can be contaminated with *L. monocytogenes*, in some cases with a high frequency. The SCVPH opinion reported a range of contamination with *L. monocytogenes* (*i.e.* % positive findings) for various foods: 7-36% for mince meat, 0-52% for meat products; 9-85% in poultry meat, 4-60% in fish products, 1-12% in vegetable salads, 2-12% for raw milk. Quantitative data were also presented showing that most contaminated food samples contained less than 100 cfu/g but a minority (e.g. 0 to 1.8% according to the food type) contained more than 100 cfu/g.

In the Community Summary Reports on Trends and Sources of Zoonoses in the EU in 2004, 2005 and 2006 (EFSA, 2005; EFSA, 2006; EFSA, 2007a), the overall ranges of contamination of foods with *L. monocytogenes* were (presence in 25 g): 0-48% in meat products, 0-40% in poultry meat product, 0-30% in fish products, 0-100% in raw milk. In addition, the Community Summary Reports provided range of contamination for other foods, not considered in SCVPH (1999): 0-38% for cheeses, 0-33% for fruit and vegetables, 0 to 33% for sandwiches.

In the 2004, 2005 and 2006 Community Summary Reports, most surveys found absence of *L. monocytogenes* for ready-to-eat dairy products, cheeses, ready-to-eat fruits and vegetables, and surveys with high frequencies of positive samples were an exception in the EU. Indeed, the

mean % of positive samples for these products was below 1% in 2005 (1.2% for cheeses in 2006). In the Community Summary Reports, the highest frequencies of positive samples in ready-to-eat foods was found in meat and fish products: 2.7% for meat product and 7.5% for fish products in 2005, 3.5% for bovine meat product, 2.7% for pork meat products and 4.9% for fish products in 2006. The prevalence of food samples containing more than 100 *L. monocytogenes* per g was in accordance with those reported in the SCVPH opinion (0 – 1.8%), except for a few surveys on meat and fish products with around 5% (meat) or even up to 20% (fish) of samples containing more than 100 *L. monocytogenes* per g. In the Community Reports from 2004 to 2006, the presence of few samples containing more than 100 *L. monocytogenes*/g was observed in all the food categories considered.

Data presented in the 2004, 2005 and 2006 Community Reports showed that ready-to-eat fish products were more frequently contaminated with *L. monocytogenes*, and contained a higher proportion of samples with more than 100 *L. monocytogenes* per g than other food categories (samples with > 100 cfu/g were found in 29% of surveys of fish products compiled in the 2005 Community Report). Ready-to-eat meat products then came next in decreasing order of contamination (Appendix I). This is consistent with a survey of 2,217 samples of fish and meat products imported to or exported from Switzerland which concluded that the highest prevalence of *L. monocytogenes* was in fish products (Jemmi *et al.*, 2002).

However, the observation that some food categories were more frequently contaminated with *L. monocytogenes* than others does not imply that these food categories are more likely to cause listeriosis. In particular, it would be necessary to estimate whether these foods support growth of *L. monocytogenes*, when sampling occurred in relation to shelf life, and whether they undergo any listericidal treatment before consumption.

The last Community report (EFSA, 2007a) gives more indications on the stage of sampling (e.g. retail), the nature of the food (e.g. already cooked, stabilized, soft versus hard cheese) and the intended use (e.g. intended to be eaten cooked). The results reported in the Community Reports 2004 and 2005 are in accordance with other surveys in EU published since 2000. For instance 0 or 2-3% samples of ready-to-use vegetables at retail contained *L. monocytogenes*, with 0 or below 1% samples containing more than 100 *L. monocytogenes*/g (Sagoo *et al.*, 2001; Sagoo *et al.*, 2003a; Sagoo *et al.*, 2003b; Francis and O'Beirne, 2006). In smoked fish at retail, 13.3% to 77.8% samples were found positive for *L. monocytogenes* with 0.3% to 11% containing more than 100 cfu/g (Dominguez *et al.*, 2001; Medrala *et al.*, 2003; Suppin *et al.*, 2006). A survey on pâté found 5.4% of samples contaminated (Dominguez *et al.*, 2001). Among dairy products, 0.4% butter samples contained *L. monocytogenes* (Lewis *et al.*, 2006). Prevalence of *L. monocytogenes* in sausage at the end of the production process was 10% in France for dry fermented sausages (Thevenot *et al.*, 2006), and 3 to 4% for raw spreadable sausages in Germany (Hechelmann *et al.*, 2002). In both cases, all contamination levels were below 100 cfu/g.

Whether the incidence of *L. monocytogenes* in ready-to-eat foods has changed since the SCVPH 1999 report is difficult to assess, in particular because of the extreme variability on the percent positive samples among surveys. In addition, sampling plans and methods used for the various surveys compiled in the different reports or presented in the scientific publications are usually not the same, which provides difficulties in comparisons of prevalences between different surveys. One observation however is that in both the Community reports 2004-2005 and the SCVPH in 1999, surveys with very high frequency of contaminated samples were reported, albeit rarely.

It may nevertheless be that the occurrence of *L. monocytogenes* in ready-to-eat foods has decreased in most instances, presumably as a result of efforts of food industries to improve

hygiene of their products. Longitudinal surveys in both France and England and Wales suggests that at least for some food types there have been improvements in the levels of *L. monocytogenes* contamination during the 1980s and 1990s (Goulet *et al.*, 2001; McLauchlin, 2006).

Tracing back foods implicated in sporadic listeriosis is rarely successful. For example, molecular typing of strains isolated from pork meat in France (Hong *et al.*, 2007), cheeses in Portugal (Malak *et al.*, 2001) and cheese in Belgium (Leite *et al.*, 2006), failed to find any relationship with strains isolated from patients.

Surveys of foods performed since the SCVPH opinion (SCVPH, 1999) have not only collected data on the prevalence and contamination levels of *L. monocytogenes* in different food types, but also revealed associations with other parameters which provide an evidence basis for control of this bacterium in the food chain. Parameters associated with the presence of the bacterium include food packaging type, preparation practices (e.g. the use of slicing machines for meat products), storage temperatures, the stage of sampling with respect to shelf life, the lack of an effective HACCP system, and the lack of education and training of food handlers (Lewis *et al.*, 2006; Lianou and Sofos, 2007; Little *et al.*, 2007; Sagoo *et al.*, 2007).

4.2. Origin of food contamination

The SCVPH 1999 opinion reported an occurrence of *L. monocytogenes* in food animals between 1 to 10%. This is consistent with most of the surveys compiled in the Community reports 2005 and 2006 (EFSA, 2006; EFSA, 2007a), except for a few surveys with a higher proportion (up to one third) of animals reported positive in 2006.

The SCVPH 1999 described the establishment of *L. monocytogenes* in food processing factory environments which becomes a source of contamination to foods during processing. This has been confirmed by several studies using molecular typing methods. In smoked fish, most strains isolated in finished ready-to-eat products were strains repeatedly isolated from processing plants (Lappi *et al.*, 2004; Nakamura *et al.*, 2004; Thimothe *et al.*, 2004). The prevalence of *L. monocytogenes* was higher in processed than in raw fish (Medrala *et al.*, 2003) indicating establishment of the bacterium and contamination in the processing plant. Similarly, in a review of *L. monocytogenes* in uncooked pork meat product, Thevenot *et al.* (2006) conclude that contamination usually increases during processing. The presence of *L. monocytogenes* on the surface of packaged frankfurters (Luchansky *et al.*, 2002) was most likely the result of post-processing contamination in the plant. Evidences of plant contamination with *L. monocytogenes* of raw milk in the dairy, and of cheese during cheese making were also reported (Waak *et al.*, 2002; Kabuki *et al.*, 2004).

Guidance for environmental sampling has been proposed to better control *L. monocytogenes* in the food processing environment (Tompkin, 2002).

4.3. Chill chain (refrigerator temperatures)

Temperature is one of the most important factors affecting growth of *L. monocytogenes* in foods and the detailed information on the temperature conditions in the food chain is a prerequisite for effective risk assessment of *L. monocytogenes* in ready-to-eat foods. The SCVPH opinion did not include any information about the temperatures undergone by food products along the chill-chain, probably because of the scarcity of such data in 1999. It is now well-known that the first steps of the chain (*i.e.* processing and distribution) are in most cases satisfactorily controlled (Afchain *et al.*, 2005) whereas some retail display cabinets and especially the home refrigerators are less controlled. Temperature control during transport,

storage and at retail is usually the responsibility of the retailer and not within the manufacturers' direct control while the temperature of domestic refrigerators depends on the diligence of the consumer.

Ready-to-eat food products may spend a considerable part of their shelf life in a domestic refrigerator, rather than in a retail or industrial chiller. Domestic refrigerator temperatures can therefore have a significant effect on the safety of chilled foods. The available data on the temperature of retail refrigerators in Europe are limited. Table 2 summarizes the results of survey studies on retail storage temperature in France, Slovenia and Greece. The mean temperature in the available surveys ranges from 3.7 to 5.6 °C.

Table 2. **Temperature survey data on retail refrigerators in Europe.**

Year reported	Country	n	T _{min} °C	T _{mean} °C	T _{max} °C	Reference
1996	France	-	-	4.0	-	(Pierre, 1996)
2001-2003	France	314	-	3.7	-	(Derens <i>et al.</i> , 2006)
2003	France	67	1.4	5.6	9.8	(Afchain <i>et al.</i> , 2005)
2006	Slovenia	1286	-2.2	4.6 (weighted)	12.2	(Likar and Jevsnik, 2006)
2006	Greece	30	-0.1	4.4	10.6	(Koutsoumanis and Angelidis, 2007)

The survey in Greece was conducted from 2004 to 2006 in 9 large retail stores and temperature was recorded with miniature dataloggers (every 20 minutes for a period of one week). Temperature ranged from -0.1 to 10.6°C with a mean of 4.4°C and a standard deviation of 2.6°C. In 66.7% of the tested refrigerators the temperature was above 4°C while in 3.6% the temperature was above 10°C.

Data from seventeen surveys of domestic refrigerator temperatures from 7 Member States are presented in Appendix II. The data are presented to facilitate comparison between surveys, although this is not always possible due to the use of different parameters and temperature ranges in the reporting of the data. Of the 11 surveys (1,924 samples) for which a mean temperature was given, this ranged from 5 to 7.2°C. The weighted mean of the means was 6.5°C. No regional or temporal trends within Europe are evident from these data. The temperatures in the refrigerators in EU are higher from those in USA as illustrated by Figure 3 which presents the cumulative distributions of 16 recent studies (in the 2000ies): 14 European studies versus 2 studies in USA.

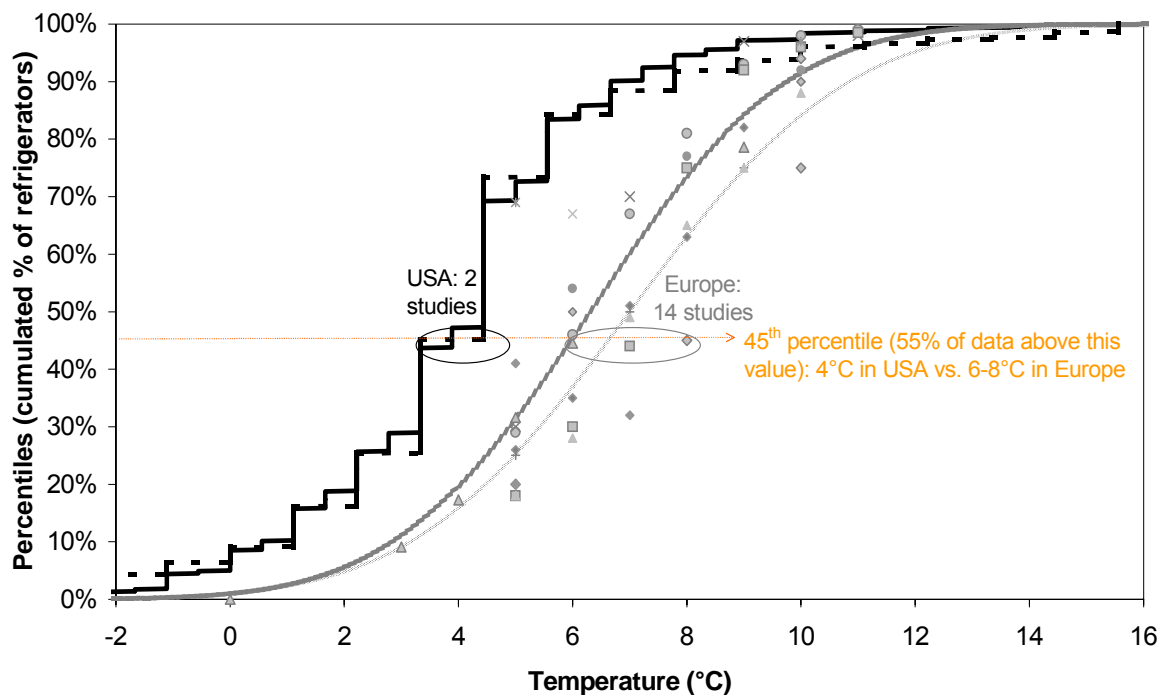


Figure 3. **Temperature distribution in domestic refrigerators in the 2000 in Europe and in USA.** [grey dots (non-parametric distributions) or S-shaped curves (parametric distributions) represent 14 studies in Europe: (AFF, 2000; Guingois, 2000; Volatier, 2000; Laguerre *et al.*, 2002; Bakalis *et al.*, 2003; Ghebrehewet and Stevenson, 2003; Afchain *et al.*, 2005; Azevedo *et al.*, 2005; Kennedy *et al.*, 2005; Taoukis *et al.*, 2005; Terpstra *et al.*, 2005; Breen *et al.*, 2006; ISSP, 2006; Xanthiakos, 2006b) and black lines (non-parametric distributions) represent 2 studies in USA: (Audits-International., 2000; Kosa *et al.*, 2007)].

In one of these surveys (Xanthiakos, 2006b), the temperature variability was recorded at the different locations in the refrigerators (Figure 4). A significantly higher mean temperature was observed in the door shelf (8.4°C) compared to the upper (7.6°C), the middle (6.3°C) and the lower shelf (6.7°C). The latter observation is important since some ready-to-eat foods such as pasteurised milk are usually stored in the door shelf of the domestic refrigerator (Xanthiakos, 2006b).

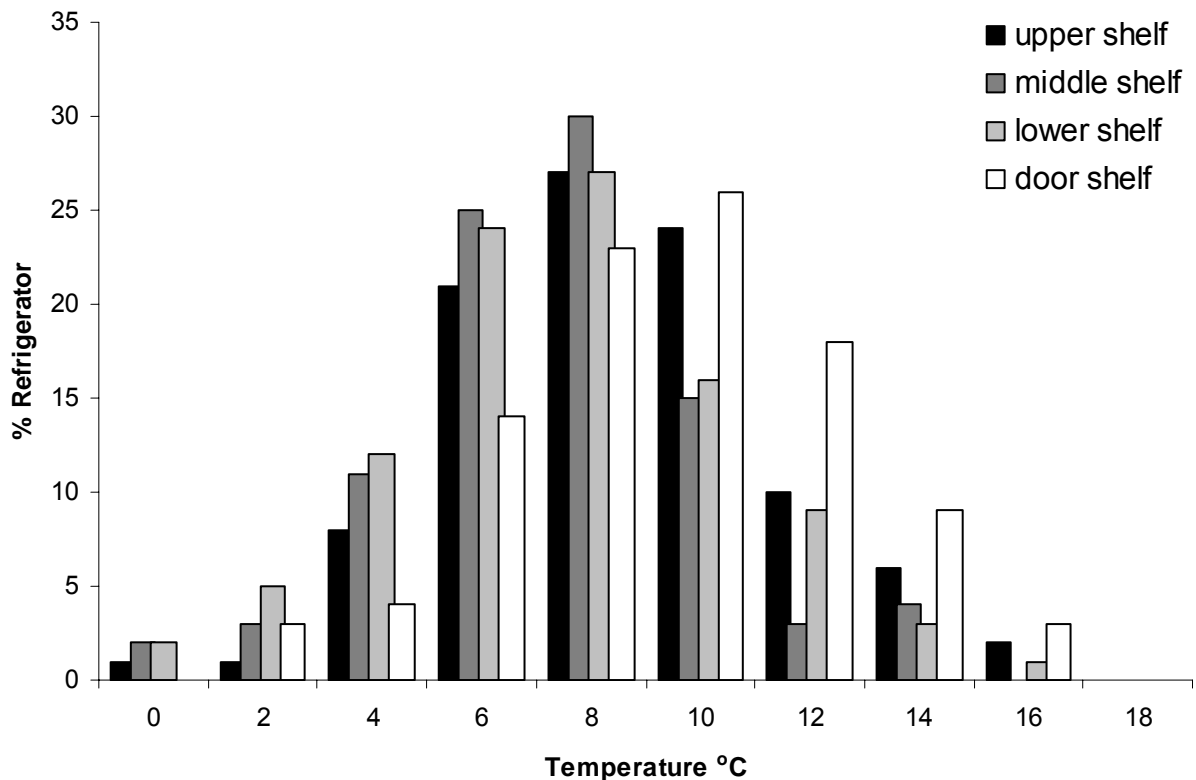


Figure 4. Examples of temperatures found in different locations of domestic refrigerators (Xanthiakos, 2006b).

The above data clearly show that storage temperature at both the retail and domestic level can vary significantly between refrigerators. Because of this variability, it is very difficult to define the “reasonable foreseeable conditions” for the chill chain as stated in the Regulation (EC) No 2073/2005. Temperature variability in the chill chain should be taken into account in both challenge tests and in the use of predictive models to establish shelf life of foods. Stochastic modelling approaches that take into account the variability of factors affecting the growth of pathogens can be more effective in evaluating compliance with the safety criteria of Regulation (EC) No 2073/2005 (Koutsoumanis and Angelidis, 2007).

The available risk assessments for *L. monocytogenes* in ready-to-eat foods (FDA/USDA/CDC, 2003; FAO/WHO, 2004) indicate inadequate refrigeration to be one of the key factors contributing strongly to the risk of listeriosis. It needs to be noted, that these risk assessment are based on data for domestic storage conditions derived from USA. Based on the available data, the temperature of the European domestic fridges is higher compared to that in the USA (Figure 3). This difference could be translated to an increased growth of *L. monocytogenes* in a ready-to-eat product stored in a domestic refrigerator in Europe compared to USA (see for details in Appendix III).

4.4. Growth curves and growth models

The SCVPH opinion reported some examples of growth rates of *L. monocytogenes* in some ready-to-eat foods. This information is still valid and illustrates the ability of *L. monocytogenes* to grow in a wide range of foods. However, recent developments, especially in the field of predictive microbiology, which is - as already stated by the SCVPH opinion - an effective tool for estimating *L. monocytogenes* growth in different food groups, have provided additional data and tools.

Since 1999, main advances concerning growth have concerned:

- the investigation of numerous growth curves, both in broth and in foods, and the creation of databases enabling all scientists to access an increasing part of them (see review of tools in Appendix IV),
- the development of tools to fit growth curves with a primary model, describing the fate of the population over time, which enable to estimate in a simple way parameters such as growth rates, and lag times (see review of tools in Appendix IV),
- the publication of new secondary models, aiming to predict the growth rate, and to a less extent of the lag time, of *L. monocytogenes* in foods as a function of the environment (see review of secondary models in Appendix V),
- the incorporation of predictive microbiology models to user-friendly application software (see review of tools in Appendix IV) that allows different users to obtain information from models in a rapid and convenient way (McMeekin *et al.*, 2006).

Despite the increase number of data and models for the growth rate and the lag time of *L. monocytogenes*, the quantitative data on the effect of the food environment on the maximum population density of the pathogen is limited. In some foods, growth of *L. monocytogenes* might be rapid initially but ends after a short growth period because of limitation in some nutrients and/or competition with the background microflora (Dalgaard and Jorgensen, 1998), which was termed the Jameson effect (Jameson, 1962; Ross *et al.*, 2000). Efforts on evaluating the Jameson effect and expressing it with quantitative terms (*i.e.* mathematical models) can increase significantly the accuracy of risk assessments (Coleman *et al.*, 2003; Pouillot *et al.*, 2007).

Another important issue in the use of predictive models is the natural variation between *L. monocytogenes* strains. Most of the available models for *L. monocytogenes* are based on growth data of a single strain. Several studies however, have reported that growth kinetics of *L. monocytogenes* can vary significantly between different strains (Begot *et al.*, 1997; Lianou and Sofos, 2007). Strain variability can be taken into account when challenge tests are performed for model validation by using inocula consisting of a mixture of strains.

Although predictive microbiology remains a valuable tool for estimating the growth of *L. monocytogenes* in foods, it is important to understand the limitation of predictive models. Most available models are developed in laboratory media, include the effect of certain environmental factors on microbial growth, and have been validated by experiments on foods. In foods, however, microbial growth can be affected by other factors which are not included in the models such as the physiological state of the cells, the food structure, the microbial interactions or the presence of antimicrobial compounds. In general, it has been observed that growth in foods is slower than growth predicted by models developed in broth media. It has also been observed that *L. monocytogenes* in naturally contaminated food grows slower than in artificially

contaminated foods (Dalgaard and Jorgensen, 1998). The above observations however, are not absolute since a large variability exists in predictions especially at conditions close to the boundary of growth (te Giffel and Zwietering, 1999). Thus the use of predictive models should be always combined with validation studies based on growth data obtained in spiked foods (by challenge-testing) or, even better, in naturally-contaminated foods (by storage trials, or durability studies).

4.5. Growth/no growth models

Modelling the behaviour of microorganisms at the growth/no growth interface is now recognised as an important component of “modern” predictive microbiology (McMeekin *et al.*, 1997; te Giffel and Zwietering, 1999; McMeekin *et al.*, 2000; McMeekin *et al.*, 2002). This aspect was still poorly known in the late 1990s, which explains why it was not fully addressed in the SCVPH opinion.

Microbial growth/no growth interface models quantify the combined effect of various hurdles on the probability of growth and define combinations of the environmental conditions at which growth ceases. These models are valuable tools in assessing the risk of growth of *L. monocytogenes* in food products as affected by formulation, process and storage conditions. To date, several growth/no growth interface models for *L. monocytogenes* have been described (Tienungoon *et al.*, 2000; Koutsoumanis *et al.*, 2004; Augustin *et al.*, 2005; Le Marc *et al.*, 2005; Valero *et al.*, 2007; Vermeulen *et al.*, 2007). The growth/no growth interface of *L. monocytogenes* at 4 and 10°C with respect to pH and a_w predicted by four different available models at a probability level of 0.1 is presented in Figure 5. It shows that the growth limits are significantly affected by the strains used for the development of the models.

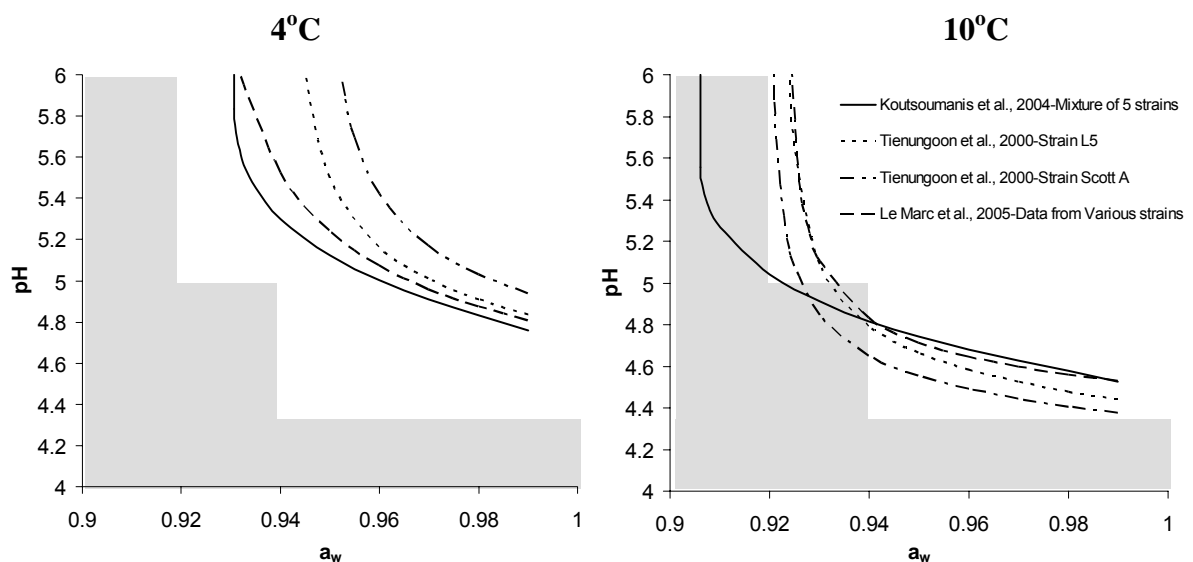


Figure 5. **Growth/no growth interface of *L. monocytogenes* at 4 °C (left) and 10°C (right) with respect to pH and a_w predicted by four different available models at a probability level of 0.1.** Note: Shaded area indicate pH and a_w combination defined in the Regulation (EC) No 2073/2005 as conditions that do not allow growth of *L. monocytogenes* ($\text{pH} \leq 4.4$ or $a_w \leq 0.92$, or $\text{pH} \leq 5.0$ and $a_w \leq 0.94$)

In Figure 5, a comparison between the growth limits predicted by the available models with the pH and a_w combination defined in the Regulation (EC) No 2073/2005 as conditions that do not allow growth of *L. monocytogenes* ($\text{pH} \leq 4.4$ or $a_w \leq 0.92$, or $\text{pH} \leq 5.0$ and $a_w \leq 0.94$) is also presented. At 4°C the pH and a_w limits for growth predicted by all the models are considerably higher than the growth limits defined in the Regulation (EC) No 2073/2005. At 10°C, growth is predicted by some models in combinations of pH and a_w which are defined in the regulation as conditions that do not allow growth. Therefore, the ability of a food to support growth of *L. monocytogenes* depends on the storage temperature.

However, these models have been developed using data obtained from liquid microbiological media. Such models do not take into account factors such as the food structure or the microbial competition that may suppress growth of the pathogen (Brocklehurst *et al.*, 1997; Dalgaard and Jorgensen, 1998; Pin *et al.*, 1999; Nguyen-the and Carlin, 2000; Wilson *et al.*, 2002; Koutsoumanis *et al.*, 2004). Indeed, it is reported that the probability of growth of *L. monocytogenes* in solid media (agar) is significantly lower than in liquid media (broth). In the latter study the minimum pH values that permitted growth at 25°C in broth and agar were 4.45 and 5.10 respectively, while the respective a_w limits were 0.900 and 0.942. Furthermore, the majority of the available growth/no growth interface models are based on a high inoculum size. Several studies have shown that with increasing the inoculum size the probability of growth also increases (Razavilar and Genigeorgis, 1998; Masana and Baranyi, 2000; Pascual *et al.*, 2001; Robinson *et al.*, 2001; Koutsoumanis and Sofos, 2005). Considering the contamination of food often occurs at very low levels it is expected that the probability of growth of *L. monocytogenes* in food is much lower than as predicted by the models.

5. Risk characterisation (update of § 4.4. of the 1999 opinion)

5.1. New information from the recent risk assessment studies

The SCVPH opinion was followed by the new approach of Microbiological Risk Analysis (CAC, 1999). The philosophy of risk assessment as a scientific basis for implementing risk management measures has been accepted worldwide. New risk assessments studies with respect to *L. monocytogenes* in foods have been elaborated.

Several formal quantitative risk assessments have been undertaken to address issues related to the relative risks of listeriosis among different ready-to-eat foods and the factors that contribute to those risks. Available governmental risk assessments currently include a comparative risk assessment of 23 categories of ready-to-eat foods (FDA/USDA/CDC, 2003), a comparative risk assessment of four ready-to-eat foods (FAO/WHO, 2004), and a product/process pathway analysis (FSIS, 2003). Moreover, several risk assessments focussing on single ready-to-eat food commodities have also been published, e.g. for raw milk cheeses (Sanaa *et al.*, 2004), smoked or gravad salmon and rainbow trout (Lindqvist and Westöö, 2000), Parma ham (Giovannini *et al.*, 2007), deli meats, focused on food handling practices at home (Yang *et al.*, 2006) and cold-smoked salmon (Pouillot *et al.*, 2007).

The governmental risk assessments, articulating concepts that countries can use to identify and categorize those ready-to-eat products that represent a significant risk of food borne listeriosis, as well as the more recent commodity-specific ready-to-eat studies have indicated that food can be categorised according to the likelihood of *L. monocytogenes* being present and its ability to grow in the food.

5.2. Focus on the FAO/WHO risk assessment conclusions

The Joint FAO/WHO Expert consultation on Risk Assessment of Microbiological Hazards in Foods (JEMRA) concluded that questions pertaining to international food safety issues can be addressed by expanding and/or adapting components of risk assessment done at a national level. They showed also that pre-existing models and data sets can serve as a basis for quantitative risk assessment efforts. The group also identified a number of areas where data gaps exist and indicated the need for improved data acquisition for prevalence and growth of *L. monocytogenes* in foods and the incidence of foodborne listeriosis.

An important part of the study related to the hazard characterisation (dose-response) is detailed in § 3.2 of this opinion.

5.2.1. Microbiological limits

The first conclusions with regard to the risk characterisation were based on theoretical calculations (generic for all ready-to-eat food products). In estimating the risk from *L. monocytogenes* in food an example was developed where the number of organisms ranges from an absence in 25g to not exceeding specified levels at the point of consumption (e.g., 1,000 cfu/g). A number of simplifying assumptions were made in developing the examples and to calculate the ingested dose the knowledge of the size of the serving was needed. A fixed serving size of 31.6 g was assumed for convenience to simplify the calculations. To calculate the concentrations for other serving sizes, the dose levels would have to be divided by the serving size (see FAO/WHO, 2004).

Dose-response curves were developed for both healthy and susceptible populations and include the entire range of ingested doses (*i.e.* not restricted to 1,000 cfu/g food). For the purposes of the example, only the dose-response curve for the susceptible population was used, and it was assumed that all cases of listeriosis were restricted to that population. The specific dose-response curve selected was that where the maximum level to which *L. monocytogenes* could grow in a food was assumed to be $10^{7.5}$ cfu/serving. For calculations, the most “conservative” dose-response model was used, *i.e.* the maximum virulence of *L. monocytogenes* was assumed.

The overall impact on the number of cases of listeriosis was estimated by multiplying the likelihood of listeriosis per serving by the total number of servings. For this calculation, the total number of ready-to-eat servings was assumed to be 6.41×10^{10} servings, *i.e.* the estimated total number of annual servings in the United States of America for the 20 classes of ready-to-eat food considered in the FDA/FSIS draft risk assessment (FDA/FSIS, 2001). The corresponding number of listeriosis cases for the susceptible population was considered to be 2,130 (FDA/FSIS, 2001).

A more realistic approach was described when employing a distribution of *L. monocytogenes* levels in foods when consumed. The overall distribution of *L. monocytogenes* levels in 20 classes of ready-to-eat foods from the FDA/FSIS risk assessment (FDA/FSIS, 2001) was used. This distribution was then used to calculate the probability of listeriosis and the predicted number of listeriosis cases. At each maximum *L. monocytogenes* level considered, the number of servings from the distribution that were above the designated value was added to that maximum level. For example, for an upper limit of 1,000 cfu/g, the number of servings was 6.23×10^7 (servings originally predicted to be at 1,000 cfu/g) + 2.94×10^7 (servings originally predicted to be at 10,000 cfu/g) + 1.39×10^7 (servings originally predicted to be at 10^5 cfu/g) +

3.88×10^6 (servings originally predicted to be at $10^{5.5}$ cfu/g) + 8.55×10^6 (servings originally predicted to be at $>10^6$ cfu/g) = 1.18×10^8 servings. The predicted annual number of listeriosis cases was then calculated and summated for each level of contamination. The predicted number of listeriosis cases for each maximum level is provided in Table 3.

Table 3. Expected annual number of listeriosis cases in the susceptible population in the USA when the level of *L. monocytogenes* was assumed not to exceed a specified maximum value and the levels of *L. monocytogenes* in the food are distributed as indicated in FAO/WHO (2004)

Level (cfu/g)	Maximum dose (cfu/serving) ^(a)	Cumulative percentage of servings when maximum level ^(b)	Estimated number of listeriosis cases per year ^(c)
0.04 (absence in 25g)	1	100	0.5
0.1	3	3.6	0.5
1	32	1.7	0.7
10	316	0.8	1.6
100	3,160	0.4	5.7
1,000	31,600	0.2	25.4

(a) Serving size of 31.6g.

(b) Number of servings in the highest *L. monocytogenes* level assumed, divided by 6.41×10^{10} times 100.

(c) Levels of *L. monocytogenes* per serving used to calculate predicted number of cases based on the overall distribution from the FDA/FSIS risk assessment (2001). A total of 6.41×10^{10} servings per year was assumed in US population.

5.2.2. Deviations from the limits

A simple “what-if” scenario was developed describing the impact on public health of the level of compliance to a microbiological limit. Two often discussed limits, 0.04 cfu/g (absence in 25 g) and 100 cfu/g, were examined in conjunction with different “defect rates” (a defect rate is the percentage of servings that exceed the specified limit). To simplify the model, a single level of *L. monocytogenes* contamination, 10^6 cfu/g, was assumed for all “defective” servings. This assumption focuses the scenario on the group of defective servings that is responsible for the majority of listeriosis cases. Data in Table 3 demonstrate that at 100% compliance, the number of predicted cases for both absences in 25g and 100/g limits is low, with an approximate 10-fold differential between them, that is 0.5 cases versus 5.7 cases.

As expected, the number of predicted cases increases with an increasing frequency of defective servings (*i.e.* serving with numbers of *L. monocytogenes* above the limits) (Table 4). At defect rates $>0.0001\%$, the microbiological limit (*i.e.* 0.04 cfu/g versus 100 cfu/g) impacts only very marginally on the number of predicted cases and a 10-fold increase in the defect rate results in an approximate 10-fold increase in the number of predicted cases, regardless of this limit. If the limit were 1,000 cfu/g, the same conclusion (*i.e.* marginal impact of the limit itself) would be drawn for defect rates above 0.001% (Tables 3 and 4). Based on the conditions and assumptions of this simple what-if scenario, the defect rate that yielded a value approximately equivalent to the baseline value of 2,130 cases used in the FDA/FSIS draft risk assessment (2001) was 0.018%. This is consistent with the defect rate (0.013%) at this contamination level estimated from FDA/FSIS (2001), and the observation that the dose-response relationship predicts that this group of defective servings accounts for most cases of foodborne listeriosis.

Table 4. Hypothetical “what-if” scenario demonstrating the effect that the proportion of “defective” servings has on the number of predicted cases of foodborne listeriosis (FAO/WHO, 2004).

Assumed percentage of "defective" servings ^(a)	Expected number of listeriosis cases ^(b)	
	Initial standard of 0.04 cfu/g (absence in 25g)	Initial standard of 100 cfu/g
0 (see Table 3)	0.5	5.7
0.00001	1.7	6.9
0.0001	12.3	17.4
0.001	119	124
0.01	1,185	1,191
0.018	2,133	2,133
0.1	11,837	11,848
1	117,300	117,363

(a) For the purposes of this scenario, all defective servings were assumed to contain 10^6 cfu/g.

(b) For the purposes of this scenario, an r-value (see section 3) of $5.85 \cdot 10^{-12}$ was employed and a standard serving size of 31.6 g was assumed. In the case of the 100 cfu/g calculations, the defective servings were assumed to be proportionally distributed according to the number of servings within each cell concentration bin.

5.2.3. Food categories

The risk characterisation in the FAO/WHO risk assessment was based on an exposure assessment for six ready-to-eat foods from initial prevalence and concentration at the retail level to final concentration in contaminated servings. Risk characterisations based on the exposure profile of *L. monocytogenes* at consumption and dose response models were used to attempt to estimate predicted cases of listeriosis per serving for each of the six foods. Regarding regional considerations, the Joint WHO/FAO expert consultation on Risk Assessment considered that quantitative data on levels of *L. monocytogenes* contamination of foods and prevalence of listeriosis should be obtained in various regions of the world. This information should be developed to determine if seasonality and/or regional differences exist and the influence of climate and season in different regions in the world. Therefore, there is no indication for referring regional considerations.

The risk assessment study estimates the risk from *L. monocytogenes* in foods that support growth and foods that do not support growth under specific storage and shelf life conditions. For foods that support growth, increases in *L. monocytogenes* cell numbers between retail and consumption would have to be assumed and there is a significant likelihood that the hypothetical criteria analysed above would be exceeded. However, this would not be the case for foods that do not support growth. Thus, for foods that do not support growth of *L. monocytogenes*, the predicted number of cases in relation to maximum dose level at retail would be the same as those depicted above for doses at time of consumption.

Three different approaches were taken to demonstrate the effect of growth of *L. monocytogenes* on the risk of listeriosis associated with ready-to-eat foods. It is apparent that the potential for growth strongly influences risk, though the extent of that increase is dependent on the

characteristics of the food and the conditions and duration of refrigerated storage. However, using the examples provided in the risk assessment, the ability of these ready-to-eat foods to support the growth of *L. monocytogenes* appears to increase the risk of listeriosis on a per serving basis by 100- to 1000-fold over what the risk would have been if the foods did not support growth. While it is not possible to present a single value for the increased risk for all ready-to-eat foods, because of the different properties of the foods, the range of values here provide some insight into the magnitude of the increase in risk that may be associated with the ability of a food to support the growth of *L. monocytogenes*.

To rank food categories according to the relative risk of listeriosis it is important to distinguish the risk per serving and the risk per annum (FDA/USDA/CDC, 2003).

- The risk per serving is a function of the rate of contamination of the food, of its ability to support growth of *L. monocytogenes*, of the shelf life, temperature and duration of the food and of the serving size. It is the risk faced by the individual consumer. This risk estimate provides the basis for risk management decisions concerning the food category.
- The risk per annum also takes into account the number of serving of the food category consumed each year. It relates to the contribution of the food category to the total number of listeriosis in the population, *i.e.* the “health impact” of the food.

For instance, in the US (FDA/USDA/CDC, 2003) frankfurters sausages (not reheated) were classified as “high risk per serving” and “high risk per annum” because it is consumed in large amounts. Pasteurised milk was classified as “low risk per serving” but “high risk per annum” because it is consumed in large amounts. In contrast, unpasteurised milk was ranked as “high risk per serving” but as “moderate risk per annum” because of the low amount consumed.

5.3. Concluding remarks from the Quantitative Microbiological Risk Assessments

The models developed predict that nearly all cases of listeriosis result from the consumption of high numbers of the pathogen. Conversely, the models predict that the consumption of low numbers of *L. monocytogenes* has a low probability of causing illness and are responsible for the minority of cases. Old age and pregnancy increase susceptibility and thus the risk of acquiring listeriosis per exposure. Likewise, diseases and medical interventions that severely compromise the immune system greatly increase the risks.

Control measures that reduce the frequencies of contamination imply proportional reductions in the rates of illness, provided the proportions of high contaminations are reduced similarly.

Control measures that prevent the occurrence of high levels of contamination at consumption would be expected to have the greatest impact on reducing the rates of listeriosis. Contamination with high numbers of *L. monocytogenes* at manufacturing and retail is rare, and foods such as ice cream and some fermented meat products that do not permit growth during storage have relatively low risks per serving and low annual risks per population. In foods that permit growth during storage, particularly if stored at higher temperatures or for longer duration, the low numbers of *L. monocytogenes* at manufacture and retail may increase during storage to levels that represent substantially elevated relative risks of causing listeriosis.

Although high levels of contamination at retail are relatively rare, improved public health could be achieved by reducing these occurrences at manufacture and retail in all ready-to-eat foods. In ready-to-eat foods that permit growth, control measures, such as better temperature control or limiting the length of storage periods, will reduce the risk due to growth of *L. monocytogenes*. Re-formulating foods so they do not support growth would also be expected to reduce the occurrence of high doses and thus reduce the risk of listeriosis. The vast majority of cases of

listeriosis are associated with the consumption of foods that do not meet current standards for *L. monocytogenes* in foods, whether the standard is zero tolerance or 100 cfu/g.

The categorisation of the ready-to-eat foods in relation to the risk of listeriosis depends on both the frequency of consumption and the prevalence and the numbers of *L. monocytogenes* in the food at consumption. The numbers of this bacterium in foods at the point of consumption depends on the ability of the food to support the growth of *L. monocytogenes*, the temperature and the duration of refrigerated/chilled food storage (FAO/WHO, 2004).

The general outcome of the available risk assessments is following:

- The greatest risk associated with ready-to-eat products is the small proportion of the products with high contamination levels of *L. monocytogenes*.
- All risk assessments agree that foods supporting the growth of *L. monocytogenes* to high levels should be the target of risk management measures.

Key component of a successful risk management program is assurance that the control measures (e.g. preventing contamination and growth of the pathogen) can be achieved consistently. All risk assessments have identified population subgroups with increased susceptibility and thus the risk of acquiring listeriosis per exposure concerns: elderly persons, pregnant women and immuno-compromised persons. In the risk assessments, at the consumer level, temperature abuse, in particular elevated refrigeration temperature, was the most significant contributor to increased risk.

6. Microbiological criteria and categories of ready-to-eat foods

In discussing possible preventive measures, the SCVPH (1999) stated that “*the critical point relates primarily to the potential for growth of L. monocytogenes in the food*”, and proposed that “*L. monocytogenes survival and multiplication conditions can be used to suggest grouping principles for food commodities and production regimes*”.

Information obtained since the SCVPH opinion still support the importance of focussing on foods that permit growth of *L. monocytogenes* (see section 5). The SCVPH opinion proposed a categorisation according to the ability of *L. monocytogenes* to grow in ready-to-eat foods, and criteria for the various categories. In 2005, the Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs laid down a more simple categorisation: foods supporting or not supporting growth and foods for infant or special medical purposes. This Regulation defined different “food safety criteria” for each categories of ready-to-eat foods: absence in 25 g throughout shelf life for foods intended for ‘at risk’ consumers (infant foods and foods for special medical purposes); below 100 cfu/g for ready-to-eat foods unable to support growth of *L. monocytogenes* during their shelf life and all products with a shelf life of less than 5 days; absence in 25 g at production and below 100 cfu/g during shelf life for ready-to-eat foods able to support growth of *L. monocytogenes*. The criteria of below 100 cfu/g during shelf life will likely be applied during retail. For foods supporting growth of *L. monocytogenes*, depending on the conditions between retail and consumption, the limit of 100 cfu/g might be exceeded at consumption.

The purpose of these food safety criteria is to prevent exposure of consumers to ready-to-eat foods with high numbers of *L. monocytogenes*. They provide harmonised standards in the EU and impact the entire food chain because the risk of recall and economic loss for food processors are a strong motivation to meet the criteria (EFSA, 2007b). However, application of microbiological criteria is only one of several management activities to ensure that ready-to-eat

foods are of low risk related to human illness. Microbiological testing alone to verify compliance with the criteria may convey a false sense of security due to the statistical limitation of sampling plans (EFSA, 2007b). Food safety criteria should not be considered without implementation of efficient control programs based on HACCP principles.

In addition to criteria, the Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs also introduced the requirement for food business operators “to conduct studies to investigate compliance with criteria throughout shelf life”, for ready-to-eat foods able to support growth of *L. monocytogenes*. These investigations should take into account the “reasonably foreseeable storage conditions” (in particular temperature and shelf life) and should consider the important variability in refrigeration temperatures observed in Europe, particularly in domestic refrigerators (see section 4.3).

In 2007, the Codex Alimentarius Commission (CAC, 2007) proposed a new document for microbiological criteria for *L. monocytogenes* in ready-to-eat foods (step 3). As in the Regulation (EC) No 2073/2005, criteria proposed for ready-to-eat foods non supporting growth of *L. monocytogenes* is below 100 cfu/g during shelf life. However, the Codex document proposed a more stringent criterion for ready-to-eat foods supporting growth of *L. monocytogenes* than the Regulation (EC) No 2073/2005, with absence in 25 g during shelf life. This criterion might lead to elimination of foods that would not have exceeded the limit of 100 cfu/g at consumption.

A limit of 100 cfu/g throughout shelf life for food supporting growth is also proposed in the Codex document only if the manufacturer can demonstrate that a positive product will not exceed 100 cfu/g throughout shelf life. However, such demonstration will be difficult to achieve with a high degree of certainty, in particular because of the important variation in domestic storage conditions (including temperature and duration) and variability of the food composition.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

ToR1: Update the scientific literature from the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods

- An increase in the number of listeriosis cases, which is predominantly a foodborne disease, has been observed in several EU countries since 2000.
- This increase concerns persons over 60 years old, especially those immuno-compromised.
- The number of large (*i.e.* more than 50 cases) listeriosis outbreaks have declined since the late 1990s and the large majority of listeriosis cases are sporadic.
- The molecular mechanisms of *L. monocytogenes* virulence are now better understood as well as the relevance of the various animal and *in vitro* models. The low virulence of some strains of *L. monocytogenes* has been demonstrated *in vitro* and *in vivo*, albeit not over the complete oral route of infection on relevant animal models. At present, no routine methods permit the differentiation between virulent and avirulent strains of *L. monocytogenes*.
- A substantial effort has been placed on investigations for the presence of *L. monocytogenes* in foods in the Member States. Information on the ability of the food to support growth of *L. monocytogenes* and information on the stage of sampling (*i.e.* at the start of shelf life or at consumption) is generally not provided. Therefore, for most investigations, it is not possible to assess the impact of contaminated samples on the risk for consumer health.
- Comparison between most investigations is not possible as sampling practice, sampling sites and randomisation during sampling may be different. In some cases, samples were collected based on the suspicion of a problem, in other cases randomised sampling may be performed. Therefore, it is difficult to discern changes in the exposure of the European consumer from one year to another.
- Surveys of foods have not only collected data on the prevalence and contamination levels of *L. monocytogenes* in different food types, but also revealed associations with other parameters including: food packaging type, preparation practices (e.g. the use of slicing machines for meat products), storage temperatures, the stage of sampling with respect to shelf life, the lack of an effective HACCP system, and lack of education and training of food handlers.
- Risk assessments published since the SCVPH opinion concluded that compliance of ready-to-eat foods to limits of “below 100 cfu/g” or “absence in 25 g” at consumption would both lead to very low numbers of listeriosis cases. It was concluded that most cases were due to consumption of ready-to-eat food able to support growth and containing levels markedly above these limits.
- Growth of *L. monocytogenes* is a function of the type of food, the storage time and the storage temperature. Storage temperature at retail and domestic refrigerators can vary significantly, especially for the domestic refrigerators. For instance, the temperature of 20-35% of domestic refrigerators in Europe was above 8°C.
- New tools to predict growth have been developed since 1999 and can be used to determine if the product will or will not support growth of *L. monocytogenes* and estimate the extent of growth during the shelf life. However, the use of predictive models should be combined with validation studies, especially for foods close to the growth/no growth boundaries.

ToR2: Provide scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods (absence in 25 g, 100cfu/g and higher levels) and the related risk for human illness.

- Application of microbiological criteria is only one of several management activities to ensure that ready-to-eat foods are of low risk for humans.
- Microbiological criteria will assist in controlling the levels of *L. monocytogenes* e.g. absence in 25 g or ≤ 100 cfu/g at the point of consumption.
- Most listeriosis cases are due to consumption of ready-to-eat foods able to support growth of *L. monocytogenes* and containing levels markedly above 100 cfu/g.
- The most recent Codex document on microbiological criteria for *L. monocytogenes* in ready-to-eat foods suggests a zero tolerance throughout the shelf life of the product for ready-to-eat foods in which growth of this microorganism can occur. Applying this criterion throughout the shelf life may prevent consumption of ready-to-eat foods representing a high risk. However, applying this criterion close to the end of shelf life could classify products as unsatisfactory, although they are of low risk.
- An additional option proposed in this Codex document is therefore to tolerate 100 cfu/g throughout the shelf life provided that the manufacturer is able to demonstrate that the product will not exceed this limit throughout the shelf life. For ready-to-eat foods that support growth of *L. monocytogenes*, it is impossible to predict with high degree of certainty that the level will or will not exceed 100 cfu/g during the shelf life of these products. Thus, applying this option may result in accepting a probability that foods with more than 100 cfu/g will be consumed. The impact on public health would depend whether the levels markedly above 100 cfu/g are reached.

RECOMMENDATIONS

- More thorough investigation of sporadic and outbreak cases of listeriosis would improve our knowledge of the foods responsible of listeriosis.
- Consumption data of ready-to-eat foods that support growth of *L. monocytogenes* are needed for a better assessment of risks.
- Comparison between studies (e.g. surveys) should be made only when similar sampling strategies are applied and studies should focus on ready-to-eat foods able to support growth of *L. monocytogenes*. The stage of sampling (e.g. at production or close to consumption) is important and should be noted to further allow better comparisons between studies.
- As the application of microbiological criteria is only one of several management activities to ensure that ready-to-eat foods are of low risk for humans, application of GHP in combination with HACCP should be consistently applied to minimise the initial contamination at manufacturing level, and/or reducing the potential for growth. This latter implies fixing a realistic shelf life in relation to realistic storage temperatures and potential growth of *L. monocytogenes*. The variability of temperatures during retail and domestic storage of the foods should be taken into account.
- The chill chain especially at the domestic level and dietary and food storage advice (particularly for the elderly) should be improved to reduce the risk of listeriosis.

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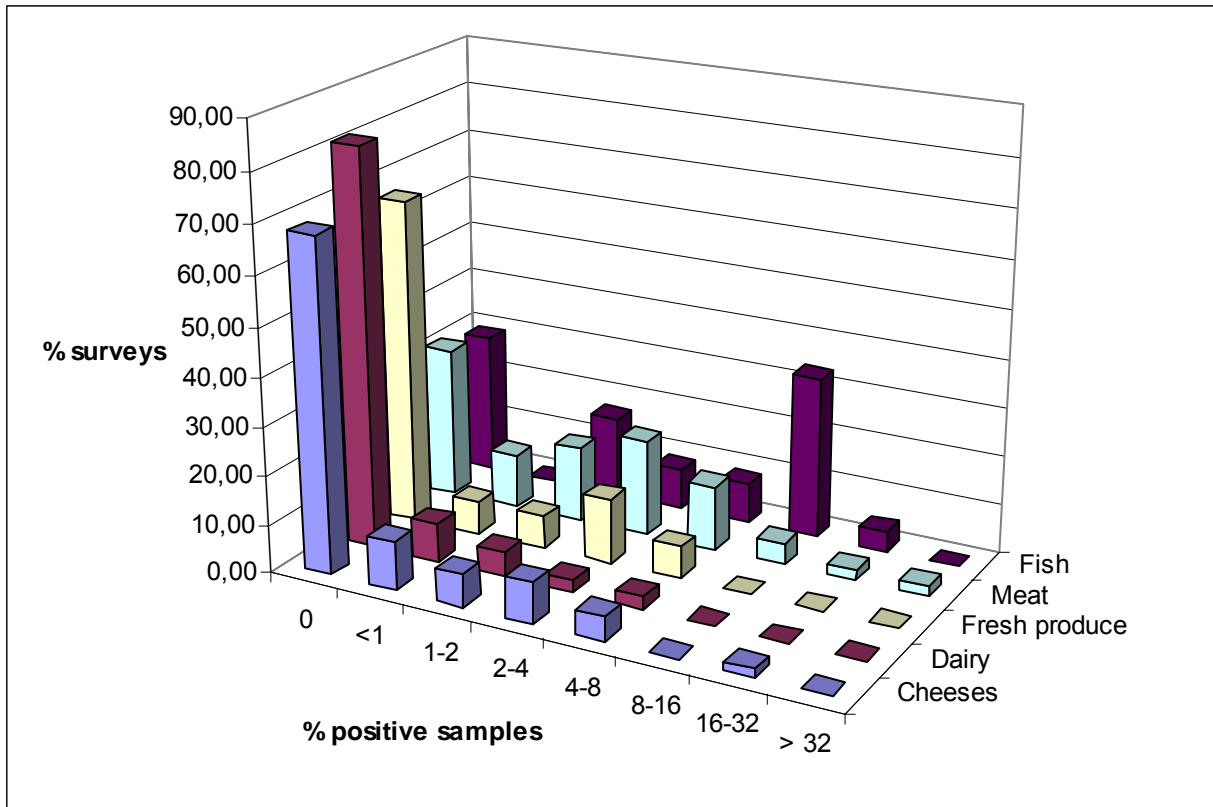
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APPENDICES

APPENDIX I

Percentage of samples positives for *L. monocytogenes* for various food categories (EFSA, 2006).



APPENDIX II

Temperature survey data on domestic refrigerators in Europe [modified from James *et al.* (2007)].

Year reported	Country	n	% refrigerators running at temp (°C)										Reference	
			T _{min} °C	T _{mean} °C	T _{max} °C	>4	>5	>6	>7	>8	>9	>10		
1990	UK	75		<5	15		6							(Rose <i>et al.</i> , 1990)
1991	UK	252	0.9	6	11.4		70							(Evans <i>et al.</i> , 1991)
1992	UK	150	0.8	6.5	12.6		71							(Flynn <i>et al.</i> , 1992)
1993	France	102			14			70						(Victoria, 1993)
1994	Netherl.	125					70		28		2			(Lezenne Coulander de, 1994)
1997	Greece	136									50			(Sergelidis <i>et al.</i> , 1997)
1997	UK	108	2	5.9	12		50							(Worsfold and Griffith, 1997)
1998	UK	645	-2	7	13		70							(Johnson <i>et al.</i> , 1998)
2002	France	119	0.9	6.6	11.4		80							(Laguerre <i>et al.</i> , 2002)
2003	France	68	0.7	7	16.8	82	74	65	49	37	18	10		(AFSSA, 2006)
2003	UK	901					31						3	(Ghebrehewet and Stevenson, 2003)
2003	Greece	110					74		46		23		8	(Bakalis <i>et al.</i> , 2003)
2004	Belgium	2803						75		50		25		(ISSP, 2006)
2005	Ireland	100	-7.9	5.4	20.7		59							(Kennedy <i>et al.</i> , 2005)
2005	Portugal	86						70						(Azevedo <i>et al.</i> , 2005)
2005	Greece	258	-2	6.3				50					10	(Taoukis <i>et al.</i> , 2005)
2005	Netherl.	31	3.8		11.5				68					(Terpstra <i>et al.</i> , 2005)
2006	Greece	100	0.2	7.2	14.6	100	82	72	51	35	25	12		(Xanthiakos, 2006b)
2006	UK	24		5				33						(Breen <i>et al.</i> , 2006)

APPENDIX III

A simulation shows that the concentration of *L. monocytogenes* in a contaminated deli meat product (pH:5.5, a_w :0.95, nitrites:50ppm, initial contamination 1 cfu/25 g) after storage of 10 days in a domestic refrigerator in USA is significantly higher compared to storage of the same period in Greece (Figure III.1). The percent of non-compliant packages to the safety criteria of Regulation (EC) No 2073/2005 (100 cfu/g) is 2.1% for USA and 19.7% for Greece. The above example indicates that improving the European chill chain, especially refrigeration conditions in domestic refrigerators, or reducing shelf life, would lead to a significant reduction of listeriosis risk and to an increased compliance of ready-to-eat food to the safety criteria.

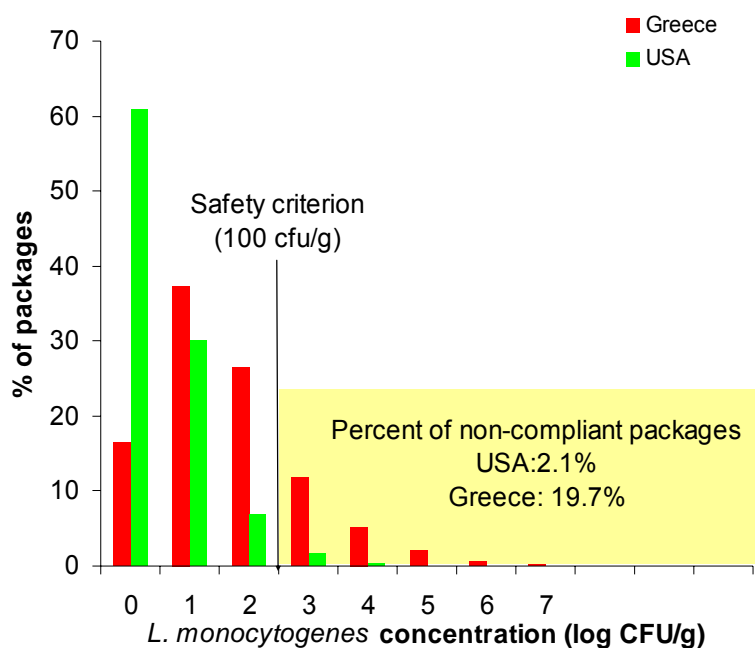


Figure III. 1. **Concentration of *L. monocytogenes* in a contaminated deli meat product (pH:5.5, a_w :0.95, nitrites:50ppm, initial contamination 1 cfu/25 g) after storage of 10 days in a domestic refrigerator in USA and in Greece.**

Growth of the pathogens was predicted using the Monte Carlo technique (30000 iterations) based on the temperature distributions presented in Figure 3 and the model of Buchanan and Phillips (Buchanan and Phillips, 2000).

APPENDIX IV

New predictive microbiology tools available

The Pathogen Modeling Program (PMP) is available free of charge (<http://ars.usda.gov/Services/docs.htm?docid=11550>) and is the most widely used predictive microbiology application software. The present version includes more than 35 models for 11 bacterial pathogens including *L. monocytogenes*.

The ComBase (<http://www.combase.cc>) is a combined database of microbial responses to food environments. It is linked to the ComBase modelling toolbox, which includes :

- ComBase Predictor, a set of 23 growth models and 6 thermal death models for food pathogenic and spoilage microorganisms including *L. monocytogenes*,
- Perfringens Predictor, an application for predicting the growth of *Clostridium perfringens* during the cooling of meats.
- DMFit, a fitting tool, for growth and inactivation curves.

The commercial software, Food MicroModel, was in several ways similar to PMP. It is no longer available, data has been integrated in the ComBase database and models have been integrated in ComBase Predictor.

Sym'previus (<http://www.symprevius.net>) a French decision support system, includes a database (in French), fitting tools for growth and inactivation curves (both in French and English), and predictive tools based on challenge testing for growth and inactivation of six pathogenic bacteria (both in French and English). Information from Sym'previus is available on a commercial basis through contact centres as indicated on the homepage cited above.

The Food Spoilage Predictor-FSP (Neumeyer *et al.*, 1997) and the Seafood Spoilage and Safety Predictor (SSSP, available at www.dfu.min.dk/micro/sssp) (Dalgaard *et al.*, 2003) are examples of more specific predictive microbiology application software. These softwares include facilities to read product temperature profiles, as recorded by data loggers, and thus predict the effect of fluctuating temperatures on growth of microorganisms. In addition to models for different seafood spoilage bacteria, SSSP includes a model to predict the simultaneous growth of *L. monocytogenes* and spoilage microorganisms in sliced and vacuum packed cold-smoked salmon (Gimenez and Dalgaard, 2004).

APPENDIX V
Table V.1 List of some recent secondary models for *L. monocytogenes* growth

Reference	Temperature range °C	Other environmental factors (ranges for polynomial models)	Type of model
(Fernandez <i>et al.</i> , 1997)	4-20	NaCl (0.5-8.0%), pH (4.0-7.2), atmosphere (CO ₂ : 0-100%)	Polynomial
(McClure <i>et al.</i> , 1997)	1-35	NaCl (0.5-11.5%), pH (4.5-7.0), Na-nitrite (0-200 ppm)	Polynomial
(Lebert <i>et al.</i> , 1998)	4-30	a _w (0.96-1.0), pH (5.4-7.0)	Polynomial
(Nerbrink <i>et al.</i> , 1999)	9	NaCl (1.0-4.0%), pH (5.5-6.5), Na-lactate (0-0.5%), Na-acetate (0-0.6%)	Polynomial
(Buchanan and Phillips, 2000)	4-37	NaCl (0.5-10.5%), pH (4.5-7.5), Na-nitrite (0-1000 ppm), atmosphere (aerobic, anaerobic)	Polynomial
(Augustin and Carlier, 2000a; Augustin and Carlier, 2000b)	-2.7-45.5	a _w , pH, lactic acid, acetic acid, citric acid, Na-benzoate, K-sorbate, Na-nitrite	cardinal
(Rodriguez <i>et al.</i> , 2000)	4-20	- (specific to green asparagus)	Arrhenius
(Devlieghere <i>et al.</i> , 2001)	4-12	a _w (0.962-0.988), pH (6.2), Na-lactate (0-3%), Na-nitrite (20 ppm)	Belerhaddek, polynomial
(Le Marc <i>et al.</i> , 2002)	0.5-43	pH, lactic acid, acetic acid, propionic acid	cardinal
(Semán <i>et al.</i> , 2002)	4	NaCl (0.8-3.6%), pH (4.55-9.61), K-lactate (0.15-5.6%) Na-diacetate (0.0-0.2%), Na-erythorbate (317 ppm), Na-nitrite (97ppm), Na-tripolyphosphate (0.276%)	Polynomial
(Pouillot <i>et al.</i> , 2003)	-2.5-45	- (specific to milk)	cardinal (Bayesian)
(FDA/USDA/CDC, 2003)	-1.2-suboptimal	- (23 models, each specific to 1 of 23 different food categories)	square root
Ross, unpublished, used by (FAO/WHO, 2004)	0.9-41.4	a _w , pH, Na-lactate, Na-nitrite	square root
Ross, unpublished, modified by (Gimenez and Dalgaard, 2004)	0.9-41.4	a _w , pH, Na-lactate, Na-nitrite, Phenolic compounds	square root
(Devlieghere <i>et al.</i> , 2001) modified by (Gimenez and Dalgaard, 2004)	4-12	a _w , pH, Na-lactate, Na-nitrite, Phenolic compounds	square root
(Augustin <i>et al.</i> , 2005)	-2-30	pH, a _w , Na-nitrite, phenolic compounds, CO ₂	cardinal and square root
(Delignette-Muller <i>et al.</i> , 2006)	-2.9-25	- (specific to cold-smoked salmon)	square root (Bayesian)
(Xanthiakos <i>et al.</i> , 2006a)	1.5 - 16	- (specific to pasteurised milk)	square root

There has been a decrease in the use of polynomial models, which have been superseded by food-specific modelling approaches.