EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

OPINION OF THE

SCIENTIFIC COMMITTEE ON VETERINARY MEASURES RELATING TO PUBLIC HEALTH

on

Listeria Monocytogenes

23 September 1999

Contents

1. BACKGROUND

With the exception of provisions for soft cheeses and pasteurised milk (absence in 25 g) and for other dairy products (absence in 1 g.)[1] current Community legislation does not provide for microbiological standards with regard to *Listeria*.

The lack of microbiological reference values has led to situations where food-products have been declared unfit for human consumption because of non-quantified demonstration of contamination with *L. monocytogenes*. The absence of agreed reference values for this zoonotic agent has laid to substantial controversy especially in cases of intra-Community trade.

2. TERMS OF REFERENCE

The Scientific Committee on Veterinary Measures relating to Public Health is requested to assess the risk to health from the presence of *L. Monocytogenes* at different levels in ready to eat foods.

In doing this, the Committee is invited to take into account the principles for the development of microbiological criteria for animal products and products of animal origin intended for human consumption and to develop a risk assessment where appropriate.

Considering the common field of interest, the Committee is invited to set up a joint working group including external experts and experts from both the Scientific Committee on Veterinary Measures relating to Public Health and from the Scientific Committee for Food.

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[1] Council Directive 92/46/EC of 16 June laying down the health rules for the production and placing on the market of raw-milk, heat-treated milk and milk-based products (OJ N° L 268, 14.09.1992, p. 1-31)

3. INTRODUCTION

During the last 15-20 years there has been an increasing concern world-wide about *L. monocytogenes* and its implications for food safety. Several large well-documented foodborne outbreaks and sporadic cases have been described and *L. monocytogenes* has been isolated from a wide range of raw and ready-to-eat meats, poultry, dairy products, seafoods and vegetables and from various food processing environments.

Table 1. Foodborne outbreaks of human listeriosis

Country	Year	Number of cases (deaths)		Food implicated	Level of L.m/g	
USA	1976	20	(5)	?Raw salad*		
New Zealand	1980	20	(5)	?Shell or raw fish*		
Canada	1981	41	(18)	Coleslaw		
USA	1983	49	(14)	?Milk*		
USA	1985	142	(48)	Soft Cheese	10^3-10^4	(R)
Switzerland	1983-7	122	(34)	Soft Cheese	10^4 -10^6	(R)
UK	1987-9	>350	(?)	Pâté	10^2-10^6	(R)
Denmark	1989-0	26	(6)	Hard and Blue Cheese		
Australia	1990	9	(6)	Pâté	10^3	(R & P)
Australia	1991	4		Smoked mussels	10^7	(R)
New Zealand	1992	4	(2)	Smoked mussels		
France	1992	279	(85)	Pork tongue in aspic	10^4 -10^6	(R)
France	1993	33		Pork rillettes	10^2 -10^4	(R)
Italy	1993	18✞		Rice salad		
USA	1994	45✞		Chocolate milk	10^9	(R)
Sweden	1994-5	8	(2)	Smoked fish	10^2 -10^6	(R)
France	1995	33	(4)	Soft cheese		
Australia	1996	4	(1)	Cooked chicken		
Italy	1997	748✞		Corn-meal	10^6	(R)
USA	1998-9	100	(>10)	Hot dogs and deli meats		
Finland	1998-9	18	(4)	Butter	10^1 -10^4	(R & P)

* = Epidemiological association only, without recovery of the implicated strain from the specific food item

✞ = Predominantly pyrexial and gastrointestinal illness

R = Food from retailer, usually unopened

P = Food from patients home, usually opened

Updated from McLauchlin, 1996

It is now widely recognised that the consumption of contaminated food is an important route of transmission of listeriosis and a wide range of food products have been shown to be associated with both outbreaks and sporadic cases. (Tables 1 and 2). The overall incidence of listeriosis increased in both Europe and North America in the 1980s, but whether this reflected a true incidence in numbers or was due to better diagnosis, reporting and/or awareness of the disease is unclear. Several more foodborne outbreaks have been reported recently and with the population most susceptible to listeriosis (the elderly and immunocompromised) increasing there is a need for continued vigilance and surveillance.

Table 2. Sporadic cases of foodborne human listeriosis

Country	Year	Patient died	Food implicated	Level of L.m/g
USA	1985	No	Turkey frankfurters	10^3 (P)
England	1986	No	Soft cheese	'High' (P)
USA	1987	NK	Raw milk	
England	1988	No	Soft cheese	10^7 (P)
England	1988	Yes	Cooked chicken	
England	1988	Yes	Rennet	
Canada	1989	Yes	Alfalfa tablets	
USA	1989	No	Sausage	
Finland	1989	No	Salted mushrooms	10^6 (P)
Italy	1989	NK	Sausage	10^6 (P)
Italy	1989	No	Fish	
Denmark	1989	NK	Smoked cod roe	
Canada	1989	No	Soft cheese	
Belgium	1989	No	Fresh and ice cream	10^3 - 10^6 (P)
Sweden	1993	No	Medwurst	
Italy	1994	NK	Pickled olives	

NK = Not known
P = Food from patients home, usually opened
Updated from McLauchlin, 1996

TABLE 3. ILLNESS CAUSED BY *L. MONOCYTOGENES*

TYPE OF LISTERIOSIS	NATURE OF INFECTION	SEVERITY	TIME TO ONSET
Zoonotic infection	Local infection of skin lesions	Mild and self-resolving	1-2 days
Neonatal infection	Infection of new-born babies from infected mother during birth or due to cross-infection from one neonate in the hospital to other babies	Can be extremely severe, resulting in meningitis and death	1-2 days (early onset) usually from congenital infection prior to birth 5-12 days (late onset) following cross-infection from another infant
Infection during pregnancy	Acquired following the consumption of contaminated food	Mild flu-like illness or asymptomatic in the mother but serious implications for unborn infant including spontaneous abortion, foetal death, stillbirth and meningitis. Infection is more common in third trimester	
Infection of non-pregnant adults	Acquired following the consumption of contaminated food	Asymptomatic or mild illness, which may progress to central nervous system infections such as meningitis. Most common in immunocompromised or elderly	Illness may occur within 1 day or up to several months
Listeria food poisoning	Consumption of food with exceptionally high levels of *L.monocytogenes*, > 10^7 / g	Vomiting and diarrhoea, sometimes progressing to bacteraemia but usually self-resolving	< 24h after consumption

From: Bell and Kyriakides, 1998

4. RISK ASSESSMENT: ESSENTIAL CONSIDERATIONS

The use of the formalised Microbiological Risk Assessment concept as defined by EU and the Codex Alimentarius would normally involve considerations of quantitative and/or qualitative data related to specific commodity groups. The output of the assessment process is an estimate of risk including uncertainties. The considerations presented here include elements of this, but do not represent a full risk assessment.

4.1. Hazard Identification

Listeria monocytogenes is a bacterial pathogen causing serious illness in humans. *L. monocytogenes* can cause a variety of infections (Table 3), but listeriosis most often affects the pregnant uterus, the central nervous system, or the blood stream. Although listeriosis can occur in otherwise healthy adults and children, the most commonly affected populations include pregnant women, neonates, the elderly, and those persons who are immunosuppressed by medications or illness.

While the incidence of human listeriosis is low (2-15 per million inhabitants), the case fatality rate (the proportion of cases that die) is reported to be between 20 and 40% (McLauchlin, 1990 a, b) in the UK, while Gelling and Broome (1989) reported a case fatality rate of around 20% in the USA. In immuno-compromised individuals Nørrung (1999) reported that case fatality rates might approach 75%. Hence, 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 immuno-compromised persons (i.e. cancer, HIV, rheumatic diseases) and pregnant women. Buchanan et al., (1997) suggest that the prevalence of immuno-compromised persons in the US population is around 20%. In Europe, North America, Japan and Australasia the proportion of elderly people (above 65 years of age) is expected to double within the next 30-35 year (United Nations Population Fund, 1999) and in countries like Germany and Italy the proportion of elderly people will approach 30%. Hence the prevalence of groups that are more susceptible to listeriosis can be foreseen to increase during the next decades.

Although other modes of transmission exist, foods have been clearly identified as a primary source of infection. The high prevalence of *L. monocytogenes* in foods in general, together with a high fatality rate of listeriosis suggests that *L. monocytogenes* represents an important hazard to human health. Consequently, the occurrence, spread, growth and survival of *L. monocytogenes* in foods and food environments has to be controlled.

4.2. Hazard Characterisation

4.2.1. The Disease

L. monocytogenes is a Gram-positive, facultatively anaerobic, non-sporeforming rod. Listeria infections most frequently result in meningitis, with or without septicaemia, or septicaemia alone. The latter form of illness is

generally confined to immunocompromised individuals and rarely has identifiable foci of infection. In pregnant women listeriosis most commonly produces a flu-like illness, characterised by fever, headache and myalgia. The most serious consequences of infections in pregnant women are to the foetus or newborn, resulting in miscarriage, stillbirth, or meningitis. Although the disease can be treated with antimicrobial drugs the use of these agents is not always successful; recurrent infections after appropriate antimicrobial treatment have also been reported.

Listeriosis, although often acquired by ingestion of contaminated food, has until recently not been recognised as causing symptoms normally attributed to the usual types of food poisoning. However, three recent documented foodborne outbreaks of listeriosis (Table 1) include many cases where the presence of high levels of *L. monocytogenes* has resulted in the rapid onset of symptoms of vomiting and diarrhoea with few apparent cases of the more classical infection.

Epidemiological information related to listeriosis is to some degree dependent on the regulatory situation regarding the reporting of *L. monocytogenes* and listeriosis. Annex 1 describes notifiability of *L. monocytogenes* in EU member states.

4.2.2. Virulence and pathogenicity

For testing the virulence of *Listeria* spp. in vivo tests using growth of the organisms in spleens of infected mice, killing of chick embryo's and in vitro assays using cell-lines are normally used. Virulence studies using mice spleen, chick embryos and in vitro assays indicate that in the Listeria genus only strains of *L.monocytogenes* show virulent properties (McLauchlin, 1997; Chakraborty et al., 1994). Studies summarised by McLauchlin (1997) and Notermans et al. (1991) demonstrated that the majority of *L.monocytogenes* strains are virulent. However, for a small number of strains (≤ 5%) no clear virulent properties were detected in spleens of infected mice. Chakraborty et al. (1994) showed that the few strains of *L.monocytogenes* which did not show a clear increase in the bacterial load of the spleens of mice (so-called attenuated strains) persisted for short periods in the infected host. Despite their reduced virulence they were as effective in generating protection in mice as highly virulent strains. Therefore, these strains may significantly contribute to the general level of resistance observed in natural populations.

Subtyping data together with epidemiological evidence may indicate that some strains are more pathogenic than other for humans. McLauchlin, (1997) found that out of 24 outbreaks reported in literature since 1966 14 outbreaks (58%) and ≈ 40% of the cases (1359/3338) were attributed to serovar 4b, while serovars 1/2 a, b were attributed to 8 outbreaks and 11% (385/3338) of the cases. Aureli (1998) reported an outbreak in Italy with fever and gastrointestinal symptoms involving 748 cases in which serovar 4 was implicated. McLauchlin (1997) noted that there is a discrepancy between the distribution of isolates from human cases and the prevalences of the serovars in food surveys, i.e. that a higher frequency of isolates of serotypes 4 and 1/2 are found in the diagnosed cases when compared to findings in food. This could indicate that some serovars could be more pathogenic than others, or

that these serovars (4b, 1/2 a, b) are isolated less easily than other serovars using the food *L. monocytogenes* detection methods. Nørrung (1999) interpreted the epidemiological evidence that human listeriosis are mainly caused by a few serovars (4b and 1/2 a, b), nevertheless, McLauchlin (1997) concluded that a wide range of strains might cause serious disease. Additionally, none of the (sub-)typing methods can be used to discriminate pathogenic from non-pathogenic or less virulent strains. Therefore, all *L.monocytogenes*, including those present in food, should be regarded as potentially pathogenic.

4.2.3. Dose/response

The infectious dose of a foodborne pathogen depends on many variables including the immune status of the host, the virulence and infectivity of the pathogen, the type and amount of contaminated foods consumed, the concentration of the pathogen in the food and the number of repetitive challenges.

Animal model studies using the mouse bioassay have demonstrated a potential physical barrier for *L. monocytogenes* introduction/infection offered by the stomach and intestines. Based on result of Notermans et al. (1998) this protection barrier in mice amounts to 4.5-5.5 $\log_{10}$ units. In addition to this barrier a non-specific protection is observed if *L.monocytogenes* is injected intravenously into non-immunological protected mice. This factor amounts to 1.0-1.8 $\log_{10}$ units. The cumulative effect of both protecting factors could amount to approximately 6.3-6.5 $\log_{10}$ units in mice. Additional experiments showed that there is also a clear adaptive response caused by immunological protection. In immunologically protected mice the total protection found was > 9 $\log_{10}$ cells of *L.monocytogenes.* It could be suggested that to contract listeriosis a scenario involving several simultaneous events must occur: a) exposure to large numbers of *L.monocytogenes* with concomitant breaching of the intestinal barrier, b) followed by a break-down of the non-specific defence and c) a delay in the onset of the immune response. However, when extrapolating from animal experiments to the situation in humans great caution should be exercised.

Because of the long incubation periods (1 to 90 days) shown by some human cases, incriminated food is rarely available from cases of listeriosis. In those instances where it is available, the levels of *L.monocytogenes* detected both from unopened foods and from food remnants obtained from the patients have usually been high (>10^3/g) (see Table 1 and 2). Although *L. monocytogenes* is widespread in the environment and can be isolated from a wide variety of foods, the reported incidence of listeriosis in humans is very low. In foods the organism is usually present in relatively low numbers (<100/g). This feature together with the limited data on the recovery of the organism from foods implicated in illness support the likelihood of a high infectious dose for infection through food. However, considerable caution is required because of the small number of cases where information is available and the likelihood of wide differences in susceptibilities to infection between individuals because of their immune status. The possibility of infection from

low numbers of *L.monocytogenes* especially among the immuno-compromised cannot be discounted.

In the study of Hitchins (1996) the incidence of foodborne listeriosis (approximately 10 per million) was consistent with the estimated exposure rate only if the susceptible population was unexpectedly small or extremely high doses were necessary for infection. Published frequencies of *L. monocytogenes* concentrations in food were used to convert occurrences to colony forming units (CFU). It was estimated that low *L. monocytogenes* concentrations (approximately 1 CFU/g) were too frequent to be responsible for listeriosis in susceptible subjects, using a one-cell threshold infection model. The probability of exposure to a higher dose (> 1.000 CFU) was large enough to account for the observed rate of listeriosis.

Notermans et al. (1998) used the *L. monocytogenes* prevalence data from Germany and consumption data from Dutch studies. From this information and human incidence data they concluded that despite the relatively high frequency of exposure to *L. monocytogenes*, human listeriosis is relatively rare, and therefore it is likely that infections are normally caused by high doses. It is also concluded that exposure of vulnerable groups to high doses of *L. monocytogenes* does not always result in illness.

The use of dose-response models have been introduced in several studies; these types of investigations are likely in the future to form the basis of a quantification of the risk. Farber et al., 1996, assuming reference ID_{10} and ID_{90} levels of response of 10^5 and 10^7 CFU, used the Weibull-Gamma (WG) dose-response model. Buchanan et al. (1997) used an exponential model combining epidemiological data with survey data on *L. monocytogenes* in foods. The studies are referred to in more detail in Section 4.4.

4.3. Exposure Assessment

L. monocytogenes is widespread in nature and can be found in soil, foliage and the faeces of animals and humans. The prevalence in food animals seems to be between 1-10% and a typical report is from Germany in 1997 where 49324 samples from food animals were analysed and 1800 samples (4%) were found positive for *L. monocytogenes*, Table 7.1.1 (Anonymous, 1999). Several studies indicate that humans can be carriers of *L. monocytogenes*, a prevalence between 5-10% has been indicated (Gledel, 1987, Kampelmacher and van Noorle Jansen, 1980). Somewhat worrying is that some investigations seem to show that *L. monocytogenes* can establish itself within a slaughterhouse or food processing factory as an in-house bacteria. Like other bacteria *L. monocytogenes* can create a biofilm on stainless steel surfaces and can be isolated from equipment, cold stores and floors (Herald and Zottola, 1988). Hence the possibility exists that food receiving a heat treatment during production can become contaminated post-heating in the production environment. In-house reservoirs of Listeria have been reported from both dairy plants (Unnerstad et al., 1996) and fish processing establishments (Loncarevic et al., 1996). Experience from fish production plants show that some production plants can function without *L. monocytogenes* problems while comparable plants have continuing problems (Johansson et al. 1999).

A cross section of published data from Europe and the rest of the World is presented in Annex 2. These data do not represent a total description of the situation in all food groups and all types of food production, but some general trends can be derived from the data:

- The general occurrence of *L. monocytogenes* in a relatively high prevalence in various food groups, including ready-to-eat products is well documented in many countries, and seem to underline the general view that *L. monocytogenes* is an ubiquitous organism. The food groups most often investigated are fresh meat, including poultry meat, meat products, salads, raw milk and dairy products and fish products.

- Not surprisingly *L. monocytogenes* occurs also in environmental habitats as well as in some production systems and related environments.

- In most countries there is a lack of quantitative data. When available, predominantly low numbers (< 100 *L. monocytogenes*/g) are reported, whereas a small and possibly significant fraction of the positive samples contain > 1000 *L. monocytogenes*/g.

It has been suggested that prepacked foods may be more critical in relation to human *L. monocytogenes* risk than foods without packaging (Teufel, 1994). Because of the ubiquitous nature of *L. monocytogenes* the physical handling of foods may lead to contamination of food products. Therefore it could be speculated that packaged food not heat treated in the final package and with a long shelf life could represent some of the more critical food groups.

4.3.1. Limits for growth

An important factor related to occurence of *L. monocytogenes* disease is the growth of this microorganism in food. Therefore techniques to inhibit or slow down this growth are important parts of the preventative efforts in this area. General techniques of food preservation are given in Annex 3.

L. monocytogenes is a psychrotrophic pathogen and is capable of growth at refrigerator temperatures. The minimum pH for growth in foods is 4.6-5.0 (Sutherland and Porritt 1997). *L. monocytogenes* can grow under aerobic, microaerophilic, and anaerobic conditions, and under vacuum (ICMSF, 1996). Growth has also been reported to be reduced by the presence of lactic acid bacteria (LAB), due to the lowered pH resulting from LAB metabolism but also in some cases by production of bacteriocins (Adam and Nicolaides 1997, and references therein). A summary of bacteria and compounds found to have inhibitory activity against *L. monocytogenes* has been reported in Farber (1992).

A summary of some of the limiting factors for growth is shown in Table 4.

Table 4. The limits for growth of *Listeria monocytogenes* (ICMSF, 1996).

	Minimum	Optimum	Maximum
Temperature (°C)	-0.4	37	45
PH	4.39	7.0	9.4
NaCl			10 %
Water activity	0.90 (glycerol, 30 °C)	–	–
Water activity	0.92 (NaCl)	–	–
Water activity	0.93 (Sucrose)	–	–

The ranges permitting growth in real foods are more restricted than those reported in Table 4 due to interactions between several of these factors. For instance, Farber and Hartwig (1996) summarised non-growth conditions in ready-to-eat foods to include 1) pH 5.0-5.5 and a_w < 0.95, 2) pH < 5.0 regardless of a_w, and 3) $a_w \leq 0.92$ regardless of pH.

4.3.2. Data on growth of L. monocytogenes in food

Specific data for growth of *L. monocytogenes* in different food commodities have been presented in detail by Ryser and Marth (1999).

L. monocytogenes has the ability to survive the manufacturing and ripening of many types of cheeses, surviving best in cheeses such as camembert and least in products such as cottage cheese (Farber and Peterkin 1991).

Table 5. Doubling times of *L. monocytogenes* in dairy products (Modified from McClure et al. 1997).

Product	Doubling time (h)	Temperature	pH	NaCl (g l^{-1})
Skimmed milk	12.5[1]	8	6.40*	0.5*
Cream	6[1]	13	6.40*	0.5*
UHT milk	18.5[2]	5	6.60	0.5*
Non-fat milk	12[1]	7	6.40*	0.5*
Camembert cheese	18[1]	6	6.10	2.4

1 Calculated by McClure et al., (1997) from graphs in the source reference.
2 Mean value of the range 13-24 h
* Value assumed, not given in source article.

In general, *L. monocytogenes* appears to be capable of survival on meat regardless of treatments such as freezing, surface dehydration, and simulated spray-chilling, and growth is highly dependent on the temperature, pH and type of meat, as well as background microflora present (Farber and Peterkin 1991). Poultry supported growth better than other meat products, whereas roast beef, summer sausage and hot dogs supported it the least, due to inhibition through pH, combined pH and water activity, and liquid smoke, respectively (Glass and Doyle 1989).

L. monocytogenes can grow on fresh produce stored at refrigeration temperatures. Growth on asparagus, broccoli, and cauliflower stored at 4 °C,

lettuce at 5 °C and chicory endive at 6.5 °C have been reported (Beuchat 1996b and references therein).

Table 6. Doubling times of *L. monocytogenes* in meat products and poultry (Modified from McClure et al. 1997).

Product	Doubling time (h)	Temperature	pH	NaCl (g l^{-1})	$NaNO_2$ (mg l^{-1})
Vac.-packed lean beef	30[1]	5.3	5.60	0.5*	0
Minced meat (cooked)	10.8[1]	8	5.80	0.5*	0
Cured raw pork	3.6[1]	15	6.30	2	40
Minced beef	18.1[1]	5	6.27	0.5*	0
Vacuum-packed ham	16.4[1]	10	6.63	2.77	170
Frankfurters	9[1]	15	5.80	3.2	156
Chicken legs	19.3[2]	6	6.52	0.5*	0

1 Calculated by McClure et al., (1997), from graphs in the source reference.
2 Calculated by McClure et al., (1997), from tabulated values in the source reference.
* Value assumed not given in source article.

L. monocytogenes survived but did not grow on raw salmon stored at 4 °C for 3 weeks or at 5°C for 6 days, whereas after a 10 day lag phase more than 1 log count growth on raw cod fillets was recorded after 17 days of storage at 5 °C (Ben Embarek 1994). Growth of *L. monocytogenes* on cold-smoked cod, cold-smoked salmon, crab meat, cooked shrimp, cooked crawfish tail meat, and canned lobster meats stored at 4-10 °C have been observed (Ben Embarek 1994). In naturally contaminated cured seafood, such as brined shrimps, surimi, oil marinated shrimps, caviar and marinated herring no growth was observed at 5 °C (Jørgensen and Huss 1998). A number of studies have shown that the growth rate of Lm in cold smoked salmon (vacuum packed, 3-5 % water-phase NaCl) stored at 5 °C is in the order of 1 to 2 log cycles per week (Huss 1997).

4.3.3. Estimations of L. monocytogenes growth in different food groups

In the following some predictions of microbiological growth are presented using predictive modelling programs. A short summary of available models is presented in Annex 4.

In a given food supporting growth of *L. monocytogenes* the key controlling factors determining the exposure to this organism is the initial numbers of bacteria, temperature and storage time. Figure 1 shows the influence of storage temperature on the predicted time for a 1 to 4 log increase in *L. monocytogenes* numbers in cold-smoked salmon. The predicted time for a 10-fold increase at 1 °C is approximately 12 days whereas at 7 °C it is less than 5 days (Figure 1). Similarly, the time for a 100 fold increase at 4 °C is around 10 days but at 7 °C it is about 5 days.

Predicted times for log increase -cold-smoked salmon

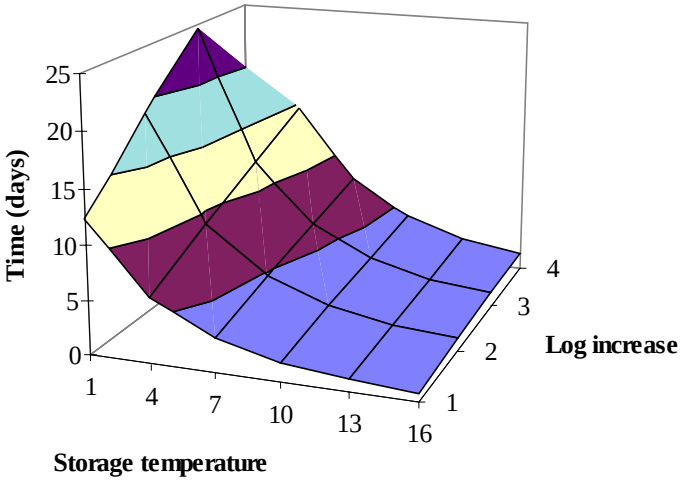

Figure 1. The predicted time for a 10 to 10000-fold increase in numbers of *L. monocytogenes* in cold-smoked salmon stored at varying temperatures. The predictions were made based on the Food MicroModel software (Version 2.5, Food MicroModel Ltd, UK), and parameters from Hudson and Mott (1993, pH=6.1, NaCl=4.41 g l^{-1}). Note: t_{10000} at 1 °C outside model range.

In table 7, the predicted time for a 10 to 10000-fold increase in four additional types of foods are presented. A comparison between lag-phases estimated with the Food MicroModel software and Pathogen Model Program (version 5.1) are shown in table 8.

Table 7. The predicted time (days) for a 10 (t_{10}) to 10000-fold (t_{10000}) increase in *L. monocytogenes* in different food types depending on storage temperatures. The predictions are based on the Food MicroModel (FMM), and the properties of the food were taken from the literature (see below). The duration of the lag-phase is included in these estimations.

	Camembert cheese [a)]					Fresh, broad-leaved endive [b)]			
T (°C)	t_{10}	t_{100}	t_{1000}	t_{10000}	T (°C)	T_{10}	t_{100}	t_{1000}	t_{10000}
1	12	19	25	Na	1	Na	Na	Na	na
4	6	9	12	15	4	7	10	14	17
7	3	5	6	8	7	3	5	7	8
10	2	3	3	4	10	2	3	4	5
13	1	2	2	2	13	1	2	2	3
16	<1	1	1	2	16	<1	1	2	2

	Vacuum-packed ham [c)]					Liquid whole egg [d)]			
T (°C)	T_{10}	t_{100}	t_{1000}	t_{10000}	T (°C)	T_{10}	t_{100}	t_{1000}	t_{10000}
1	27	45	Na	Na	1	9	15	21	26
4	12	20	26	34	4	4	7	10	12
7	6	9	12	16	7	2	4	5	6
10	3	5	6	8	10	1	2	3	4
13	2	3	4	4	13	<1	1	2	2
16	1	2	2	3	16	<1	<1	1	1

Na = not applicable. Outside model range.

a) Camembert cheese assumed to have pH=6.1, NaCl=2.4 g l^{-1} (McClure et al., 1997), using FMM Lm (lactic) model.
b) Fresh broad-leaved endive in modified atmosphere assumed to have pH=6.0, NaCl=0.5* g l^{-1}, CO_2 = 10 % (Carlin et al. 1996), using FMM Lm (CO_2) model.
c) Vacuum-packed ham assumed to have pH=6.63, NaCl=2.77 g l^{-1}, NO_2=170 ppm (McClure et al., 1997), using FMM Lm (nitrite) model.
d) Liquid whole egg assumed to have pH=7.0, NaCl =0.5 (McClure et al., 1997), using FMM Lm (lactic) model.

Table 8. Lag-phases (days) of *L. monocytogenes*, in food types stored under different conditions predicted by the Food MicroModel (FMM) software and the USDA Pathogen Modelling program (PMP version 5.1). The model used is shown in parentheses. The minimum NaCl concentration in the PMP model was 0.5 % and this was used for all predictions.

	Camembert			Fresh, broad-leaved endive	
T (°C)	FMM(lact.)	PMP	T (°C)	FMM(CO_2)	PMP[1]
1	6	Na	1	Na	Na
4	3	3	4	4	3
7	2	2	7	2	2
10	1	1	10	1	1
13	<1	<1	13	<1	<1
16	<1	<1	16	<1	<1

	Vacuum-packed ham			Liquid whole egg	
T (°C)	FMM(Nitr.)	PMP[2]	T (°C)	FMM(lactic)	PMP
1	8	Na	1	3	Na
4	4	3	4	2	3
7	2	2	7	1	2
10	1	1	10	<1	1
13	<1	<1	13	<1	<1
16	<1	<1	16	<1	<1

	Cold-smoked salmon				
T (°C)	FMM(lact.)	PMP			
1	7	Na			
4	3	3			
7	2	2			
10	1	1			
13	<1	<1			
16	<1	<1			

Na = outside model range
[1] CO2-level is not included in the model
[2] Nitrite concentration was set to 150 ppm, which is the maximum in the software.

The availability of suitable food composition data is important when the consumption of energy, nutrients, or other food constituents or contaminants are being investigated. Many regional and national tables of food consumption are available, but in general considerable differences exist among these tables (Périssé, 1982). The most relevant ways to collect food consumption data are described in Annex 5.

Food consumption data are collected for a variety of purposes, among these: food and nutrition planning; nutritional studies; and toxicological aspects of the food supply. Intake of food components may be estimated from food consumption data that describe the relative contribution of individual food items or food groups to the diet. Estimation of the average consumption of additives or contaminants by different population groups requires collection of food consumption data at the individual level. This data would also provide information about maximum and minimum levels of intake. The use of food consumption data in the food additives and contaminants areas have been instrumental in toxicological (chemical) risk assessment as an important part of the exposure assessment. Traditional food consumption data systems cannot be used directly in microbiological exposure assessment, but the original data could to some degree be used to give an overall picture. If more specific data are needed, separately designed studies are likely to be necessary until an increase in the need for microbiological exposure data influence the national food consumption monitoring programs towards basic changes. The most obvious, direct need in this area would be inclusion of some sort of food frequency data.

It has not been possible to find in the open literature an example of the use of traditional and valid, direct food consumption data, in a Listeria monocytogenes risk assessment. There are a number of examples of the use of consumption estimates from other sources, such as production or importation statistics. Some examples of the use of food consumption estimates for specific food commodities are discussed in the Risk characterisation section.

Hitchins (1996) used survey data on the frequency of foodborne occurrence and dietary exposure to *L. monocytogenes* to estimate the minimal mean per person annual rate of exposure in the United States during the late 1980s. The estimate was restricted to ready-to-eat (RTE) foods. The mean amount of each food type per *L. monocytogenes* occurrence was calculated in about 100 sources, and dietary intake data were used to calculate the mean number of occurrences of *L. monocytogenes* consumption per person per year. The dietary intake data used were obtained from the published literature, primarily from USDHHS/USDA, 1989, and USDA 1992. The mean number of occurrences of exposure to L. monocytogenes consumed annually per person was determined to be 10 to 100, when the proportion of RTE food out of the total dietary intake was estimated to values of 2 to 20%. (see original article Table 1 and 2).

Like most of the other data to be used in the exposure assessment, food consumption data should relate to national conditions, and it is difficult to extrapolate from one country to another. However there are some trends, relevant to *L. monocytogenes* exposure, which could probably describe the situation in most, if not all, of the EU Member States. The proportion of RTE food bought at the retail level has increased over the last decade and will probably increase even further in the future. In some of these foods the general flora has been controlled, in order to prolong the shelf life of the product, which could result in a change in the potential for Listeria growth in the product. The general increase in RTE foods with extended shelf lives

should result in a consideration of a future potential for an increase in the *L. monocytogenes* exposure and thereby also in the foodborne risk.

4.4. Risk characterisation

4.4.1. Human incidence and risk factors

Based on the Community Zoonosis Report (Anonymous, 1999) 342, 533 and 614 human cases were reported during 1995, 1996 and 1997, respectively. The Community annual incidence was between 1-2 cases per million inhabitants for these years. In the individual Member States (MS) the annual incidence varied between 0.5 and 16. In the Community zoonosis reports, during 1995, 1996 and 1997; 8, 10 and 9 MS reported on the findings in animals, 10, 11 and 12 MS reported on the findings in food, 11, 9, 10 MS reported on the findings in humans, respectively. In the USA Tapper et al., (1995) reported an annual incidence of 4 to 5 cases per million inhabitants. In other papers (Danielsson-Tham and Tham, 1999; and Farber and Peterkin, 1991) the annual incidence is reported to be between 2 and 15 per million inhabitants. According to Bille (1996) and Hitchings (1995) there is reason to believe that the true incidence is higher since not all clinically suspect cases are examined bacteriologically. In many references the population at increased risk is referred to as the YOPI fraction (Young, Old, Pregnant, Immuno-compromised)

The annual incidence is composed of cases related to outbreaks and sporadic cases. Whereas very little information relating sporadic cases to specific food commodities is available, outbreak data can be used to highlight risk related foods. The picture emerging from outbreak investigations is that the food implicated has undergone some preparation process and is ready to eat after a period in cold storage (Tham and Tham, 1999 and Gilbert, 1995). The California outbreak in 1985 (Linnan et al., 1988) was an instructive example in this regard where in the USA Mexican style cheese made of un-pasteurised milk was kept in cold storage for a period of weeks, while in Mexico this cheese is eaten fresh within 1-2 days. It resulted in an increased incidence of sporadic cases of listeriosis (abortions), which appeared to be an epidemic first at the community level. The conclusion appears to be that the introduction of cold storage to prolong the shelf life opened up the ecological window for the Listeria bacteria.

In outbreak investigations the prolonged incubation periods of Listeria of up to 3 months, might introduce a recall bias for the cases with the longest incubation period, resulting in less precise or even biased risk estimates for factors associated with the cases having long incubation periods. Hall et al. (1995) reported a case-control study on sporadic listeriosis cases. In this study in which 124 cases and 459 controls were compared, the consumption of ready cooked chicken eaten cold, crustaceans and mussels and eating out were the identified risk factors. A recent example of an observational study is the outbreak investigation of a Finnish outbreak in hospitalised patients (Lyytikäinen et al., 1999), the source of infection was most likely butter from a particular plant. The victims were cancer patients who were given butter as an extra energy source, studies of the distribution of butter from that plant,

genotyping (pulsed field gel electrophoresis) and contamination analysis were used to clarify the source of infection.

When assessing the outbreak histories referred to in Tables 1-2, neither in-house contamination nor contamination from the primary production can be excluded.

4.4.2. Risk quantification

Buchanan et al. (1997) presented a study using data on listeriosis in Germany combined with levels of *L. monocytogenes* in smoked fish. The assumption was that a conservative dose-response relationship (i.e. erring on the safe side) could be estimated on the basis of comparing available epidemiological data with food survey and consumption data for a ready-to-eat product, i.e. smoked fish. A survey of food surveillance data in Germany indicated that smoked fish could be a major source of *L. monocytogenes* in the German diet because a significant percentage of the product contained elevated levels. There are an estimated 200 cases of Listeriosis per year for a population of around 80 million. In reaching what the authors believe to be a conservative risk estimate the study assumes that all *Listeria* cases are foodborne, cases are generally restricted to the high risk population, annual consumption per capita of smoked fish = 1 kg, and dose-response relationship for *L. monocytogenes* infection fit the exponential dose-response model. In summary, it is claimed that from disease incidence and actual *L. monocytogenes* levels in food combined with consumption data, it is possible to estimate the relationship between exposure and morbidity. The authors noted that quantitative data on the actual levels of *L. monocytogenes* in food would allow frequency distributions to be used in assessing pathogen levels. Thus more advanced stochastic simulation techniques such as Monte Carlo simulations could then be used. The conclusions of these types of studies rely heavily upon the assumptions, which are often taken because of a lack of data. Some of the important assumptions of this study are: a) the dose response considerations are derived only from a combination of (relatively poor) exposure and incidence data, which are in origin non-correlated, leading to a presumptive dose-response relationship not related to relevant observations, b) the potential for microbial growth in the food is not considered, which is a very realistic possibility with important implications, and c) there is no consideration of uncertainty.

Farber et al. (1996) considered the steps in a risk assessment process using *L. monocytogenes* in paté and cheese as examples. Canadian *L. monocytogenes* policy directs inspection activities towards ready-to-eat products capable of supporting growth of the organism. These foods are usually linked to listeriosis and within these food types highest priority is given to foods with a shelf life of greater than 10 days. The authors define (arbitrary?) reference values to which the dose-response model should approximate (ID_{10} and ID_{90}), i.e. dose causing illness in 10 and 90% of the population respectively. In considering the exposure characteristics, the average incidence data for *L. monocytogenes* in Canada for meats and dairy products is 4.4 and 1.2% respectively. According to the authors accurate data on Canadian consumption patterns are not available, but it is estimated from existing disappearance data (i.e. produced amount minus residual amount) from

Statistics Canada that 55 servings of 100g of soft cheese per capita per year are consumed - disappearance data for paté does not exist. The authors used an assumption that 10-20% of the cases of *L. monocytogenes* could be attributed to exposure through cheese consumption, and used these figures to present a calculation of the average probability of acquiring human listeriosis in Canada from soft and semi-soft cheese consumption. The authors conclude that there is a substantial level of consistency between reported data and assumptions of this risk assessment model.

Again the Farber study relies heavily upon certain assumptions because of the usual lack of solid data. Some of the important assumptions of this study are: a) a dose causing illness in 10 and 90% of the general population and of the 'high risk' fraction of the population separately was introduced in the model without basis in dose-response data, b) the study does not include a full characterisation of the hazard in all relevant food types, and as usual the fraction of actual disease cases attributable to the chosen food types can not be estimated, c) the possibility of occurrence of *L. monocytogenes* strains with different virulence characteristics is lacking. In general the principle of estimating the reliability of one of the factors in a multifactorial model with a number of relatively uncertain, and even qualitative, data, through a comparison with actual incidence data, merits further consideration and especially a thorough estimation of the uncertainty involved, which is not presented. Another interesting point missing is the general question of differences in the population susceptibility to *L. monocytogenes*. It could be relevant to scrutinise further the use of only two subpopulations: general and 'high risk'. Likewise a discussion of the merit of a two-log difference between the stipulated infective doses for the two groups would be interesting.

Smoked fish data (Teufel and Bendzulla, 1993) were used to estimate the risk of foodborne listeriosis in individuals with increased risk in Germany (Van Schothorst, 1995). It was assumed that all cases of listeriosis in Germany (estimated 300 cases of listeriosis for a population of 83 million – different to the 200 cases estimated by Buchanan et al. (1997)) were attributable to ready to eat smoked fish containing >10,000 cfu *L. monocytogenes*/g, that the normal serving size is 100g, and that up to 20% of the population may belong to the high-risk group at any time. Based on these assumptions van Schothorst (1995, 1996) estimated the risk of an immunosuppressed individual acquiring listeriosis from such a heavily contaminated portion of smoked fish at 1 in 6000. The corresponding estimated risk for a product containing <100 cfu/g would be 1 in 100,000. Buchanan et al. (1997) stated that this latter value was over-conservative due to the exponential character of dose-response relations, and that the probability of acquiring listeriosis from a serving of smoked fish containing 100 cfu/g was less than 1 in 1,000,000. It should be noted that both estimates of risk are based on a series of conservative assumptions and the actual risk of acquiring listeriosis is likely to be even less by one or more orders of magnitude. Again the lack of data, the nature of the assumptions and the lack of uncertainty presentation or discussion makes it difficult to use these risk estimates in further modelling.

Bemrah et al. (1998) attempted a quantification of the microbial risk in soft cheese made from raw milk. Since quantitative data could only be found for *L. monocytogenes*, this hazard was used as an example. The complete process of cheese making was modeled, from milking to consumption. The probability of milk contamination and the percentage of cheese with a predicted concentration of *L. monocytogenes* greater than 100 CFU g-1 was suggested. Individual annual cumulative risk of listeriosis, in a population each consuming 50 servings of 31 g, ranged from 1.97 x 10(-9) to 6.4 x 10(-8) in a low-risk sub-population and 1.04 10(-6) to 7.19 10(-5) in a high-risk sub-population. This study suffers from a lack of solid dose-response data, and the basis for the exposure (consumption) estimates are not clearly presented.

The overall risk to the human population for the contraction of listeriosis appears to be around 1 to 10 per million per year based on internationally published incidence data. Even though a recent studies by Miettinen et al. (1999) point at the use of pulsed-field gel electrophoresis to suggest cause-effect relationship in a food-borne outbreak of *L. monocytogenes*, it is at the moment difficult to stratify the risk relative to the relevant food commodities. At present no sub-typing systems seem to open the possibility to relate specific human strains to specific food types. This would correspond to the pathogen account principle applicable to Salmonella spp. It has been suggested that packaged food which is not heat treated in the final package and with a long shelf life could represent some of the more critical food groups (Teufel, 1994). It has not been suggested, however, what fraction of the *L. monocytogenes* risk relates to this food group.

4.4.3. L. monocytogenes risk in the future

Several factors might result in an increased incidence of listeriosis in the future:

(1) The increasing proportion of susceptible people be it due to old age or immunosuppresive treatments and/or diseases;

(2) The increased use of cold storage to prolong the shelf life of foods;

(3) The possible emergence of non-classical listeriosis, such as diarrhoea.

5. PREVENTATIVE MEASURES

It seems clear from the data presented in Section 3 that certain food commodity groups represent a special risk scenario in relation to food-borne listeriosis. The critical point relates primarily to the potential for growth of *L. monocytogenes* in the food. Therefore the *L. monocytogenes* survival and multiplication conditions can be used to suggest grouping principles for food commodities and production regimes relative to *L. monocytogenes* conditions.

Additional to any preventative or management initiative the importance of risk communication and consumer education in this area must be stressed.

5.1. Grouping of food commodities relative to *L. monocytogenes* conditions

For the control of *L. monocytogenes* in a particular food, consideration must be given to the potential for growth based on criteria such as pH, water activity, the presence of preservatives and the shelf life of the product. This is especially important in foods that have not received any listericidal treatment or which are handled after such a treatment which may increase the possibility for recontamination. The use of predictive growth models may be helpful to initial assessments of the exposure to *L. monocytogenes* under different storage regimes, but these assessments must be confirmed experimentally.

If the initial number of *L. monocytogenes* in a food is known, it may be combined with simulated growth data as those described in Table 7, to come up with a first estimate of the tolerable storage regime (time and temperature) for a batch of food. The acceptable storage regime would be based on a tolerable level of *L. monocytogenes* on the day of consumption. The initial number of *L. monocytogenes* can be obtained from quantitative analyses or, in the absence of positive samples, it may be possible to estimate it based on the sampling plan or other information.

Management initiatives, including recommendations or criteria for tolerable levels of *L.monocytogenes* in ready to eat foods have been introduced in a number of countries (Gravani 1999). Among these, USA and Italy require absence of *L. monocytogenes* in 25 g of foods ('zero tolerance') while other European countries (i.e. Germany, Netherlands and France) have a tolerable level of 100 or 1000 *L. monocytogenes*/g at the point of consumption. Finally some countries, i.e. Canada and Denmark, have a tolerance of below 100/g for some foods and a zero tolerance for others, especially those which are supportive of growth and have extended shelf lives.

Current Community legislation only provide for microbiological standards with regard to *Listeria* in provisions for soft cheeses and pasteurised milk and for other dairy products. The SCVPH has recently finalised a general report discussing the use of Microbiological Criteria in Community legislation.

Several countries have concluded that a complete absence of *L. monocytogenes* for certain ready to eat foods is an unattainable and

unrealistic requirement that would restrict food production and consumption without having a positive impact on public health. Consequently such risk management action might detract resources form other potentially more efficient measures against *L. monocytogenes*.

A few examples of suggested food groupings are presented here:

A recently suggested approach in Germany (Bartelt et al. 1999) includes four food categories:

I. Technology assuring death of *L. monocytogenes*, no recontamination potential

II. Foods, which could be contaminated by *L. monocytogenes*, but not promoting growth of *L. monocytogenes*

III. Foods, which could be contaminated by *L. monocytogenes* and which may allow growth of *L. monocytogenes*

IV. Foods, which are not ready to eat, to be heated before consumption

The Danish grouping approach has been described by Nørrung et al. (1999)

I. Foods, which have been heat treated in the final package.

II. Heat treated foods, which have been handled after heat treatment. The products support growth of *L. monocytogenes* during the shelf life. Typically the shelf life of the products is above 1 week.

III. Lightly preserved, not heat treated, ready to eat products. The products support growth of *L. monocytogenes* during the shelf life. Typically the shelf life of the products is above 3 weeks.

IV. Heat treated foods, which have been handled after heat treatment. The products are stabilized against growth of *L. monocytogenes* within the shelf life. Products which have a shelf life less than 1 week are regarded as stabilized

V. Lightly preserved not heat treated ready to eat products. The products are stabilized against growth of *L. monocytogenes* during the shelf life. Products with a shelf life less than 3 weeks are regarded as stabilized.

VI. Raw, ready to eat foods.

The Canadian approach has been described by Farber et al. (1996):

I. Foods, which have been causally linked to outbreaks of listeriosis

II. Foods, which are capable to support growth of *L. monocytogenes*, having a shelf life of >10 d

III. Foods supporting growth of *L. monocytogenes* with a shelf life < 10d

IV. Foods not supporting growth of *L. monocytogenes*

The data presented in Section 3 supports the consideration of a differentiation of food groups relative to the potential for *L. monocytogenes* contamination and growth. The three national grouping schemes presented above all suggest a separation along such lines. Thus the separation should relate to:

- the capacity of the food technology to kill *L. monocytogenes* and
- the capacity of the food technology to prevent recontamination with *L. monocytogenes*,
- the potential of *L. monocytogenes* to grow in the food commodity.

If ready-to-eat foods are divided in two groups according to each of the above three criteria, it would result in eight groups. However two of these eight groups can logically be included in some of the other groups, resulting in the six group system described in Table 9.

Table 9. Grouping of ready-to-eat food commodities relative to the control potential for *Listeria monocytogenes*. *) *Ranking does not relate to risk magnitude*

A.	Foods heat-treated to a listericidal level in the final package.
B.	Heat-treated products that are handled after heat treatment. The products support growth of *L. monocytogenes* during the shelf life at the stipulated storage temperature.
C.	Lightly preserved products, not heat-treated. The products support growth of *L. monocytogenes* during the shelf life at the stipulated storage temperature.
D.	Heat-treated products that are handled after heat treatment. The products are stabilized against growth of *L. monocytogenes* during the shelf life at the stipulated storage temperature.
E.	Lightly preserved products, not heat-treated. The products are stabilized against growth of *L. monocytogenes* during the shelf life at the stipulated storage temperature.
F.	Raw, ready to eat foods.

*) Examples of products:
Groups B and D: meat products such as cooked ham, wiener sausages or hot smoked fish, soft cheese made from pasteurized milk.
Groups C and E: cold smoked or gravad fish and meat, cheese made from unpasteurized milk
Group F: tartar, sliced vegetables, sprouts.
Separation between groups B and D, and C and E respectively based on the technology used.

The impact of food technologies on *L. monocytogenes* survival and growth is described in Annex 2. However, it should be considered that several studies conclude, that the response of *L. monocytogenes* to single preservative factors in experimental studies may be different compared to data from „field“ studies in foods, even if the same preservative factors are studies. This makes the predictivity of bacterial pattern in foods difficult.

A number of investigations, including data from Dalgaard & Jørgensen (1998), show that experimental data may not be sufficient to evaluate the *L. monocytogenes* pattern in various food commodities. This may be due to the observation, that in most foods more than one factor of preservation would be applied and the sum of each of them will additionally influence the whole preservative action.

Accepting that the above-mentioned uncertainties do exist, Table 9 presents a suggested division of food processing types and their relation to *L. monocytogenes*, resulting in three categories of food processing:

5.2. Microbiological levels

Microbiological criteria should be developed according to the "Principles for the development of Microbiological Criteria for Animal Products and Products of Animal Origin Intended for Human Consumption" (EU Commission paper, 1997). The considerations presented here are not meant as Criteria considerations, but will only discuss the *L. monocytogenes* levels, which could be relevant in a possible further effort to lower the incidence of food-borne listeriosis.

Based on the data presented in Section 3 a concentration of *L. monocytogenes* not exceeding 100/g of food at the point of consumption could be considered to be of low risk to the consumers. Because of the uncertainty related to the estimation of this risk and because the potential growth of *L. monocytogenes* in food seem very important in listeriosis case developments, levels lower that 100/g may need to be applied for those foods in which growth can occur. It is likely that an intensified effort related to the critical food commodity groups could lead to a better and more consistent risk management of *L. monocytogenes* in food.

Following this rationale and recognising the characteristics of foods belonging to the different groups in Table 9, the following levels may be considered.

1. Food groups D, E, and F

    - *L. monocytogenes* levels should be < 100 cfu per gram at the time of consumption, and therefore throughout the shelf life of the commodity.

From outbreak and sporadic cases data (Tables 1 and 2), it appears that a level greater than 100 cfu per gram is commonly found in the implicated foods. Based on these observations this level may be discussed as a tolerable level *L. monocytogenes* at the time of consumption.

2. Food groups A, B, and C

   - *L. monocytogenes* should not be detected in 25 gram at the time of production.

For food groups A, B, and C that are heat-treated in the final package and/or support growth, more stringent levels may need to be applied. Due to the large uncertainties regarding growth and storage, analytical methods, sampling etc, a more conservative approach may be warranted. Furthermore, detection of *L. monocytogenes* in food belonging to group A indicates a failure in the thermal processing.

However, it should be emphasised that it is the ingested dose, not the level, that constitutes the hazard and, thus, that the consumption pattern of a specific food is important. Also, in food intended for more vulnerable consumers other levels may be warranted.

The degree of protection suggested by the above levels will depend upon the management initiatives, they will be part of, and notably by the parameters chosen in the final sampling plan if microbial criteria are applied.

In a further effort towards reducing the shelf life of critical food commodities limits for total shelf-life/temperature combinations could be considered.

The nature of this report does not allow for in-depth discussions of microbiological metods. It is clear that any development of microbiological criteria will have to include method considerations.

5.3. Further developments

Pending risk management considerations as to the tolerable level of risk for listeriosis, it is likely that future risk management options will have to be considered.

When considering such options it could be relevant to use the grouping of food commodities presented in Table 9 as a basis. Epidemiological and outbreak data have pointed out commodities from these groups as especially problematic in relation to food-borne listeriosis.

5.3.1. GHP and HACCP

Application of general principles of food hygiene and in particular of the HACCP- concept as laid out in the Food Hygiene Directive 93/43/EEC[2] as well as in the "Fishery Products" Decision 94/356/EC[3] could form the basis for an improved effort to control *L. monocytogenes* and hence to prevent

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[2] Council Directive 93/43/EEC of 14 June 1993 on the Hygiene of feedstuffs (O.J. L175, 19.07.93, p. 1)

[3] Commission Directive of 20 May 1994 laying down detailed rules for the application of Council Directive 91/483/EEC, as regards own health chicks or fishery products

listeriosis. Timely action, taken in case of a deviation at a critical control point (CCP) will reduce the risk that defective products reach the consumer. Analysing samples of end products may provide some additional information concerning the microbiological status of the product but will not guarantee their safety. The importance of handling procedures, such as slicing, packing of fish, mincing of meat, in relation to *L. monocytogenes* contamination underline the environmental origin and persistence of the bacteria (Cortesi et al., 1997). Also the diligence of particular workers within a given production (i.e. smoking of salmon) have been shown to have a strong impact on the prevalence of *L. monocytogenes* (Rorvik et al., 1997). The potential of *L. monocytogenes* 'nests' in the final products, resulting from biofilm contamination should be considered. And the implications of the experience from fish production plants showing that some plants can function without *L. monocytogenes* problems while comparable plants have continuing problems should be further considered (Johansson et al., 1999).

In the present food microbiology regulatory environment many countries have introduced HACCP thinking throughout most of their food production chains. Even if HACCP is functioning, the production units still need guidance from the authorities as to what level of protection should be aimed at. In the future this guidance could take the form of Food Safety Objectives.

6. SUMMARY AND CONCLUSIONS

(1) *L. monocytogenes* is ubiquitous, and can be present in all food types that have not been exposed to treatments during production, which are listericidal.

(2) The introduction of cold storage to prolong the shelf life of a specific food commodity has opened an ecological window for the growth of *L. monocytogenes.* Because of the ubiquitous nature of *L. monocytogenes* the physical handling of foods may lead to contamination of food products. Packaged, long shelf-life food, which is not heat-treated in the final package, represents the most critical food commodity group.

(3) Several features support the likelihood of a high infectious dose for *L. monocytogenes* infection through food. However, the possibility of infection from low numbers of *L. monocytogenes*, especially among the most susceptible population groups (young, old, pregnant, immunocompromised) cannot be discounted.

(4) According to outbreak data available it would seem that the presence of *L. monocytogenes* in food represents a very low risk for all population groups, when the *L. monocytogenes* concentration is below 100cfu/g. The implications of this statement do not relate to a dose but only to a concentration. The limit of 100cfu/g is not based upon formal dose-response formulas. Likewise it should be born in mind that the consumption pattern for relevant foods are not directly available and have therefore not been considered.

(5) *L. monocytogenes* levels above 100cfu/g may be reached after in-food growth. Therefore risk management efforts should be focused on those food commodity types where *L. monocytogenes* can multiply. The potential for accidents in the production should not be neglected.

(6) The potential for growth of *L. monocytogenes* can be minimised in food through pH, water activity, preservatives and the shelf life of the product combined with temperature and storage time. Predictive growth models are helpful when assessing this potential, but experimental confirmation is often lacking and is necessary.

(7) There are indications that *L. monocytogenes* can establish itself within a food processing factory as an in-house bacteria. It is also noteworthy that some production plants seem to be able to function without *L. monocytogenes* problems while comparable plants have continuing problems.

(8) In addition to published *L. monocytogenes* prevalence and concentration data from food, it is essential for the exposure assessment to obtain frequency based intake data for relevant foods.

(9) Three factors might result in an increased incidence of listeriosis in the future:

- The increasing proportion of susceptible people be it due to old age or immunosuppresive treatments and/or diseases;

- The increased use of cold storage to prolong the shelf life of foods;
- The possible emergence of non-classical listeriosis, such as *L. monocytogenes* food poisoning resulting in diarrhoea.

7. RECOMMENDATIONS:

(1) Management options to control and/or lower the risk of human listeriosis from food consumption must be implemented in view of the high case-fatality rate of this infection, despite the relatively low incidence of human disease.

(2) An objective must be to keep the concentration of *L. monocytogenes* in food below 100cfu/g and to reduce the fraction of foods with a concentration above 100 *L. monocytogenes* per gram significantly. This objective should be expressed as a Food Safety Objective. The effect of initiatives to this end must be evaluated through surveillance investigations of food, especially including quantitative investigations, as well as efficient monitoring of human listeriosis.

(3) The potential for growth of *L. monocytogenes* must be the focus of attention when designing management options. In particular the following must be considered:

a) The grouping of foods according to *L. monocytogenes* growth potential (see Table 9);

b) The presentation of production dates on all products;

c) Appropriate temperature and storage time combinations;

d) The potential of shelf life limitations;

e) Identifications of relevant *L. monocytogenes* limits for the different food groups (see Section 5.2).

(4) Since some production plants operate without *L. monocytogenes* problems while comparable plants have recurrent problems underline the necessity of improvements in production hygiene. HACCP and GMP must be geared to reduce/eliminate *L. monocytogenes* colonisation of production environment. The potential for real time monitoring for *L. monocytogenes* at the production line must be considered. Further research must be initiated towards control of 'house strains' in food production facilities.

(5) Strategies for risk communication must be implemented. Apart from advice to the general public, special attention should be addressed to consumer groups at increased risk (i.e. young, old, pregnant, immunocompromised) which represent a considerable and growing section of the total population.

(6) Technological changes in food production and food storage regimes must be evaluated with regard to *L. monocytogenes* prevalence and growth.

(7) Experimental data on *L. monocytogenes* growth are lacking for a number of specific commodities. Research to acquire this information must be implemented to support predictive model estimations of growth potential.

8. GLOSSARY

In this text the following definitions have been used:

- **Raw materials**: food which has not been processed or only processed through chilling and (depending on national definitions) freezing, cutting or mincing.

- **Processing**: every technical procedure exceeding the procedures mentioned under the above definition of raw materials.

- **Ready to eat**: food intended for consumption without any further processing (e.g. heating).

- **Growth / multiplication of bacteria**: the status of population size or cell mass increase of a bacterial population under advantageous environmental circumstances.

- **Death of bacteria**: status of a single cell where it has been injured irreversibly resulting in non-potential to divide, death in bacterial populations normally follow an exponential decay model (constant fraction of the population dying per time unit).

- **Listericidal**: characteristic of a technical procedure or a microbial environment resulting in the killing of *L. monocytogenes.*

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Annex 1

Notifiability of *L. monocytogenes* in EU member states
(Based on the Zoonosis Report for 1997 – Anonymous, 1999)

(1) In animals

In most EU Member States listeriosis is not a notifiable disease but in Sweden and Germany listeriosis is notifiable based on clinical symptoms in food animals (Anonymous, 1999). Other Member States have ongoing monitoring programs of varying intensity. Between eight and ten member states provided information on the listeriosis frequency in animals during 1995-1997.

(2) In food

Listeriosis is not notifiable, however several Member States have monitoring of the findings in varying foodstuffs, while the sampling strategies and microbiological procedures are not harmonised. Belgium is continuously monitoring the Listeria prevalence in meats and the Netherlands monitors the prevalence in cheese.

Between ten to twelve Member States gave some information on the Listeria frequency during 1995-1997.

(3) In humans

Listeriosis is a notifiable disease in Sweden and Denmark, while in other Member States the reporting is based on laboratory findings. Between nine and eleven Member States provided reports on the listeriosis occurrence in humans. Listeriosis is a disease with several manifestations (see Table 3), among these skin lesions, infections during pregnancy with abortions, pre and post natal infections, CNS symptoms and food poisoning. This could result in a downward bias in the reported incidence of human cases, based on clinical symptoms alone.

Annex 2

PREVALENCE OF *L. MONOCYTOGENES* IN FOOD COMMODITIES: FIELD STUDIES

GENERAL SURVEYS:

Ben Embarek (1994) reviewed the literature on seafood. The following contamination status *with L. monocytogenes* in the environment and the product was reported:

- Freshwater, seawater and sediments: 8 references, contamination prevalence between 0 (3 references) and 62 %;
- Live fish and shellfish: 7 references, contamination prevalence between 0 (5 references) and 11 %;
- Fresh, frozen, processed sea foods: 36 references, contamination prevalence between 0 (15 references) and 50 %.

Little data is available for frozen foods. In a German study, Mangold et al. (1991) found *L. monocytogenes* most frequently in fish products, meat products, soups and broths, food containing poultry meat and meat meals. It should be noticed, that for food groups with final production steps involving direct handling the prevalence of *L. monocytogenes* was high, i.e., there could be a risk factor related to the process of handling foods.

L. monocytogenes occurs commonly in sewage and related substrates (Beuchat 1996). Palumbo et al. (1999) found *L. monocytogenes* in reconditioned water at a local meat processing plant and a slight increase in the number in non-chlorinated water, whereas the presence of chlorine lead to a decline in viable counts. In sewage sludge, the highest frequency of *L. monocytogenes* was found in activated sludge samples (53.3 % positive samples with a mean MPN concentration of 1300 *L. monocytogenes*/g dry matter) and the lowest in dewatered sludge (8.3 % positive samples with a mean MPN concentration of 29 *L. monocytogenes*/g dry matter) (de Luca et al., 1998).

SURVEILLANCE DATA (QUALITATIVE) FROM PARTICULAR REGIONS WORLD WIDE:

Extensive data on *L. monocytogenes* in foods have been reported over the years. The following data may serve as a guide to the *L. monocytogenes* burden in foods. It should not be regarded as an exhaustive review of the literature.

The following numbers in various commodities have been found. They demonstrate the ubiquitous occurrence of *L. monocytogenes* in foods and food processing plants

(data from Beuchat 1996b; Breuer & Prändl 1988; Fries & Müller-Hohe 1990; Genigeorgis et al. 1990; Gitter 1976; Guyer & Jemmi 1990; Hartung 1997; Jay 1996; Jemmi 1990; Johansson et al. 1999; Jörgensen & Huss 1998; Kozak et al. 1996; Loncarevic et al. 1996; Noack & Jöckel 1992; Ozari & Stolle 1990; Pini & Gilbert 1988; Rorwik & Yndestad 1991; Rorwik et al. 1995; Skovgaard & Morgen 1988; Steinmeyer 1989; Varabioff 1990; Weise 1987).

Fresh meat	0 – 8 %
Minced meat	7 - 36 %
Meat products	0 – 52 %
Poultry (Broilers)	9 - 85 %
Fish products	4 – 60 %
Vegetables, Salads	1 - 12 %
Milk, milk products	
Ice cream	22 %
Raw milk	2 - 12 %
Restaurant	
(ready to eat)	9 %
(heat treated, intended to be cooked again)	3 %

QUANTITATIVE DATA:

Loncarevic et al. (1996) found that ten of 16 positive samples of 150 vacuum packed fish in Sweden were contaminated with more than 100 cfu/g *L. monocytogenes.* In a study of Guyer & Jemi (1990) from Switzerland the concentration of *L. monocytogenes* in positive samples of smoked salmon was in all cases <1/g. Kozak et al. (1996) stated, that in frozen products only low numbers (< 10 cfu/ml) had been found. According to Jay (1996), raw meat tends to contain numbers <100/g *L. monocytogenes*, whilst processed meat and poultry meat contain higher numbers.

In Germany, Teufel and Bendzulla (1994) assessed the prevalence of *L. monocytogenes* in different commodities quantitatively. Most numbers were found in meat products followed by fish products, salads and cheese. The numbers are as follows:

Concentration/g:	0.04 – 1	$1 – 10^2$	$10^2 – 10^4$	$> 10^4$
Meat products	13.7 %	7.8 %	1.4 %	0.2 %
Fish products	6.0 %	2.2 %	0.6 %	0.6 %
Cheese	1.8 %	0.7 %	0.5 %	0.1 %
Salads	3.1 %	1.9 %	0.2 %	0

Results from a survey performed in 1994 and 1995 show the following prevalences and quantitative groupings for *L. monocytogenes* in retail foods in Denmark (Andersen and Nørrung, 1995)

	No of samples (percent) *L.m.* positive in a 25 grams sample	No of samples (percent) with L.m. between 10-100 per g.	No of samples (percent) with more than 100 *L.m.* per gram	No of samples investigated
Preserved fish products (not heat treated)	35 (10.8%)	11 (3.3%)	6 (1.8%)	335
Preserved meat products (not heat treated)	77 (23.5%)	6 (1.8%)	2 (0.6%)	328
Heat treated meat products	45 (5.0%)	12 (1.5%)	11 (1.4%)	772
sub total	157 (11.9%)	29 (2.0%)	19 (1.3%)	1435
Raw fish	33 (14.2%)	6 (2.6%)	1 (0.5%)	232
Raw meat	106 (30.9%)	30 (8.7%)	12 (3.6%)	343
Total	296 (14.7%)	65(3.2%)	32(1.6%)	2010

Annex 3

THE IMPACT OF FOOD TECHNOLOGIES ON *L. MONOCYTOGENES* SURVIVAL AND GROWTH

Field studies of survival and growth in food commodities

Guyer & Jemmi (1990) found a significant increase of *L. monocytogenes* during refrigerated storage of marinated, cold-smoked (26 - 30 °C) salmon, leading the authors to conclude that an increased storage time for cold-smoked salmon would lead to an increase in *L. monocytogenes* risk. Rorvik et al. (1991) reported similar findings for storage under vacuum and at 4 °C. Ben Embarek & Huss (1993) found different D_{60}- values between 1.95 and 4.48 depending on *L. monocytogenes* strain and fish type (cod and salmon). Schaik et al. (1999) demonstrated a protection of pH adapted (pH 5.5) *L. monocytogenes* against some bacteriocins (nisin, lacticin).

Experimental studies of survival and growth in food commodities

The response of *L. monocytogenes* in a variety of food commodities has been investigated experimentally by Glass and Doyle (1989). Growth was closely related to the pH of the product, e.g. *L. monocytogenes* grew well on products near or above pH 6 and poorly or not on products near or below pH 5. *L. monocytogenes* survived but did not grow on summer sausage, grew only slightly on cooked roast beef, and grew well on some wiener Frankfurters products (but not on all of them), ham, „bratwurst„ (uncured sliced meat intended to be roasted), sliced chicken and turkey. Palumbo et al. (1993) reported D_{60} values for *L. monocytogenes* quoting several authors from 1.62 min. (ground beef) to 8.32 min. (ground beef slurry).

In the following table some detailed experimental studies are listed:

Commodity	***L. monocytogenes* inoculation number and results**
Fermented sausage	
German Tea sausage	8 x 10^6/g and 6.5 x 10^2/g; *L. monocytogenes* survived but concentration decreased rapidly during the first four days of ripening (Buncic et al. 1991)
Frankfurter Type Sausage	dipped into a suspension (10^2/ ml), dried and packaged; initial load increased 30-fold after 10 d, by 420 times after 20 d (Buncic et al. 1991)
Liver sausage	contamination rate of 10^9/ g, temperature of 155° F for 150 min, hereafter *L. monocytogenes* was below the detection limit (Palumbo et al. 1993)
Fish products	
(smoked salmon)	salmon (technologically different treated: salted, smoked, dried) inoculated, stored at 4 °C over 30 d. In smoked samples no significant growth was observed (Niedziella et al. 1998)
lobster meat	combined effect of nisin and moderate heat (60°C/5 min and 65 °C/ 2 min) resulted in 3-5 log decimal reduction compared to 1-3 log reduction using separately nisin respective heat (Budu-Amoako et al. 1999).
Egg products	

Egg white	Tryptic Soy Broth with lysozyme: a prolonged lag phase of *L. monocytogenes* was observed. Lowering the pH from 7.2 to 5.5, the lag phase was 9 d. If additionally using Lysozyme, the lag phase was prolonged to 70 d (all data at 5 °C; Johansen et al. 1994)

L. monocytogenes is a poor competitor and is often overgrown by other bacteria present in foods (Adams and Nicolaides, 1997; Jay, 1996).

Annex 4

MODELS - PREDICTIVE MICROBIOLOGY

Several predictive mathematical models describing growth of *L. monocytogenes* in foods have been developed (Table 4). These models describe the effects of extrinsic parameters such as temperature, CO_2, and aerobic/anaerobic conditions, and intrinsic parameters including NaCl/a_w, pH, $NaNO_2$, acetic acid, lactic acid and phenol. However, no single model contains more than four of these controlling factors (Dalgaard and Jørgensen 1998).

Summary of some models describing the growth rate of L. monocytogenes. The table was modified from te Giffel and Zwietering (1999) and also includes references cited in Dalgaard and Jørgensen (1998).

Model	Controlling factors	Type of model
Gamma	T, pH, a_w	Square root
Pathogen Modelling Program	T, pH, a_w	Polynomial (2^{nd} order)
Food Micro Model	T, pH, a_w, nitrite, lactate, CO_2	Polynomial (2^{nd} order)
Grau and Vanderlinde 1993	T, pH	Modified Arrhenius
Duffy et al. 1994	pH, aw	Polynomial (2^{nd} order)
Farber et al. 1996	T, pH, CO_2	Polynomial (2^{nd} order)
Patterson et al. 1993	T, irradiation	Polynomial (3^{rd} order)
George et al. 1996	T, pH, acetic and lactic acids	Polynomial (2^{nd} order)
Membré et al. 1997	T, NaCl, phenol	Exponential/polynomial
Murphy et al. 1996	T, pH, NaCl	Polynomial (3^{rd} order)
McClure et al. 1997	T, pH, NaCl, $NaNO_2$	Polynomial (2^{nd} order)

Several of these models have been evaluated by comparisons of predictions with literature data against challenge studies in foods using graphical and statistical methods (Dalgaard and Jørgensen 1998, te Giffel and Zwietering 1999). In general, published growth rates are slower than those predicted, i.e. predictions err on the side of consumer safety, although a larger variability exists in predictions at near growth-limiting conditions (te Giffel and Zwietering 1999). It has also been observed that *L. monocytogenes* in naturally contaminated food grows slower than in artificially contaminated foods (Dalgaard and Jørgensen 1998). Another limitation of the predictive models is the natural variation between *L. monocytogenes* strains. Begot et al. (1997) divided *L. monocytogenes* strains into 5 groups depending on their growth responses in a meat broth. There were less variation in generation times than in lag phases between the groups, and lag phases ranged from 4 h to 4 days at 10 °C, pH 7.0 and a_w 0.96.

Based on their validation of predictive models, te Giffel and Zwietering (1999) concluded that small differences between the models were observed and that predictions were accurate within a factor of two to four depending on the product. Thus, predictions are not absolute and it is important to understand the limitations of a predictive model. To improve the prediction in a specific food additional controlling factors may need to be included and during the development naturally contaminated food should be included (Dalgaard and Jørgensen 1998).

Annex 5

FOOD CONSUMPTION DATA

The availability of suitable food composition data is important when the consumption of energy, nutrients, or other food constituents or contaminants are being investigated. Many regional and national tables of food consumption are available, but in general considerable differences exist among these tables (Périssé, 1982).

Food consumption data normally derive from food consumption analyses, which are performed in two different ways: retrospectively and prospectively. The prospective investigations include a registration of consumption 'as it happens', i.e. the participants register at every meal what and how much is eaten. Such investigations will normally continue for very short periods, such as days or weeks. The retrospective studies can be divided into three main groups: 24 h recall, dietary history and frequency analysis. In the 24 h recall method everything consumed over the last 24 hours (or longer) is recalled. The dietary history investigations look back over a longer period, up to one year, and the time unit for consumption recollection could be days, weeks or months, questions normally relate to 'usual' or standard consumption. Frequency analysis questions the frequency of consumption (sometimes including quantity estimation) of specified, selected foods over a specified time period, varying from days to months.

As stated, the most relevant information needed for microbiological exposure assessment, is food frequency data. Originally the food frequency method was developed because of inherent problems of using the 24-hour recall studies to classify subjects into high, medium, or low consumers of certain foods, which is relevant in many food-related epidemiological studies (Wiehl and Reed, 1960). Initially, the questionnaires did not include quantitative estimates other than as so many servings or portions per day/week/month. To overcome this gross assumption in relation to quantitative consumption estimation, direct quantitative questions have been included. Depending on the purpose of the study, information is sought only on those foods, which are relevant to the aim(s) of the study.

The food frequency method has been used in studies investigating possible associations between diet and health risk (Bjelke, 1975; Byers, 1984; Räsänen, 1982). The relative validity of food frequency questionnaires estimating a limited number of dietary components is, in general, better than the relative validity of the food frequency questionnaires trying to estimate total diet. (Byers et al., 1983; Chu et al., 1984) Very few studies have examined the precision or reproducibility of food frequency questionnaires.

More recently, case-control studies related to microbiological risk in food have used estimations comparable to food frequency analyses, notably a French study of risk factors for sporadic listeriosis in France (De Valk et al., 1998). In this study consumption frequencies were presented for the case group of: smoked fish 9 times/year, patés and cold cuts 208 times/year and cheeses 266 times/year. The corresponding frequencies for the control group were not presented in the abstract, which is the only open reference to this study.

A more prevalent, only partially food consumption related outcome of case-control studies is the identification of dietary risk factors for Listeria monocytogenes infection. For example, Schwartz et al. (1990) in a case-control study involving 82 cases and 239 controls identified uncooked hot dogs and undercooked chicken as potential risk factors, since case-patients were significantly more likely than controls to have eaten such products. The authors caution that the ability of a case-control study to detect an association is limited when an exposure is very common, when an exposure is very rare and when the magnitude of the increased risk is small. These types of studies do not present or use direct food consumption data, but could be used to guide the selection of food types relevant for future frequency analyses and/or risk assessments.

The investigation of dietary consumption of vulnerable groups can give information useful for comparison to more general dietary consumption data. However, when presented in isolation such data is very difficult to interpret in a risk assessment context. Manasse et al. (1992) presented interim results (466 out of potentially 1723 completed questionnaires analysed) of an investigation into dietary habits of pregnant women. From these results it could be seen that 62% never eat take-away food or eat it less than once/week, whereas 77% never eat cooked and chilled meals or eat it less than once/week, and 4% state that they eat food after the eat-by date. The study was primarily geared towards an investigation of the level of food-Listeria knowledge in this population group, and it was concluded that ‘safe eating’ during pregnancy was not a conscious priority and that potentially pregnant women should be better informed about diet-related risk factors.