Risk assessment of *Listeria monocytogenes* in fish products: some general principles, mechanism of infection and the use of performance standards to control human exposure

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Abstract

Risk assessment is increasingly used as a scientific process to assess the potential for adverse health effects to occur and as a basis for management of unacceptable risks. For each risk assessment activity, the purpose of the assessment should be clearly stated. For *Listeria monocytogenes*, the purpose of risk assessment may be providing information on the relative contribution of listeriosis to infectious diseases. For control purposes, the emphasis may be put on factors contributing to the risk of occurrence in a food or to inform risk managers that they should be setting food safety objectives. For an adequate risk assessment of *L. monocytogenes*, sound scientific data are necessary. This especially applies both to exposure assessment and hazard characterisation. Surveillance data indicates that cold storage to prolongs product shelf-life has opened an ecological window for the growth of *L. monocytogenes*. Assessment of dose–response relationship is often regarded as a key element in risk characterisation. Due to the large variability of the current assessed dose–response data, their contribution to assessing risks is low. The use of epidemiological data on incidence rate, types of food involved in listeriosis, etc. may be good alternatives. The use of performance standards or criteria, such as inactivation by heat or by fermentation, combined with processes that prevent outgrowth of the organism should be reconsidered. Presently, performance standards can simply be assessed since mathematical tools for their calculations are becoming increasingly available. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords*: *Listeria monocytogenes*; Risk assessment; Virulence; Performance standards

1. Introduction

Risk assessment is the science of understanding adverse health effects, how likely they are to occur and the consequences if they do occur. It also includes the identification and quantification of factors contributing to the frequency of occurrence of adverse effects. Since risk assessment is a scientifically based process, general principles should be taken into account to guarantee that the process is not spoiled by perception, incomprehension and miscommunication. Table 1 comprises some general
Table 1
General principles for microbial risk assessment of food products

- Risk assessment for microbial hazards must be based on sound science
- There must be a functional separation between risk assessment and risk management
- A structured approach must be used when conducting a risk assessment of microbial hazards
- A risk assessment of microbial hazards must clearly state both the purpose of the assessment and the form of the risk estimate that will be the output communication between risk assessors and risk managers
- Risk assessment must be transparent.
- The risk estimate must contain a detailed description of uncertainty and where this arose during the risk assessment process
- Data must be of sufficient quality and precision such that uncertainty in the risk estimate is minimized
- Where, appropriate, a risk assessment of microbial hazards must consider the fate of the hazard(s) in food(s) and the disease process following infection
- Risk estimates, where possible, must be re-evaluated over time against human health data and when new data become available

principles for microbial risk assessment which can be of benefit in guaranteeing the use of sound science, consistency, etc. It is generally agreed that risk assessment comprises the following steps: (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment, and (iv) risk characterisation. These steps should be proceeded by a statement of purpose of risk assessment and finished by production of a formal report. Fig. 1 presents the scheme for risk assessment of foodborne microbiological hazards.

The recommended scheme provides a working agenda. However, the stages listed may not necessarily be considered in sequence but rather in an orderly manner, as suggested in Fig. 1. For microbial risk assessment, it might even be that information about the actual final probability of adverse health effects by a pathogen is already known. This may be the case if, for example, epidemiological data are available. In such cases, identification and quantification of factors contributing to the risk of occurrence of adverse health effects may be the primary goal of the risk assessment. In this document, general principles are provided for the risk assessment of Listeria monocytogenes in fish products. In addition, basic information on the mechanism of infection is presented as part of a hazard characterisation as well as the use of performance standards to control human exposure.

2. General principles of risk assessment of L. monocytogenes in fish products

2.1. Statement of purpose of risk assessment

For each risk assessment activity, the specific purpose of the risk assessment should be clearly stated. The output form and possible output alternatives should be defined. During this stage, the cause of concern, the goals, breadth and focus of the risk assessment should be defined. As well, the entry
into a risk assessment should be triggered, for example, by:

- Emerging and re-emerging pathogens
- Public concern
- The need to establish or to evaluate control options

Depending on its purpose, a risk assessment process may be focussed on either the agent, the food vehicle or the treatment process of a food product. The output might be, for example, an estimate of:

- Annual occurrence of illness
- The effect of control options on the annual rate of illnesses

In the case of *L. monocytogenes*, two types of adverse health effects are described; one with severe clinical manifestation known as listeriosis (Hof, 1998) and the other which is characterised by temporary and self limiting gastro-intestinal complaints (Dalton et al., 1997; Aureli, 1998). For listeriosis, the clinical characteristics are well known as are the high risk groups. There also exists relatively reliable epidemiological data about the frequency of occurrence of *L. monocytogenes* in foods; human exposure data are available. The statement of purpose should take all these aspects into account.

The purpose of a risk assessment might be to provide information to risk managers to set food safety objectives. In such a case, and taking the existing knowledge into account, the objective could be the identification of factors that contribute to human exposure and the quantitative effects of these factors on exposure.

2.2. Hazard identification

Hazard identification is the identification of whether *L. monocytogenes* is an organism which is capable of causing adverse health effects and which may be present in a particular food or group of foods. Due to the research efforts of the last two decades, it can be concluded that *L. monocytogenes* is a well documented hazardous organism that frequently occurs in a wide variety of food products. The organism is the cause of the severe disease called listeriosis. Information about the self-limiting gastro-intestinal type of illness is limited, i.e. up until now, a very limited number of cases of gastro-intestinal listeriosis have been described (Hof, 1998).

2.3. Exposure assessment

Exposure assessment is the qualitative and/or quantitative evaluation of the likely intake of *L. monocytogenes* via food as well as exposure from other sources, if relevant. The ultimate goal of exposure assessment is to evaluate the level of *L. monocytogenes* in the food at the time of consumption. It may also include an assessment of actual or anticipated human consumption. *L. monocytogenes* is widespread in nature and can be found in soil, foliage, and feces of humans and animals. The organism appears to be a transient resident in the intestinal tract of humans and about approximately 5–10% of the general population are carriers of the organism (Gledel, 1987; Kampelmacher and van Noorle Jansen, 1980). As a result of the ubiquitous character of *L. monocytogenes*, the organism easily enters the food chain. Numerous reports have been published dealing with the occurrence of *L. monocytogenes* in food products (Farber and Peterkin, 1991; Morris and Ribeiro, 1991). These studies show that the organisms can be detected in a wide variety of raw food products. In general, food is an ideal substrate for *L. monocytogenes* to proliferate. Processing, especially heat processing, will inactivate the organisms present. Other processes, e.g. the addition of preservatives, are designed to prevent the outgrowth of *L. monocytogenes*.

Information about the initial contamination of raw materials, the effect of processing, the potential for cross-contamination and re-contamination, storage conditions, the characteristics of the food, etc., are all factors of interest in the evaluation of exposure assessment. Identification of these factors and an assessment of their contribution to the exposure are essential activities in the exposure assessment. This especially applies to the assessment and evaluation of human exposure to *L. monocytogenes*. To gain information about exposure, several techniques may be used, e.g. data collection by surveillance testing, storage tests, challenge testing, and the use of predictive models.
To assess human exposure information on consumption patterns and habits (so-called ‘dietary information’) is also important. This information includes among other things, food preparation and storage practices, consumption data, frequency of consumption, susceptible groups in a given population and age distribution of the consumers.

In most cases, control of human exposure is the most obvious management activity to control the risk of foodborne microbiological hazards. In the case of \textit{L. monocytogenes}, exposure control can be exercised by adequate processing (for example by the use of performance standards) or by education of high risk groups.

### 2.4. Hazard characterisation

Hazard characterisation is the evaluation of the nature of the adverse effects associated with \textit{L. monocytogenes} present in food. A dose–response assessment may be part of this activity. For \textit{L. monocytogenes}, the virulence factors have largely been elucidated. In addition, the clinical aspects of listeriosis are well described. The complete mechanism of infection, however, is still not completely clear.

As already indicated, a key aspect of the hazard characterisation is establishing a dose–response relationship. It is the process of obtaining quantitative information on the probability of human illness following exposure to a hazard; it is translation of exposure into harm. Recently, several attempts have been made to establish dose–response curves for \textit{L. monocytogenes}. One of them has been established by linking exposure data with epidemiological data (Buchanan et al., 1997). Animal models have also been used to establish dose–response curves (Notermans et al., 1998). Although the dose–response established by using animal models provides data which can explain certain aspects of the mechanism of infection, translating animal dose–response data to man is questionable. It is doubtful whether the current data on dose–response relationships are useful in any microbial risk assessment on \textit{L. monocytogenes}. The dose–response relationships established to date have shown large variations and may only have value in estimation the relative effect of several control options.

### 2.5. Risk characterisation

Risk characterisation is the estimation, including attendant uncertainties and variabilities, of the probability of occurrence and severity of the adverse health effects and is based on hazard identification, hazard characterisation and exposure assessment. It is an integration of the previous stages of risk assessment into an estimate of the likelihood of the adverse effects occurring in a given population. In the case of listeriosis, data on adverse effects and severity are made available by many countries. They are collected using epidemiological techniques. Linking exposure data and the epidemiological data reveals that despite the frequency of exposure to \textit{L. monocytogenes}, listeriosis is a relatively rare disease. Studies performed by the CDC in the USA have established a yearly baseline incidence of four to seven cases per $10^6$ persons (Gellin and Broome, 1989; Gellin et al., 1986). In Europe, the yearly incidence varies for each country and range from 0.1 to 11 cases per $10^6$ persons (Gledel, 1987; Ralovich, 1987). Recent results indicate a decreased incidence of human listeriosis in several countries, including the US (Tapper et al., 1995). Authorities from several countries, including the European Union, and international bodies such as WHO and FAO are asking for risk assessment studies for \textit{L. monocytogenes}. In all probability, these risk assessment studies have been requested in order to reduce the incidence of listeriosis to some lower level.

### 2.6. Production of a formal report

The risk assessment should be fully and systematically documented. To ensure transparency, the final report should indicate in particular any constraints and assumptions relative to the risk assessment. The report should also be made available to independent parties on request.

### 3. Basic information on the mechanism of infection

#### 3.1. Exposure rate

Humans are frequently exposed to \textit{L. monocytogenes}, and high numbers may be ingested during
consumption of certain types of food. In an earlier study by Notermans et al. (1998), the rate of human exposure was estimated based on data from Teufel and Bendzulla (1994). In this report, quantitative test results of 14,329 food products for the presence of L. monocytogenes are described. Meat products, fish products, cheese, and salads, were the products that were most heavily contaminated. Three of these products are cold-stored products with an extended shelf life. Based on these data and the estimated yearly consumption frequencies for meat products, fish products, cheese, and salads of 200, 50, 100, and 100, respectively, exposure frequencies have been determined (Table 2). In these calculations, it is assumed that portions of 100 g of product were consumed. These calculations show that the average yearly exposure to $3 \log_{10}$, $5 \log_{10}$, and $>6 \log_{10}$ L. monocytogenes occurs 19.3, 3.8, and 0.8 times, respectively.

However, epidemiological investigations show that listeriosis is a rare disease. Several facts may help to explain these observations. One explanation may be that because of differences in virulence not all strains of L. monocytogenes present in food may cause disease. Another reason could be that just by virtue of being in a ‘high-risk’ group, does not necessarily mean that an individual exposed to the organism will become ill. The existence of such groups has indeed been confirmed by epidemiological data (Gellin and Broome, 1989; McLaughlin, 1990). The elderly (over 65 years of age), unborn children, and immunocompromised persons are members of the vulnerable groups. Although these groups are also frequently exposed to large numbers of L. monocytogenes, the prevalence rate of listeriosis in these groups is not high. For example, for the vulnerable group of persons aged over 70 years, the risk of contracting severe listeriosis is 1:40,000; for pregnant women the risk is 1:8000 (Gellin and Broome, 1989). These findings indicate that additional mechanisms contribute to susceptibility to the disease.

3.2. Virulence of Listeria strains

Tests used to determine whether a particular strain of Listeria spp. is virulent include: (i) the growth of the organisms in the spleen and liver of infected mice; (ii) killing of chick embryos and (iii) killing of cells of several tissue culture lines.

Notermans et al. (1998) studied the ability of several Listeria strains to grow in the spleen of mice and in chick embryos. All L. monocytogenes strains tested showed rapid increases in the spleens of mice. However, for a very small minority of strains, no clear increase in the bacterial load in the spleens of infected mice was observed. None of the other Listeria spp. multiplied in the spleens of mice. Comparable results were observed in the chick embryo test. These studies showed that the large majority of L. monocytogenes are virulent.

3.3. Dose–response studies in animals

In the dose–response studies of Notermans et al. (1998), L. monocytogenes strain EGD was administered either intravenously (i.v.) or orally to mice. The infectious dose (ID) was expressed in 50% (ID$_{50}$), as was the lethal dose (LD) in 50% (LD$_{50}$) (Table 3). Non-protected mice (not previously exposed to L. monocytogenes) are sensitive to i.v. injected organisms. The ID$_{50}$ level was approximately $1.8 \log_{10}$ of L. monocytogenes. Non-protected mice were much more resistant to orally administered L. monocytogenes.

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Consumption (frequency/person/year)</th>
<th>Exposure frequency (times/person/year)</th>
<th>No. L. monocytogenes exposed to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10$^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$&gt;10^3$</td>
</tr>
<tr>
<td>Meat products</td>
<td>200</td>
<td>27.4</td>
<td>16.6</td>
</tr>
<tr>
<td>Fish products</td>
<td>50</td>
<td>3.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Cheese</td>
<td>100</td>
<td>1.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Salads</td>
<td>100</td>
<td>3.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Total yearly exposures</td>
<td></td>
<td>35.5</td>
<td>19.3</td>
</tr>
</tbody>
</table>

Table 2
Estimated exposure frequency per person per year (Notermans et al., 1998 and adapted from Teufel and Bendzulla, 1994)
genes vs. i.v. injected, i.e. the LD₅₀ value was approximately 6.5 log₁₀ organisms. Protection by previous injection with *L. monocytogenes* resulted in an i.v. ID₅₀ of 5.6 log₁₀ organisms and no infection was observed after oral administration of 9.0 log₁₀ organisms. Immune suppression (by injection of carrageenan) did not affect the oral ID₅₀ of non-protected mice. Furthermore, it was observed that the LD₅₀ in most cases was approximately a factor of 10–100-fold above the ID₅₀.

These studies indicate that a clear physical barrier is offered by the intestines. This barrier is finite and is not clearly affected even when the immune system is suppressed. The experiments indicate that two components contribute to protection: a nonadaptive response offered by the physical status of the intestinal barrier, and an adaptive response of the immune system. These systems act independently, and their total effect is the product of each one separately. It is evident that both mechanisms also protect human beings exposed to *L. monocytogenes* and support the findings that listeriosis is a rare disease in humans. Hence, to contract listeriosis, a scenario involving several simultaneous events must occur: (i) exposure to large numbers of organisms with concomitant breaching of the intestinal barrier, followed by (ii) lowered nonspecific defenses and (iii) a delay in the onset of the immune response. These findings explain the fact that even exposure of vulnerable groups to high doses of *L. monocytogenes* does not always result in disease.

### Table 3

Sensitivity of mice to *L. monocytogenes* (EGD) in log₁₀ colony-forming units (Notermans et al., 1998)

<table>
<thead>
<tr>
<th>Mice treatment</th>
<th>ID₅₀ i.v.</th>
<th>Oral</th>
<th>LD₅₀ i.v.</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-protected mice</td>
<td>1.8</td>
<td>6.5</td>
<td>3.2</td>
<td>&gt;9.0</td>
</tr>
<tr>
<td>Protected mice’</td>
<td>5.6</td>
<td>&gt;9.0</td>
<td>5.8</td>
<td>&gt;9.0</td>
</tr>
<tr>
<td>Immunosuppressed non-protected mice</td>
<td>1.0</td>
<td>6.3</td>
<td>2.3</td>
<td>&gt;8.0</td>
</tr>
<tr>
<td>Immunosuppressed protected mice</td>
<td>0.8</td>
<td>7.9</td>
<td>3.2</td>
<td>&gt;8.0</td>
</tr>
</tbody>
</table>

*Mice were protected by previous exposure to low numbers of *L. monocytogenes.*

4. **The use of performance standards to control human exposure**

In order to produce safe food, the industry makes use of so-called performance standards. Such a standard means operating a process to obtain a certain level of reduction of a particular number of microorganisms (Baird-Parker, 1994). This approach makes use of some aspects of quantitative risk analysis. The starting point is the level of microbial contamination in the raw materials used. Conditions of processing and distribution of the product are such that an acceptable limit is achieved at the time of consumption for a specific organism of concern. The approach is not new, having been used since 1920. A well-known example is the performance standard for thermally processed low-acid (pH > 4.6) canned foods. In this case, the standard is the so-called botulinum cook, which is a heat treatment designed to reduce the probability that *Clostridium botulinum* will survive in the food to one in 10¹². The reason for choosing 10¹² as the performance standard is the assumption that, on average, one spore of *C. botulinum* could be present in each can of food prior to processing. With cans treated in the manner described above, the risk of any one can containing botulinum toxin is one in 10¹⁰. If the global consumption of canned food amounts to 20 cans per capita per year and the world population is 5 × 10³, then over a 10-year period only one case of botulism would be expected from this type of food.

Similar performance factors have been developed for a number of other food products, including those made from pasteurised milk and, more recently, chilled foods. For *L. monocytogenes* and chilled food products, a reduction of 10⁶ has been suggested as a performance standard. An acceptable processing standard for chilled foods is a probability that contamination with vegetative cells of pathogenic bacteria is less than one in 10⁶. Risk assessment carried out for *Salmonella* in pasteurised milk, for example, has shown clearly that the performance standard results in an acceptable, safe product (Notermans and Mead, 1999).

Using performance standards to control human
exposure to *L. monocytogenes* is not necessarily limited to those products which are heat processed. Inactivation caused by fermentation processes and even processes which prevent outgrowth of the organism are also of interest. This latter component applies especially to products made from raw materials having a low level of background contamination. Performance standards can now simply be assessed since mathematical models which have been developed or are under development, take into account both the initial contamination and the distribution of organisms in the product itself. Additionally, even within a sub-population of cells, there will be a certain ‘distribution’ in terms of a cell’s growth, lag phase, etc. These variations can be accounted for in some of the newer risk assessment software packages. With these models, the effect of the performance standard on final human exposure can easily be assessed and if necessary adapted to the level of protection wanted with a high degree of security. The use of adequate performance standards to control human exposure to *L. monocytogenes* should be considered. It makes processing for safety possible and reasonable.

References


