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IN FOODS**

BACKGROUND PAPER

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**Development of Practical Risk Management Strategies based on
Microbiological Risk Assessment Outputs**

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**Case Study: *Listeria monocytogenes* in Smoked Fish
"Development of Risk Management Metrics for Food Safety"**

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1 BACKGROUND

Listeria monocytogenes is a gram positive, facultatively anaerobic, psychrotrophic bacterial species that is capable of causing life-threatening septicaemia and meningitis in adult humans, and infections in foetuses and neonates that can lead to spontaneous abortions, foetal death, and septicaemia. This is typically a disease of specific high risk subpopulations with depressed or altered immune responses due to age, pregnancy, medical interventions, or chronic, immunosuppressive diseases (e.g., diabetes, HIV infections). In the past 20 years it has been established that the primary route of transmission for this pathogenic microorganism is food, with ready-to-eat foods that support the growth of the bacterium representing the greatest risk to the consumer; i.e., foods with high levels of *L. monocytogenes* are much more likely to cause listeriosis than low levels (FDA/FSIS, 2003; FAO/WHO, 2004).

One class of the products identified as a potential vehicle for *L. monocytogenes* is smoked seafood products, particularly smoked finfish. This reflects a combination of factors associated with this product class including a relatively high prevalence of initial contamination (i.e., prevalence immediately after final packaging), the ability of the product to support the growth of the bacterium, a production process that has multiple opportunities for contamination or recontamination, and an extended shelf life at refrigerated conditions. This is further exacerbated by the ubiquity of the bacterium in food processing environments, its ability to grow at refrigeration temperatures and its relative resistance to heating, acidic environments, elevated salt levels, and inhibitory compounds used to control foodborne microorganisms. Various surveys have indicated that this pathogenic microorganism occurs in smoked fish at a relatively high prevalence rate, (FDA/FSIS, 2003). Early surveys of the prevalence of *L. monocytogenes* in smoked seafood suggested that its presence, at least at low levels, was common; isolation rates of > 10% were not unusual (Jemmi, 1990a, 1990b; Hatemink and Georgsson, 1991; Dillon et al., 1992). However, in more recent years surveys have indicated that the average prevalence of *L. monocytogenes* is in the range of 0 – 6% (Gonzalez-Rodriguez et al. 2002; Gudbjornsdottir et al., 2004), though occasional reports of high prevalence are observed in specific locations or fish types (Jeyasekaran, Karunasagar and Karunasagar, 2002; Basti et al., 2006). These rates also appear to differ widely among manufacturers. Typically, the levels in contaminated smoked fish are low (< 1 CFU/g); however, occasional samples at retail have had 10⁴ to 10⁶ CFU/g (Gombas et al., 2003; FDA/FSIS, 2003). Smoked seafood products can support the growth of *L. monocytogenes* at refrigeration temperatures, with some studies observing relatively rapid generation times (Jørgensen and Huss, 1998; Dalgaard and Jørgensen 1998; Cortesi et al. 1997). There are some reports that suggest that *L. monocytogenes* grows slower in naturally contaminated smoked fish (Guyer and Jemmi, 1991; Jemmi and Keusch, 1992; Jørgensen and Huss, 1998).

1.1 Manufacture, Marketing and Consumption of Smoked Fish

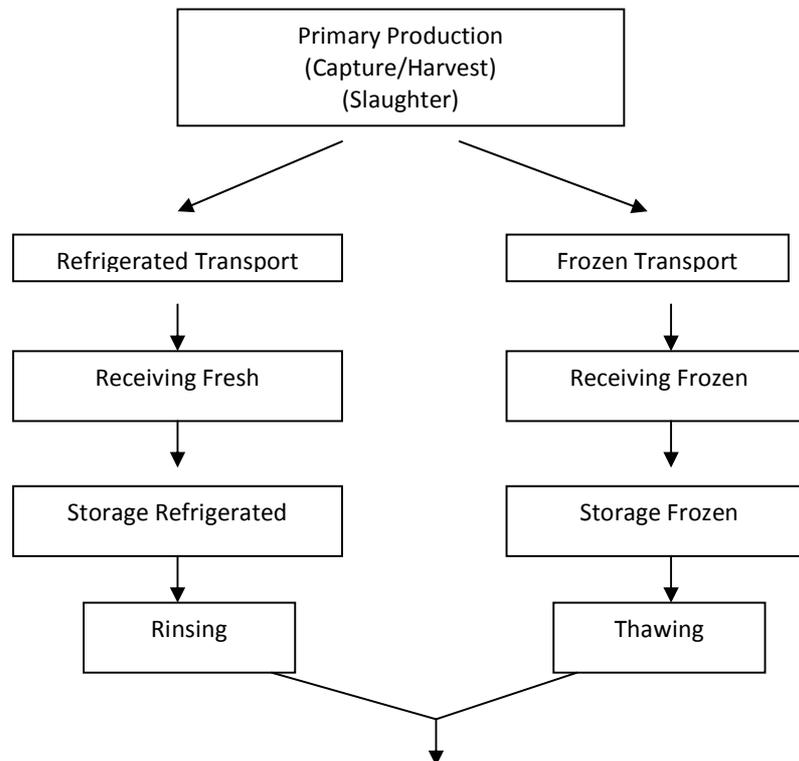
Smoked seafood includes a wide variety of products including both smoked finfish and smoked bivalve shellfish. Within the smoked finfish, smoked salmon is the most widely marketed species in most parts of the world, and for that reason will be the focus of the current document. However, a variety of other fresh water and marine species (e.g., trout, cod, sable, perch, white fish, and sturgeon) are manufactured and consumed regionally or internationally.

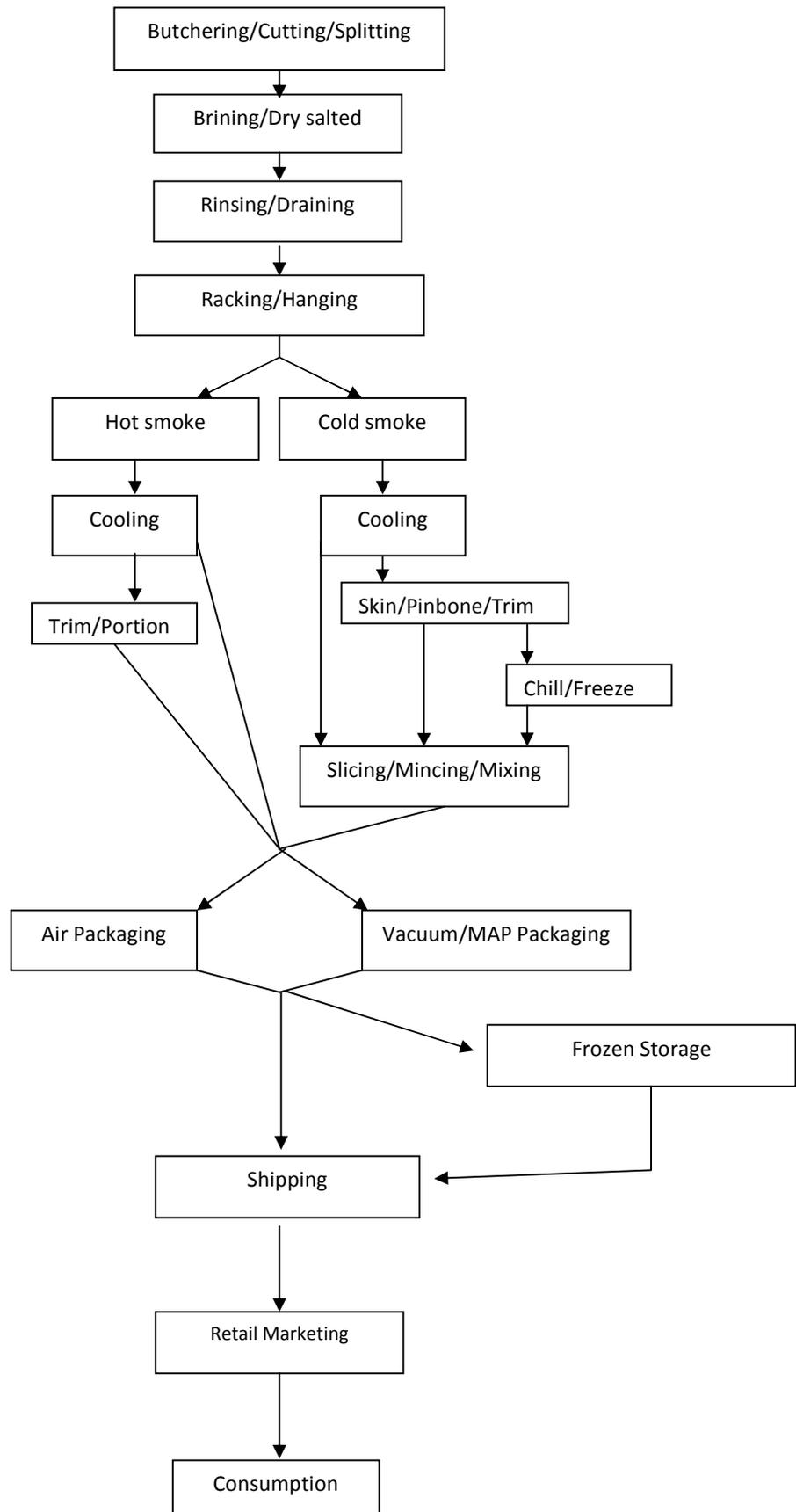
Smoked salmon is manufactured and marketed in two primary forms, cold-smoked and hot-smoked product. The temperatures used to cold-smoke salmon are insufficient to inactivate *L. monocytogenes*; however, the cold-smoking step will typically reduce the levels of the

microorganism by 90 – 99% if present on the raw salted fish. There are no steps in cold-smoked salmon production after cold-smoking that would reduce or eliminate the pathogen. The typical temperatures used during cold-smoking are 22 - 28°C. Conversely, hot-smoking reaches temperature that should be sufficient to inactivate *L. monocytogenes* (Ben Embarek and Huss, 1993). A typical hot-smoking process would involve exposing the product to drying temperatures of 30-40°C and then to a hot-smoking period of 2-3 h at 60-70°C followed by a second drying period. However, surveys of cold-smoked and hot-smoked products have typically found similar prevalence's of *L. monocytogenes* in the two product types, suggesting that the hot-smoked product is contaminated after the heat treatment.

A generic flow chart for the manufacture and marketing of cold-smoked and hot-smoked salmon that will be used in the current exercise is depicted in Figure 1. There is substantial variation between manufacturers in terms of the details of the individual unit operations. For example, the specific timing of butchering, trimming, skinning, and slicing can vary. The salting process differs markedly between different processors; some use dry salting, others brine injection, and others submersion in brine. The characteristics of the fish differ substantially with hot- and cold-smoking. As such the hot-smoked product is typically marketed as portions or “chunks” whereas the cold-smoked product is marketed primarily as a sliced product. In some instances the cold-smoked product is distributed initially as whole fillets for subsequent slicing at the retail establishment. However, for the current purpose only cold-smoked product sliced at the manufacturing plant will be considered. A portion of the hot- and cold-smoked salmon is further processed to produce “minces”, “spreads”, and “seafood salads”. These products will not be considered further in this exercise.

Figure 1. Flow chart for the manufacture of hot and cold smoked salmon.





2. GENERAL APPROACH FOR THE SELECTION OF PERFORMANCE OBJECTIVES AND THE DERIVATION OF FOOD SAFETY OBJECTIVES AND APPROPRIATE LEVELS OF PROTECTION

The general approach of the working group to develop the risk management metrics was to:

- Identify locations of POs within the food chain
- Identify the value range of POs to be considered at each of the selected locations, starting with the PO at the end of manufacture and calculating the other POs based on current literature, expert advice, and risk assessment principles, using risk assessment and predictive microbiology modelling techniques.
- Calculate the FSOs (and ALOPs) that should be achieved through the application of the corresponding POs.
- Derive the appropriate Performance Criteria, Microbiological Criteria, and Process Criteria that would be required to achieve and verify that the POs are achievable and being achieved.
- Consider how the issue of “compliance” will impact the effectiveness of the approach and discuss different approaches for evaluating the degree of compliance and providing strategies for assessing the effectiveness of implementing the risk management program.
- Consider the effectiveness of microbiological criteria as a control measure or as a means of verifying efficacy of food safety system.

Many of the hygienic practices needed for the control of *L. monocytogenes* in hot-smoked and cold-smoked salmon are similar but the two product classes do differ in relation to the presence of a clearly listeriocidal treatment. As such the POs selected for the two product classes do differ.

For hot-smoked salmon, unless the level of *L. monocytogenes* on the fish prior to smoking was extraordinarily high, an adequate heat treatment should eliminate the pathogen. Thus, any *L. monocytogenes* subsequent to the hot-smoking step is the result of recontamination of the product. The extent of this contamination, both in terms of the frequency of contamination and the level of the pathogen present, will subsequently be dependent on how the product is handled and maintained during final manufacturing, distribution, marketing, and use by the consumer. This will largely be a function of the extent of cross contamination prior to final packaging and the adequacy of the cold chain, both in terms of time and temperature. Three locations within the food chain were identified for the potential establishment of POs: (1) immediately after hot-smoking to establish the stringency required of the heat treatment, (2) the extent of contamination immediately after final packaging to establish the stringency of the good hygienic practices needed to control recontamination, and (3) at retail to establish the stringency of the cold chain that is required to ensure minimal increases in *L. monocytogenes* levels between manufacture and consumption. The inclusion of a PO that would address the handling, use, and potential contamination of “leftovers” in the home refrigerator was discussed but considered impractical at the current time due to the lack of specific information on contamination rates and storage times.

While there are a number of steps within the manufacture of cold-smoked salmon which influence the frequency and extent of *L. monocytogenes* contamination, there is no distinctly listeriocidal treatment, although the drying/cold-smoking steps often achieve a reduction in the levels of the microorganism. *L. monocytogenes* contamination is dependent, in part, on the incidence of the pathogen on the incoming fish. However, the primary factors affecting the frequency and extent of *L. monocytogenes* contamination in cold-smoked product appear to be the

degree of contamination from the manufacturing environment, the degree of cross contamination during manufacturing prior to final packaging, and the adequacy of the cold chain during manufacturing, distribution, marketing, and consumption. Again, three locations along the food chain were identified for the potential establishment of POs: (1) the frequency of contamination of incoming raw fish and/or fillets entering the manufacturing facility to establish the degree of stringency required to minimize the extent of contamination inherent to the fish (and also limit the recontamination of the manufacturing environment), (2) the extent of contamination immediately after final packaging of the product as a means of establishing the degree of stringency required of good hygienic practices to prevent amplification of contamination, and (3) at retail to establish the stringency of the cold chain that is required to ensure minimal increases in *L. monocytogenes* levels between manufacture and consumption.

The locations of the POs along the food chain are diagrammatically summarized in Figure 2.

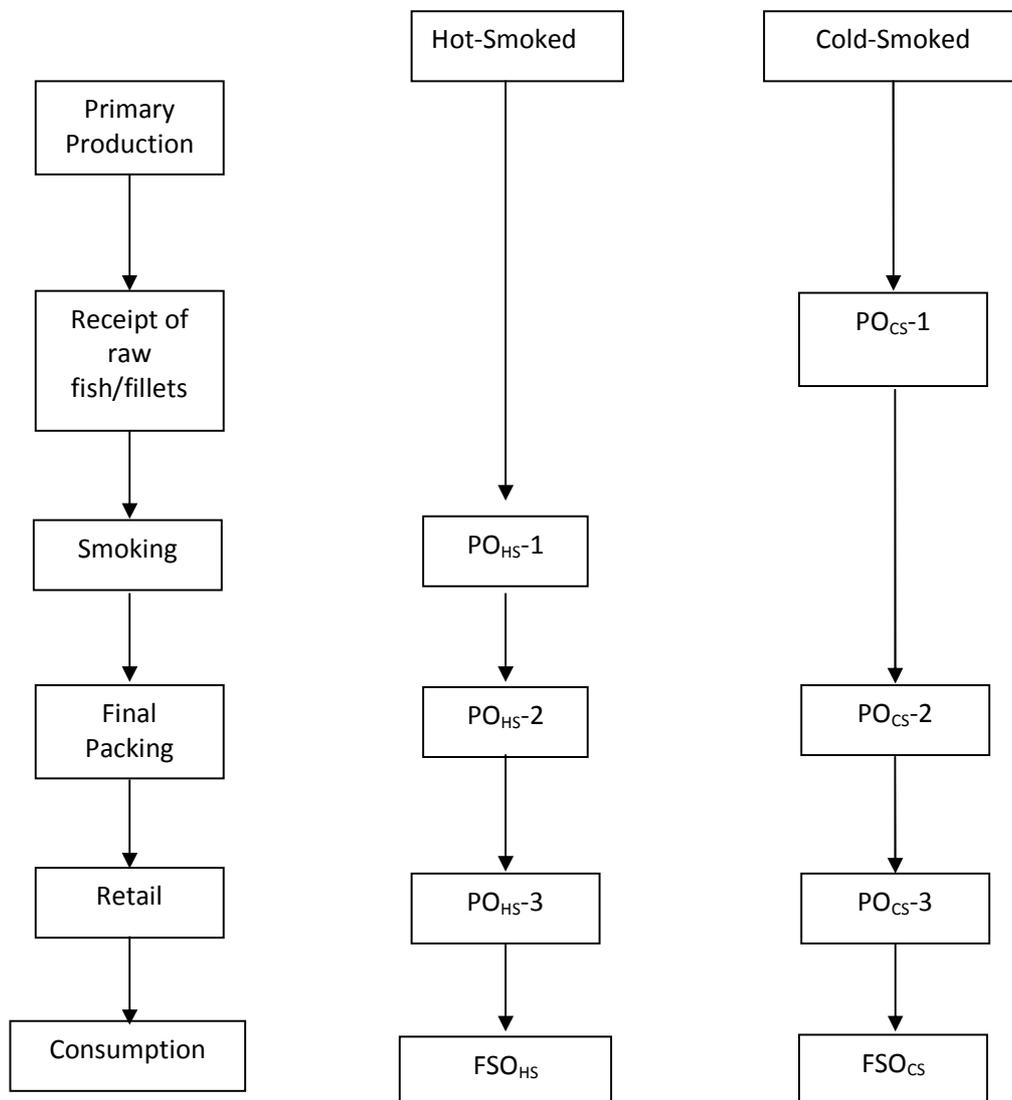


Figure 2. Location of performance objectives (POs) along the production to consumption chain for smoked fish

As indicated above, the approach used in this exercise was to start by selecting a series of potential POs values at the point immediately after final packaging. While this decision was somewhat arbitrary, it was based on a clearly definable step and the availability of substantial data related to the frequency of contamination. Basing the PO values on either a unit mass basis or a serving size basis was considered. The former was selected because serving size is a variable; the FDA/FSIS risk assessment (2003) reported that the mean serving size was 57 g and the 5 to 95% interval was approximately 20 to 140 g. A second underlying decision in developing the PO values is that the mean log concentration of *L. monocytogenes* (i.e., the mean of the levels of the bacterium observed after the values have been converted to log numbers) would be employed, and not the frequency of contamination. The underlying assumption is that if a large enough sample of a population of smoked salmon servings could be assayed that the microorganism would be detected and a concentration calculated. Thus, despite the fact that at low levels of contamination individual servings may be considered free of *L. monocytogenes*, there is a finite mean concentration in the population of servings if every serving could be analyzed. The reasons for this assumption are that it avoids attempting to differentiate “true zeros” from values below the limit of detection, and is necessary to use the PO at final packaging to derive the PO value at retail. This assumption is not likely to have a significant impact in relating the POs and FSO to the ALOP. The potential PO values evaluated in this exercise for cold-smoked and hot-smoked salmon are provided in Table 2a and 2b, respectively.

Table 2. The PO values after final packaging considered for (a) cold-smoked and (b) hot-smoked salmon

a.

| PO _{CS} -1 | PO _{CS} -2 [Log(CFU/g)] | PO _{CS} -3 | FSO _{CS} | ALOP _{CS} |
|---------------------|-------------------------------------|---------------------|-------------------|--------------------|
| | 3.00 | | | |
| | 2.00 | | | |
| | 1.00 | | | |
| | 0.00 | | | |
| | -1.00 | | | |
| | -2.00 | | | |
| | -3.00 | | | |

b.

| PO _{HS} -1 | PO _{HS} -2 | PO _{HS} -3 | FSO _{HS} | ALOP _{HS} |
|---------------------|---------------------|---------------------|-------------------|--------------------|
| | 3.00 | | | |
| | 2.00 | | | |
| | 1.00 | | | |
| | 0.00 | | | |
| | -1.00 | | | |
| | -2.00 | | | |
| | -3.00 | | | |

The approach taken to subsequently derive the other corresponding PO values was to utilize the conceptual equation of the ICMSF (ICMSF, 2002) in combination with the data available from the FDA/FSIS (2003) and FAO/WHO (2004) risk assessments and the scientific literature. The ICMSF conceptual equation depicts the fact that the ability to achieve a PO (or an FSO) is dependent on the sum of the initial contamination burden (H_0), the factors that increase extent of

contamination either by permitting growth or contamination (ΣI), and the factors that decrease the level of contamination (ΣR). Thus, a PO will be achieved when:

$$H_0 + \Sigma I - \Sigma R \leq PO$$

When considering a product production pathway, the steps with the pathway can each be described by the same equation applied only to the preceding portion of the pathway. Thus, the PO for one segment of the manufacturing process becomes the H_0 for the subsequent stage of manufacturing. This conceptual equation can be used to describe each of the POs for the two smoked salmon products being considered:

2.1 Hot-smoked salmon

If the calculation of product's POs and FSO starts with the product immediately after final packaging (i.e., PO_{HS-2}), the maximum level of *L. monocytogenes* that could be in the product immediately after the heating steps would be H_0 for this stage of the manufacturing process, and the level of stringency required would be described by,

$$H_0 + \Sigma I - \Sigma R \leq PO_{HS-2}$$

where H_0 is the level is the mean log concentration of *L. monocytogenes* immediately after the heat treatment and ΣI is the increase in mean concentration as a result of growth of surviving cells plus increases in pathogen levels as a recontamination and subsequent growth due to the presence of the pathogen in the manufacturing environment of the pathogen. Since there are no inactivation steps between the heating step and final packaging that would reduce the mean log concentration, the ΣR for this segment of the product pathway is zero and equation becomes:

$$H_{0-2} + \Sigma I \leq PO_{HS-2}$$

Since the stringency achieved in the section of the product pathway is largely a function of the rate of recontamination and subsequent regrowth, under ideal conditions ΣI would approximate zero. However, absolute elimination of *L. monocytogenes* from the processing environment is not likely to be feasible at the current time. A realistic rate of recontamination and growth during this phase of production will be determined by using data acquired from the literature that describes the level of contamination observed in facilities where the contamination of product is "under control". Potentially, the degree of thermal processing that would be required (i.e., the PO_{HS-1} of the first stage of processing which becomes the H_0 for the second phase) to achieve the PO_{HS-2} for the second stage of the product pathway if ΣI could be controlled to a high degree. However, current available data indicates that that possibility is not likely at the current time.

The third stage in the product pathway encompasses all the steps between final packaging of the product and the purchase of the product by the consumer. This includes the storage, distribution, and marketing of the product to the point of sale. The H_0 for this phase of the product pathway is the level of *L. monocytogenes* in the product at the point of final packaging. Thus, the PO_{HS-2} for the second stage of the product pathway becomes the H_0 for the third stage. This is again expressed by the ICMSF conceptual equation as,

$$H_{0-3} + \Sigma I - \Sigma R \leq PO_{HS-3}$$

where $H_{0-3} = PO_{HS-2}$. Since there are no steps during the third stage that are likely to consistently reduce the levels of *L. monocytogenes*, the $\Sigma R = 0$ (i.e., $H_{0-3} + \Sigma I - \Sigma R \leq PO_{HS-3}$). The primary factors determining the mean log concentration of the pathogen in hot-smoked salmon during this phase will be the time and temperature of storage. Models for the likely growth of *L. monocytogenes* in smoked seafood were developed for the FDA/FSIS risk assessment (FDA/FSIS, 2003).

The calculation of the fourth and final stage of this product pathway is the likely growth that will occur between the point of sale and the time of consumption. Again, the PO from the prior stage becomes the H_0 for the current stage. Again, the mean log concentration of *L. monocytogenes* in the product at the end of this stage (i.e., consumption) is dependent on the time and temperature of storage between the point of purchase and the consumption of the product. Since the final step in this stage is consumption, the metric calculated is the FSO, and the ICMSF equation for this stage becomes,

$$H_{0-4} + \Sigma I \leq FSO_{HS}$$

where $H_{0-4} = PO_{HS-3}$ and $\Sigma R = 0$. Again, models for calculating the effects of storage conditions on *L. monocytogenes* growth during this final phase are available in the FDA/FSIS risk assessment (FDA/FSIS, 2003). Conversion of the FSO_{HS} to the $ALOP_{HS}$ can also be achieved by using the hazard characterizations and risk characterization available through the FDA/FSIS (FDA/FSIS, 2003) and FAO/WHO (FAO/WHO, 2004) risk assessments.

2.2 Cold-smoked salmon

The approach taken for cold-smoked salmon is similar to that described above for hot-smoked salmon; however, the calculation of PO_{CS-1} , PO_{CS-3} , FSO_{CS} , and $ALOP_{CS}$ based on establishing a performance objective immediately after final packaging (PO_{CS-2}) is more complicated due to the lack of a clearly listeriocidal step in the product pathway. The most difficult calculation is PO_{CS-1} because of the multiple means for introducing *L. monocytogenes* into cold smoked fish. For this reason, it is unlikely that a national government would establish a PO at this level. However, such a value may be useful to a manufacturer and is considered as an example of how industry could use these tools for their own applications.

In developing the approach it is assumed that the manufacturing facility is separate from the facility slaughters and performs the initial cleaning and in some cases filleting of the salmon. While there are some exceptions, this is the primary means that cold-salmon are now processed, particularly with the advent of large aquaculture facilities that are often located substantial distances from the manufacturing facility, (e.g., Chilean salmon is the raw material for much of the cold-smoked salmon produced in the United States). This assumption allows the establishment of a PO at receipt of the raw ingredient at the manufacturing plant.

As indicated above, the availability of a PO for the raw fish entering the manufacturing plant would have significant utility for the cold-smoked fish manufacturers, allowing the establishment of scientific-based, risk-based specification. However, relating the mean log concentration at final packaging to the mean log concentration of raw fish or fish fillets entering the plant represents a significant challenge. The mean log concentration of *L. monocytogenes* in cold-smoked salmon at final packaging is dependent on contamination associated with two potential sources of the microorganism, the *L. monocytogenes* present on the raw fish entering the facility and the *L. monocytogenes* endemic to the manufacturing environment. A diagrammatic model of

the sources of contamination is depicted in Figure 3. Ideally, calculation of PO_{CS-1} from PO_{CS-2} would be based on a clear understanding of the interactions among each of the model compartments. However, that is well beyond the scope of the current exercise and would likely have to be done on an individual plant basis. However, an attempt will be made in the current exercise to use available survey data to establish the level of control associated with “in control” plants to estimate the value. As before, the PO_{CS-1} for the first stage of the product pathway is the H_0 for the second. In the first stage, *L. monocytogenes* contamination and growth in the raw fish and fillets would be expected to be influenced by the method of harvest and slaughter, the level of sanitation in the facility and means of transport, the use of rinses to reduce bacterial loads, and the times and temperatures of refrigerated or frozen storage and transport. This initial stage could be describe using the ICMSF equation; however, relatively little data are available. Accordingly, for the purposes of the current exercise, no attempt will be made to determine steps for controlling those factors, but will instead assume that PO_{CS-1} is determined by periodic verification testing at the manufacturer’s facility.

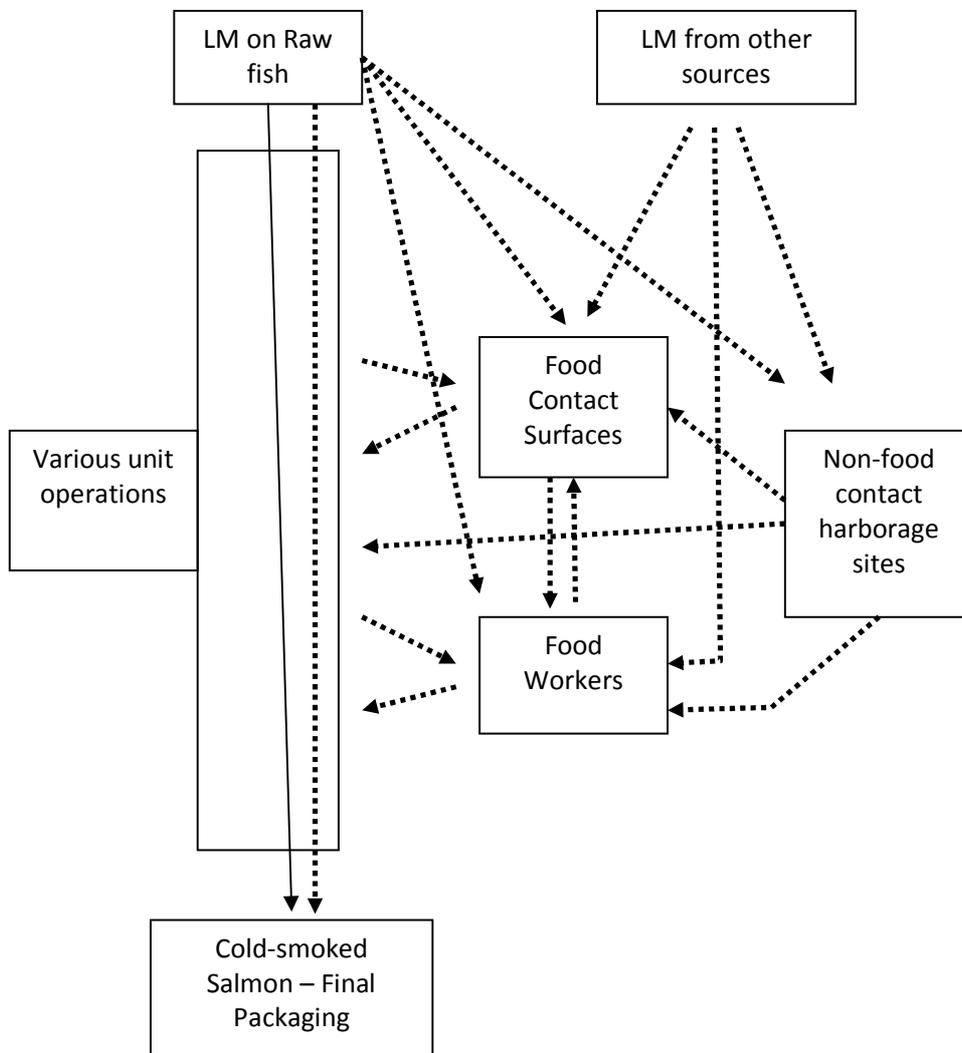


Figure 3. Potential sources of *Listeria monocytogenes* contamination of cold-smoked salmon during manufacturing.

As before, the ICMSF conceptual equation is used to calculate the POs along the product pathway. With the PO immediately after final packaging as the starting point, it can be described as,

$$H_0 + \Sigma I - \Sigma R \leq PO_{CS-2}$$

where $H_{0-2} = PO_{CS-1}$ = maximum mean log concentration of *L. monocytogenes* present on the raw fish or fillets; ΣI is the increase in mean log concentration due to the growth of the pathogen on fish contaminated prior to entering the facility plus the contamination and cross-contamination and subsequent growth of *L. monocytogenes* acquired from the manufacturing environment; and ΣR is the combined reductions in mean log concentrations resulting from unit operations such rinsing, drying, and cold-smoking that produce relatively small, but reproducible decreases. Each of these parameters will be estimated based on available studies and expert judgment.

The third stage and fourth stages of the product pathway for cold-smoked salmon are essentially the same as those for hot-smoked product. Accordingly the same approach will be used, i.e.

$$H_{0-3} + \Sigma I - \Sigma R \leq PO_{CS-3}$$
$$H_{0-4} + \Sigma I \leq FSO_{CS}$$

with H_{0-3} and H_{0-4} being equal to PO_{CS-2} and PO_{CS-3} , respectively. The appropriate values for the parameters for these equations are based on published data and expert judgment when necessary.

2.3 Establishment of other metrics

Where appropriate, other metrics such as performance criteria, process criteria, and microbiological criteria will be calculated based on achievement of the corresponding PO. For example, the extent of the reduction required of the heating step for hot-smoked salmon to achieve the required reduction in mean log concentration (a performance criterion) can be described as a specific heating time and temperature, i.e., a process criterion. In calculating these metrics the ability of address the variability and uncertainty associated with the behaviour *L. monocytogenes* will be dealt with by assuming that a 95% level of confidence has been agreed upon when verifying that a PO is being achieved. The impact of “compliance” will not be calculated but will be discussed later in the document.

3 SAMPLE CALCULATION OF PO, FSO, AND ALOP VALUES FOR COLD-SMOKED SALMON

The decision to initiate the calculation of POs, FSO, and ALOP for cold-smoked salmon based on arbitrarily considering different values for PO_{CS-2} and then calculating the resultant values for PO_{CS-3} , FSO_{CS} , and $ALOP_{CS}$ proved reasonably straight forward since there is a reasonable level of data available for how the pathogen is likely to behave in the product from that point of manufacture. In developing these values, the initial approach was to perform the calculations deterministically, i.e., point estimates, using a mean or 95% value depending on the parameter being considered. The impact of taking a probabilistic approach to estimating risks will be discussed later in the paper.

In developing these calculations, the following assumptions have been made based on available scientific data and/or expert opinion.

- Mean serving size is 57 g (FDA/FSIS, 2003). The decision to use the mean value was selected because it was assumed that the underlying distribution was highly skewed and since no log transformation was used for this parameter, the mean would actually take into account a substantial portion of the total consumption. This could be specifically considered if a probabilistic model was employed.
- 95% of the product will be sold within 14 days of manufacture.
- When handled appropriately, the product is maintained at $\leq 3^{\circ}\text{C}$ between the point of manufacture and the time of sale and that the 95% of the product will be sold with 14 days of manufacturer unless some other means is used to arrest the growth of *L. monocytogenes* (FDA/FSIS, 2003).
- The mean exponential growth rate (EGR) of *L. monocytogenes* in cold-smoked salmon is 0.070, 0.152, and 0.226 [Log(CFU/g)]/day, at 3°, 5°, and 7°C, respectively.
- The *L. monocytogenes* dose-response relationship for the population with increased susceptibility can be described with an exponential model with an r-value of 1.06×10^{-12} (FAO/WHO, 2004).
- Once purchased, the product is taken to the consumer's home and refrigerated for a period of time before it is consumed. The duration of the maximum storage time within the home is affected by the temperature of the home refrigerator, which influences the quality of the product. It is assumed that 95% of the storage times in a home with a 5°C refrigerator are ≤ 14 days, whereas this value drops to ≤ 7 days in homes with a 7°C refrigerators.

The PO at retail, PO_{CS-3} , is calculated from PO_{CS-2} by determining the maximum extent of growth that should be observed if the product is handled appropriately in terms of maintaining the integrity of the cold chain and ensuring the timely sale of the cold-smoked salmon. Based on the above assumptions, the mean log concentration of *L. monocytogenes* present at the time of manufacture would not be expected to increase greater than [14 days X 0.070 (Log(CFU/g))/day = 0.98 Log(CFU/g)]. Thus, if handled appropriately both in terms of storage time and temperature, it would be expected that the product would not exceed $PO_{CS-2} + 0.98$ (Table 3).

Table 3. POCS-3 values for cold-smoked salmon.

| PO _{CS} -1 | PO _{CS} -2 [Log(CFU/g)] | PO _{CS} -3 [Log(CFU/g)] | FSO _{CS} [Log(CFU/g)] | FSO _{CS} - Serving [Log(CFU/ serving)] | ALOP _{CS} |
|---------------------|-------------------------------------|-------------------------------------|-----------------------------------|---|--------------------|
| | 3.00 | 3.98 | | | |
| | 2.00 | 2.98 | | | |
| | 1.00 | 1.98 | | | |
| | 0.00 | 0.98 | | | |
| | -1.00 | -0.02 | | | |
| | -2.00 | -1.02 | | | |
| | -3.00 | -2.02 | | | |

The calculation of the FSO based on POCS-3 must take into account the likely changes in the levels of *L. monocytogenes* in the product between the time of purchase and consumption. As discussed earlier, since there are no steps between purchase and consumption, the primary consideration is the likely increase in the mean log concentration of *L. monocytogenes* that will occur when the product is handled “normally” by the consumer. In this case it has been assumed that the product will be consumed within 14 days of purchase in homes that have 5°C refrigerators. Based on the EGR values provided above, this time and temperature combination would be expected to result in an increase of (14 days X 0.152 (Log(CFU/g)/day) = 2.13. Thus, the FSO in terms the mean log concentration of *L. monocytogenes* would be PO_{CS}-3 + 2.13 (Table 4). However, the conversion of the FSO to an ALOP requires that total dose of *L. monocytogenes* be considered. Thus, two forms of the FSO must be used, a FSO_{CS} based on mean log concentration and a FSO_{CS}-Serving, that takes into account the likely serving size consumed so that the total dose can be calculated. In the current example, the FSO_{CS}-Serving value was calculated by adding the Log(serving size) to the FSO_{CS} value by the mean serving size, 57 g (Table 4). The ALOP is then calculated by substituting the FSO_{CS}-Serving value in the exponential model according to the formula:

$$P = 1 - e^{-r10^{(FSO-Serving)}} = 1 - e^{(-1.06E+12)10^{(FSO-Serving)}}$$

Where P is the probably acquiring listeriosis per serving of food consumed containing the corresponding level of *L. monocytogenes*, and -r is the probability of a single cell of *L. monocytogenes* causing listeriosis.

Table 4. Food Safety Objective values calculated for cold-smoked salmon.

| PO _{CS} -1 | PO _{CS} -2 [Log(CFU/g)] | PO _{CS} -3 [Log(CFU/g)] | FSO _{CS} [Log(CFU/g)] | FSO _{CS} - Serving [Log(CFU/ serving)] | ALOP _{CS} |
|---------------------|-------------------------------------|-------------------------------------|-----------------------------------|---|-------------------------|
| | 3.00 | 3.98 | 6.11 | 7.86 | 7.7 X 10 ⁻⁵ |
| | 2.00 | 2.98 | 5.11 | 6.86 | 7.7 X 10 ⁻⁶ |
| | 1.00 | 1.98 | 4.11 | 5.86 | 7.7 X 10 ⁻⁷ |
| | 0.00 | 0.98 | 3.11 | 4.86 | 7.7 X 10 ⁻⁸ |
| | -1.00 | -0.02 | 2.11 | 3.86 | 7.7 X 10 ⁻⁹ |
| | -2.00 | -1.02 | 1.11 | 2.86 | 7.7 X 10 ⁻¹⁰ |
| | -3.00 | -2.02 | 0.11 | 1.86 | 7.7 X 10 ⁻¹¹ |

While it is not likely that a food control agency would establish a PO for raw salmon coming into a smoked fish plant, such a PO would often be among the tools employed by manufacturers as part of any overall food safety risk management program. However, to be fully effective the PO should be linked to the portion of overall management of the risk. The derivation of PO_{CS}-1 from the PO_{CS}-2 is difficult because contamination of the raw material (salmon or salmon fillets) is only one of several potential means by which the final product, cold-smoked salmon becomes contaminated. The available literature does not definitively establish (nor does it rule out) a relationship between incoming levels of *L. monocytogenes* on incoming raw salmon and that on the final cold-smoked product. However, among a number of industry experts and scientific investigators there is an underlying assumption that control of raw materials is ultimately an important step in the control of *L. monocytogenes* in cold-smoked salmon (Eklund et al., 1995; Rorvik, 2000). This conclusion appears to be based on the assumption that currently the primary direct source of *L. monocytogenes* is the extent of harbourage sites within cold-smoked fish manufacturing facilities and the temporal delay in cross contamination leading to the dispersion of a *L. monocytogenes* isolated newly introduced on the raw, incoming salmon. However, as the degree of sanitation and related control measures are introduced in a facility increases and the number of harbourage sites is decreased, it is anticipated that the relative importance of the raw fish being a source for *L. monocytogenes* in the final product would increase.

Since the current literature is confounded in relation to the contribution of *L. monocytogenes* on incoming raw fish to the mean log concentration of the microorganism on the final product, it was decided to consider a scenario of a potential approach to establishing a PO based will be explored based on the underlying overall assumption that the prevalence on the raw fish and final product are related. The available literature suggest that in a well managed fish slaughter operation the frequency of contamination of the raw fish can be variable, 2 to 100% (Guyer and Jemmi, 1990; Eklund et al., 1995; Autio et al., 1999; Rørvik, 2000), largely restricted to the skin (Eklund et al, 1995) with the level of contamination being in the range of 1 – 10 CFU/g (Zukerrman and Avraham, 2002). Once in the processing plant there is a high potential for cross contamination during thawing and brining (Autio et al, 1999; Rørvik, 2000; Aguado, Vitas and Garcia-Jalon, 2001).

For the purpose of the current illustrative exercise, it has been assumed that the percentage of contaminated raw fish entering the plant is 10%, these fish are contaminated at a level of 1 CFU/g ($\text{Log}(\text{CFU/g}) = 0$), and that during processing cross contamination distributes this contamination evenly amongst all the fish in a batch. While it is realized that the only a portion of the *L. monocytogenes* attached to a fish are available for potential cross contamination, for the sake of simplicity the scenario will assume that all cells on the contaminated fish are available for transfer among fish, that transfer is homogeneous, that the mean log concentration of all of the fish after cross contamination can be calculated by multiplying the mean concentration of the contaminated fish by the portion of fish contaminated. Thus, the level of contamination in the current example after cross contamination during the early phases of the manufacturing process would be $1 \text{ (CFU/g)/contaminated fish} \times 0.1 \text{ contaminated fish/total fish} = 0.1 \text{ (CFU/g)/total fish}$. As always, care must be taken to ensure that log values and arithmetic values are clearly differentiated. If performing this calculation with log values the calculation would be: $\text{Mean Log}(\text{CFU/g}) = \text{Log}(\text{CFU/g}) \text{ for contaminated fish} + \text{Log}(\text{frequency of contamination}) = 0 + (-1) = -1 \text{ Log}(\text{CFU/g})$. Again for the sake of simplicity, it has been assumed for this example that any *L. monocytogenes* cell on the fish could potentially be in the finished product, and that the total increase in the mean log concentration of *L. monocytogenes* between cross-contamination, final packaging is 2 log cycles (i.e. $\Sigma I = 2$). Based on these simplifying assumptions, PO_{CS}-1 values that provide the appropriate PO_{CS}-2 values were determined (Table 5). For example, in this

scenario, the achievement of an ALOP = 7.7×10^{-9} cases/serving would require that the original frequency of contaminated raw fish be $\leq 0.1\%$ with the contaminated fish having a mean log concentration of 0.0 Log(CFU/g). After cross contamination, this would lead to a mean log contamination of -3.0 Log(CFU/g) and an FSO of 3.86 Log(CFU/g).

Table 5. Illustrative example of the determination of POCS-1 values for cold-smoked salmon based on a consideration of a simplified scenario.

| PO _{CS} -1 [Log(CFU/g)] / %Contam. | PO _{CS} -2 [Log(CFU/g)] | PO _{CS} -3 [Log(CFU/g)] | FSO _{CS} [Log(CFU/g)] | FSO _{CS} - Serving [Log(CFU/ serving)] | ALOP _{CS} |
|---|-------------------------------------|-------------------------------------|-----------------------------------|--|-----------------------|
| 1.0 ^b | 3.00 | 3.98 | 6.11 | 7.86 | 7.7×10^{-5} |
| 0.0/100% | 2.00 | 2.98 | 5.11 | 6.86 | 7.7×10^{-6} |
| -1.0/10% | 1.00 | 1.98 | 4.11 | 5.86 | 7.7×10^{-7} |
| -2.0/1% | 0.00 | 0.98 | 3.11 | 4.86 | 7.7×10^{-8} |
| -3.0/0.1% | -1.00 | -0.02 | 2.11 | 3.86 | 7.7×10^{-9} |
| -4.0/0.01% | -2.00 | -1.02 | 1.11 | 2.86 | 7.7×10^{-10} |
| -5.0/0.001% | -3.00 | -2.02 | 0.11 | 1.86 | 7.7×10^{-11} |

^a This scenario assumes that the mean log concentration of *L. monocytogenes* present on the contaminated fish was initially 0.0 Log(CFU/g) and the indicated value is the mean concentration on all fish after transfer of the microorganism among the contaminated and uncontaminated fish during the early phases of manufacture (see text).

^b This specific scenario requires the mean level of the contaminated raw fish increase by 10-fold from 1 CFU/g to 10 CFU/g.

It must be emphasized that the PC_{CS}-1 was based on a number of simplifying assumptions as a means of providing an example. For example, the scenario presumes that the manufacturer has a totally effective way of determining the portion of fish that are contaminated and that the level of contamination among contamination fish is the same. It may be feasible for an individual plant or group of plants to develop at appropriate values for PC_{CS}-1 based on their experience and data acquired for both their manufacturing facility and suppliers.

4. SAMPLE CALCULATION OF PO, FSO, AND ALOP VALUES FOR HOT-SMOKED SALMON:

The calculation of PO_{HS-3} , FSO_{HS} and $ALOP_{HS}$ for hot smoked salmon starting with arbitrarily established values for the PO at the point of packaging of the final product (PO_{HS-2}) is essentially the same as that used with cold-smoked salmon. The underlying assumptions are similar, if not identical, consisting of:

- Mean serving size is 57 g (FDA/FSIS, 2003).
- 95% of the product will be sold within 14 days of manufacture ().
- When handled appropriately, the product is maintained at $\leq 3^{\circ}C$ between the point of manufacture and the time of sale and that the 95% of the product will be sold with 14 days of manufacturer unless some other means is used to arrest the growth of *L. monocytogenes* (FDA/FSIS, 2003).
- The mean exponential growth rate (EGR) of *L. monocytogenes* in cold-smoked salmon is 0.054 and 0.207 [Log(CFU/g)]/day, at 3° , 5° , and $7^{\circ}C$, respectively ().
- The *L. monocytogenes* dose-response relationship for the population with increased susceptibility can be described with an exponential model with an r-value of 1.06×10^{-12} (FAO/WHO, 2004).
- Once purchased, the product is taken to the consumer's home and refrigerated for a period of time before it is consumed. The duration of the maximum storage time within the home is affected by the temperature of the home refrigerator, which influences the quality of the product. It is assumed that 95% of the product is consumed with 7 days in a refrigerator that is a $7^{\circ}C$. This is a slightly more conservative model that that employed for cold-smoked salmon which included an additional lower temperature with a longer time.

Following the same logic pattern as described above for cold-smoked salmon, the calculated values for PO_{HS-3} , FSO_{HS} and $ALOP_{HS}$ for hot smoked salmon are presented in Table 6.

Table 6. Derived FSO_{HS} and $ALOP_{HS}$ values for hot-smoked salmon based on arbitrarily set PO_{HS-2} values.

| PO_{HS-1} | PO_{HS-2} [Log(CFU/g)] | PO_{HS-3} [Log(CFU/g)] | FSO_{HS} [Log(CFU/g)] | FSO_{HS} - Serving [Log(CFU/ serving)] | $ALOP_{HS}$ |
|-------------|-----------------------------|-----------------------------|----------------------------|---|-----------------------|
| | 3.00 | 3.76 | 5.21 | 6.97 | 9.8×10^{-6} |
| | 2.00 | 2.76 | 4.21 | 5.97 | 9.8×10^{-7} |
| | 1.00 | 1.76 | 3.21 | 4.97 | 9.8×10^{-8} |
| | 0.00 | 0.76 | 2.21 | 3.97 | 9.8×10^{-9} |
| | -1.00 | -0.24 | 1.21 | 2.97 | 9.8×10^{-10} |
| | -2.00 | -1.24 | 0.21 | 1.97 | 9.8×10^{-11} |
| | -3.00 | -2.24 | -0.79 | 0.97 | 9.8×10^{-12} |

The establishment of the PO_{HS-1} requires knowledge of the magnitude of the increase in the mean log concentration of *L. monocytogenes* in the product that occurs immediately after the heat treatment and final packaging. This increase reflects a combination of any *L. monocytogenes* that (a) survived the heat treatment, (b) were reintroduced as a result of recontamination, and (c) the

growth of both. A typical hot-smoking process (65°C for 30 min) has been shown to achieve at least a 7 – 8 log reduction during inoculated fillet studies (Jemmi and Keusch, 1992). Furthermore, comparison of the heating process against published D-values, suggest that greater than a 15 – 30 log reduction could be anticipated, thus virtually ensuring the elimination of the pathogen. If cooling to proper refrigeration temperatures is part of the good hygienic practices, then, the extent of recontamination is the only significant factor affecting the level of *L. monocytogenes* after the hot smoking step.

If the heat treatment employed was mild enough such that surviving cells had to be anticipated, than the extent of heat treatment would need to be sufficient to reduce the levels of *L. monocytogenes* below the level of contamination that could grow in the conditions between the heat treatment and final packaging that would exceed PO_{HS-2} . However, in this instance the heat treatment is for a different purpose, and reduces the concentration well below the level of detection. Thus, for the purposes of this exercise we will use a notional PO_{HS-1} of -3.00 to indicate that product at this point needs to be processed to reduce the level of contamination on the fish to a point where a miniscule portion of fish coming from the smokehouse would be expected to have any *L. monocytogenes* (Table 7). An alternative way of looking at this is that for hot-smoked salmon to have a PO_{HS-2} of 3.00, the mean log concentration of the product would have to have increased at least 6 orders of magnitude, i.e., $PO - H_o = \Sigma I = 3.00 - (-3.00) = 6.00$. Conversely, achieving a PO_{HS-2} of -3.00 would require a high degree of control of recontamination.

Table 7. Inferred PO_{HS-1} Based on the Ability of Typical Hot-smoking Process to Effectively Eliminate *Listeria monocytogenes* from Salmon. The value depicted should be considered notional and is based on the approximate lower limit of detection for the detection of *Listeria monocytogenes* in experimental heating trials.

| PO_{HS-1} | PO_{HS-2} [Log(CFU/g)] | PO_{HS-3} [Log(CFU/g)] | FSO_{HS} [Log(CFU/g)] | $FSO_{HS} -$ Serving [Log(CFU/ serving)] | $ALOP_{HS}$ |
|-------------|-----------------------------|-----------------------------|----------------------------|---|-----------------------|
| -3.00 | 3.00 | 3.76 | 5.21 | 6.97 | 9.8×10^{-6} |
| -3.00 | 2.00 | 2.76 | 4.21 | 5.97 | 9.8×10^{-7} |
| -3.00 | 1.00 | 1.76 | 3.21 | 4.97 | 9.8×10^{-8} |
| -3.00 | 0.00 | 0.76 | 2.21 | 3.97 | 9.8×10^{-9} |
| -3.00 | -1.00 | -0.24 | 1.21 | 2.97 | 9.8×10^{-10} |
| -3.00 | -2.00 | -1.24 | 0.21 | 1.97 | 9.8×10^{-11} |
| -3.00 | -3.00 | -2.24 | -0.79 | 0.97 | 9.8×10^{-12} |

5. DEALING WITH VARIABILITY AND UNCERTAINTY

As indicated earlier, the above calculations were based on point estimates (i.e., a deterministic risk assessment), which provide an estimate of the relationship between levels of control at different stages in the production, marketing, and consumption of hot-smoked and cold-smoked salmon. These values are, in turn, dependent on the selection of values for various pathogen, food, and host factors (e.g., D-values for thermal resistance, r-value for dose-response relationship) that affect the level of the pathogen in the products and the susceptibility of the consumers. However, each of these factors is actually a variable and has a distribution of values. The selection of which value to employ with a point estimate influences the proportion of the distribution included and thus the stringency of the food safety system in relation to risk mitigation. For example, the 95% value for the duration of home storage of cold-smoked fish (i.e., 14 days at 5°C or 7 days at 7°C) over estimates the duration of home storage for most homes, and thus is a conservative assumption. Conversely, selection of the mean duration would underestimate the home storage duration half of the time. The normal tendency for risk managers is to select conservative values for each of the variable factors. This should be approached with a great deal of caution, particularly when dealing with a complex system where there are a series of steps. It can quickly result in unrealistically stringent requirements.

A more accurate means of evaluating the level of risk control achieved through the establishment of various PO and/or FSO values is to use a probabilistic approach that considers the entire distribution of values. This increase in accuracy is at the heart of increased use of probabilistic risk assessment tools. However, this does not in any way obviate the need for risk managers to ultimately make a decision about the level of risk which is deemed tolerable. At some point, a decision must be made regarding whether a food is acceptable to be consumed, which is inherently a yes or no decision. Hopefully, the risk analysis paradigm allows the risk managers to make decisions based on a better understanding of both the potential public health and trade impacts.

It is worth noting that even when working with a deterministic approach, one must be cognizant of the types of distributions underlying the parameters being considered. We are generally used to describing biological systems as normal distributions where the mean and the median values are the same, i.e. 50% of the distribution is above and below the mean. However, if an underlying distribution is highly “skewed” the mean can be substantially larger than the median, e.g., 95% of the distribution could fall below the mean. This is always important in microbiological considerations since one of the general assumptions made by microbiologists is that microbial populations are log normally distributed, i.e., the mean value is greater the median value.

In addition to the inherent variability of biological systems, the extent of knowledge is often incomplete or “uncertain.” This is typically handled in risk assessments by including an additional factor within the probabilistic approach that estimates the impact on the distribution of values for the various contributing factors. Thus, a highly uncertain parameter is likely to have any even greater distribution than one where the uncertainty is small. However, when the uncertainty is substantial this should not be interpreted as indicating that the distribution is infinite. Again, this quickly leads to unrealistically restrictive requirements that are not reflective of the actual risk. Instead, when such situations arise, an attempt should be made to determine the “upper bound” of the effect of the uncertain factor, and appropriately consider its impact on the overall risk and subsequent risk management decisions.

6. DEVELOPMENT OF PERFORMANCE CRITERIA (PC)

A PO establishes the level of a microbiological hazard that should not be exceeded at a specified point within the food chain, but does not specify what must be done to achieve that goal. The role of the PC is to describe the change in pathogen levels that must be achieved to obtain the PO. A PC is typically used to describe the degree of stringency required of a control measure, either in terms of the required reductions (ΣR) or the increases (ΣI) that must be limited. With the current pathogen/product pair this would require information on the initial levels of *L. monocytogenes* (H_0) at the stage of the process under consideration and the contribution that the control measure makes to the overall achievement of the PO. For example, suppose that (a) raw salmon has a mean log contamination rate of 1 CFU/g ($H_0 = 0.0$), (b) that if uncontrolled this level would increase to 1000 CFU/g if left uncontrolled ($\Sigma I = 3.0$), (c) cold-smoking can only be relied upon to achieve a 10-fold reduction ($R_1 = 1.0$), and (d) the required PO for this product at final packaging is 1 CFU/g ($PO_{CS-2} = 0.0$). In order to achieve this PO the inclusion of an additional reduction step (R_2) has been required. As depicted below, this would require an intervention capable of decreasing the concentration of *L. monocytogenes* 100-fold.

$$PO = H_0 + \Sigma I - \Sigma R = PO = H_0 + \Sigma I - (R_1 + R_2)$$

$$R_2 = PO - H_0 - \Sigma I + R_1 = 0.0 - 0.0 - 3.0 + 1.0 = 2.0$$

Thus, in this example, the PC, the amount of change in *L. monocytogenes* concentration required of the intervention selected, is a $\text{Log}(\text{CFU/g}) = 2.0$, or a 2-D inactivation. As discussed above the specific value of the PC would have to appropriately take into account the variability in the ability of *L. monocytogenes* to resist the intervention step and any uncertainty that we would have about the efficacy of the intervention. It should be kept in mind that when dealing with such interventions the levels of *L. monocytogenes* are in the linear portion of the dose-response curve used in the ALOP calculations above, each 1-D reduction is equivalent to a 10-fold reduction in risk.

7. DEVELOPMENT OF PROCESS CRITERIA

Process Criteria (PrC) are a more traditional food safety metric which is used to describe the specific actions that must be taken to achieve a desired food safety outcome. PrC describe how a specific step in the manufacture, distribution, or marketing can be carried to assure that a PC is achieved. For example, suppose that it was decided that establishment of a PC for the heat treatment received by hot-smoked fish would be beneficial, and that the magnitude of the treatment should reduce *L. monocytogenes* levels by 7 orders of magnitude, i.e., 7 log cycles. Furthermore, assume that available scientific information indicates that 95% of the *L. monocytogenes* strains have $D_{60^{\circ}\text{C}}$ -values and $D_{62^{\circ}\text{C}}$ -values of ≤ 5.3 and ≤ 1.8 minutes, respectively. Given this information the corresponding PrC criteria that would provide a high degree of confidence that the PC was being achieved would be heating for 37.1 minutes at 60°C or 12.6 minutes at 62°C. The advantage of employing PrC is that they provide small businesses and developing countries, which generally do not have the resources to conduct extensive laboratory trials, with clear guidance how to produce a safe product. However, as stated earlier it is unlikely that a PrC would be established for the heat-treatment of hot-smoked salmon since the cooking times and temperatures used to manufacture the product in relation to quality attributes produces reductions well in excess of those that would be needed to control *L. monocytogenes*.

8. DEVELOPMENT OF MICROBIOLOGICAL CRITERIA

The implementation of microbiological criteria (MC) is a control measure wherein microbiological testing is used to determine if a previously established microbiological limit for a food is being achieved, thereby verifying that a stated or an implied FSO, PO, or FSO is being met. Traditional MC have been based on the testing of individual lots where it is assumed that the examiner has no previous knowledge of the lot. This is often termed “lot-by-lot testing” or “within-lot testing,” wherein sufficient testing is performed to be able to state the likelihood that a lot is free of the pathogen based on a set of negative findings. More recently there has been increased use of process control testing which involves the “between-lot testing” or “process control verification testing” of multiple lots produced by a single manufacturing facility; a system of microbiological testing that is well suited for a facility working under a HACCP program. The focus of this type of testing is the verification that a food safety system is functioning as intended and is based on the assumption that the loss of control will lead to an observable change in the microbiological profile of lots. This type of MC requires extensive knowledge of the product and how it was manufactured. For the purposes of the current exercise, the relationship between MC and PO will be examined using lot-by-lot testing.

As soon as one attempts to determine if a PO is being achieved by microbiological testing, of necessity one moves from a PO to a MC. This reflects the fact that a PO establishes a boundary above which the food should not exceed, whereas a MC is a technological means of testing that not only specifies a microbiological limit, but also specifies the methods and sampling plans that are to be employed and the actions that are to be taken when the limit is exceeded. Standard references on the types of microbiological testing programs and the statistical basis for sampling plans are available (e.g., ICMSF, 2002). For the current examples, a 2-class attribute sampling plan would be used most often. Such plans are used in conjunction with presence/absence data or with “binned” quantitative data such as < 100 CFU/g vs. ≥ 100 CFU/g. Presence/absence attribute testing involves taking a specific number of samples (n) of a specific size (s) and testing them independently for the presence of the pathogen. The criteria include a term, c , which indicates the number of samples that can be positive and still have the lot considered acceptable. Typically, the c for an infectious agent such as *L. monocytogenes* is $c = 0$ (i.e., any positive sample is sufficient to reject the lot).

The establishment of a microbiological criterion requires information on the distribution of the contamination, the variability associated with that distribution, the microbiological limit, and the required level of confidence (i.e., the likelihood that the negative result would occur by chance alone) that the limit is not being exceeded. Typically, a log normal distribution (or normal distribution of the log values) is assumed which means that the distribution can be described by mean log concentration of the hazard and the standard deviation of the distribution. By specifying the microbiological limit, the level of confidence required, the standard deviation, and the sampling plan, it is then possible to calculate the mean log concentration that would have to be achieved to assure that the microbiological limit with the level of confidence required.

As an example of how a microbiological criterion could be calculated based on a PO, a microbiological criterion to verify PO_{CS-2} was calculated based on the assumption that contamination in a lot is log normally distributed with a standard deviation of 0.6 and the a sampling plan was designed to ensure that one could be 95% confident that a lot that had units that exceeded PO_{CS-2} value were detected 99.9% of the time (Table 8). This was calculated using the ICMSF attribute sampling plan tool, with the underlying assumption that the probability of detecting a lot that exceeds the PO is 99.87% (rounded to 99.9%) could be established using the

relationship, $\mu = PO - 3\sigma$, where μ = mean log concentration. The value for μ in Table 8 can be considered the mean log concentration (MLC) of *L. monocytogenes* that would need to be achieved to ensure that the PO was not exceeded at least 99.9% of the time. Examples of potential sampling plans that would verify that level of stringency are also provided.

Table 8. The mean log concentration (μ) that a manufacturer would need to achieve to ensure that microbiological testing prevented lots have greater than the specific PO_{CS-2} at 95% confidence, assuming that the standard deviation = 0.6.

| PO _{CS-2} [Log(CFU/g)] | Mean log concentration (μ) that should be achieved to be 95% confident of detecting a lot that has more than 0.1% of its units exceeding PO _{CS-2} [Log(CFU/g)] | Potential Sampling Plans | |
|------------------------------------|--|---|--|
| | | Plan 1 | Plan 2 |
| 3.00 | 1.20 | n = 32 m = 2.00 [Log(CFU/g)] ^a s = 0.010g ^b | n = 9 m = 1.52 [Log(CFU/g)] ^a s = 0.033g ^b |
| 2.00 | 0.20 | n = 32 m = 1.00 [Log(CFU/g)] s = 0.10g | n = 13 m = 0.70 [Log(CFU/g)] ^a s = 0.20g ^b |
| 1.00 | -0.80 | n = 60 m = 0.20 [Log(CFU/g)] s = 0.63g | n = 5 m = -0.72 [Log(CFU/g)] s = 5.24g |
| 0.00 | -1.80 | n = 10 m = -1.40 [Log(CFU/g)] s = 25.0 g | n = 31 m = -1.00 [Log(CFU/g)] s = 10.0g |
| -1.00 | -2.80 | n = 32 m = -2.00 [Log(CFU/g)] s = 100.0g | N = 5 m = -2.70 [Log(CFU/g)] s = 500.0g |
| -2.00 | -3.80 ^c | --- | --- |
| -3.00 | -4.80 ^c | --- | --- |

^a. Effective lower limit of sensitivity of the microbiological method employed.

^b. The size of the samples required if the method was 100% effective.

^c. Due sample sizes or sample numbers, it is not likely that microbiological testing would be practical or effective.

The ability for a microbiological sampling plan to discriminate between lots that do or do not exceed a PO is dependent on the standard deviation of the distribution of the pathogen in the lot. For example, if the PO_{CS-2} was 0.00 Log(CFU/g), there is a 99.9% likelihood that a 2-class sampling plan of n= 10, c = 0, and s = 25.0 g, would indicate that the PO had not been achieved if the manufacturer exceeded mean log concentration of -3.00, -2.40, -1.80, -1.20, -0.60, and -0.30 Log(CFU/g) if the standard deviation of the distribution was 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1, respectively. Thus, when the level of variability within a lot is reduced, the manufacturer can produce closer to the PO without exceeding it. The impact that the standard deviation and mean log concentration has on selecting appropriate sampling plan is large, and would have to be taken into account to ensure that the microbiological sampling program was effectively assessing that the PO was being achieved. This could be handled by manipulating the sample size which effectively modifies the m for the sampling plan (Table 9).

Table 9. Effect of standard deviation on the mean log concentration that would be needed to ensure that a PO = 0.00 was not exceeded 99.9% of the time and a sampling plan that could be used to identify lots that exceed the PO.

| Standard Deviation | Mean log concentration (μ) that should be achieved to be 95% confident of detecting a lot that has more than 0.1% of its units exceeding POcs-2 [Log(CFU/g)] | Sampling plan c/n/m/s |
|--------------------|--|--------------------------|
| 1.0 | -3.00 | 0/10/-2.35/224g |
| 0.8 | -2.40 | 0/10/-1.90/79g |
| 0.6 | -1.80 | 0/10/-1.40/25g |
| 0.4 | -1.20 | 0/10/-0.95/8.9g |
| 0.2 | -0.60 | 0/10/-0.47/3.0g |
| 0.1 | -0.30 | 0/10/-0.24/1.7g |

9. LESSONS LEARNED

Considering that the working group was developing this approach with a minimum of precedents to draw upon, one could consider that the entire exercise was a learning experience. However, several specific items jump to mind in terms of areas that were difficult to find solutions or for which decisions on assumptions or data had to be made. Several of them are made below.

1. There is a great deal of variability in the way that hot-smoked and cold-smoked salmon is manufactured, distributed, and marketed. Without simplifying assumptions or consideration of how the key steps are handled by a majority of the industry, one could easily get lost in the details. This may be easier to avoid by using more sophisticated probabilistic approach but this is likely to increase the difficulties in explaining the rationale for the calculation to stakeholders.
2. The measurement of variability and uncertainty will have to be dealt with ultimately, either in a simplified manner using a deterministic assessment as in the current example or more explicitly using a probabilistic approach. Both variability and uncertainty ultimately have to be considered in more detail to get better estimates of the risk reductions that would be achieved by different PO values and the various control measures that might be considered to mitigate that risk.
3. The techniques for relating MCs to POs are still in its infancy and consensus on approaches has not been reached. The FAO/WHO should encourage and support the development of “user-friendly” tools by member nations or international scientific advisory organizations (e.g., ICMSF) to make it possible for a broader range of risk managers to perform these calculations.
4. Making consistently conservative assumptions in a multiple step food processing series has the potential for producing POs and FSOs that are unrealistically stringent.
5. The approach of providing a series of potential PO, FSO, and ALOP was found to be a useful approach, providing the risk managers with a series of options and avoiding having the risk assessors make the risk management decision.
6. There are often multiple combinations of control measures that can achieve an FSO. For example, if cold-smoked salmon was shipped frozen to retail markets, then growth would be reduced by approximately 1 Log(CFU/g). Conversely, an intervention step at final packaging that reduced *L. monocytogenes* by 1 Log(CFU/g) would give an equivalent degree of risk reduction.

10. CONCLUSIONS

The current example is only one of several approaches that could have been employed. The approach selected after initial discussions with risk managers on different potential approaches indicated that arraying the information in this manner effectively communicated the options to a group of risk managers, assisted them in reaching decision, provided a transparent basis for the communicating the scientific basis for the decision to stake holders, and did not lead to “hidden risk management decisions” being made by the risk assessors. While risk managers are increasingly becoming more sophisticated in regard to probabilistic approaches, they are generally more comfortable in translating decisions into deterministic consideration and ultimately to yes/no criteria.

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