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Quantitative risk assessment for *Listeria monocytogenes* in smoked or gravad salmon and rainbow trout in Sweden

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Abstract

The objective of the present work was to develop a quantitative risk assessment model in which the exposure and risk of acquiring listeriosis from consumption of packaged smoked or gravad salmon and rainbow trout were estimated. An Excel spreadsheet model was constructed in which variables were represented by distributions based on surveys of *L. monocytogenes* in these food products, and on demographic and consumption data. Growth or inactivation was not included in the model. The model was run through Monte Carlo simulations using the @Risk software (Palisade Corporation). The probability of illness per serving was calculated using two dose-response models from the literature. The first was an exponential model in which the species specific constant *R*, that helps define the dose-response curve, previously has been estimated to be 1.18×10^{-10} based on German data (GR). In this study, *R* was estimated to 5.6×10^{-10} based on Swedish data. The second model was a flexible Weibull–Gamma model (WG), with different coefficients for high- and low-risk groups. The exponential model (GR), although conservative and generally overestimating the risk, still predicted a lower probability of illness than the WG-model. The estimated mean risk per serving was 2.8×10^{-5} (GR, high-risk group), 2.0×10^{-3} (WG, low-risk group) and 0.016 (WG, high-risk group), respectively. The average number of reported listeriosis cases in Sweden is 37 per year. In comparison, the mean number of annual cases predicted by the risk assessment model was 168 (range 47 to 2800, GR, high-risk group), and 95 000 (range 34 000 to 1.6×10^{6}, WG high-risk group), respectively. If 1 to 10% (uniform distribution) of strains, instead of all, were considered virulent, the mean number of predicted cases would decrease to nine (GR) and 5200 (WG), respectively. The mean annual cumulative individual risk in the high-risk group based on a monthly exposure was estimated to be 4.0×10^{-4} (range 8.0×10^{-8} to 5.4×10^{-3}, GR). This risk increased to 1.5×10^{-3} (range 1.7×10^{-5} to 9.2×10^{-3}, GR) based on a weekly exposure. The risk assessment model was most sensitive to the input distribution describing the level of contamination and to a lesser degree on the prevalence of *L. monocytogenes*, the proportion of virulent strains, and serving sizes. A lack of data on the prevalence and concentration of *L. monocytogenes* in these products, dose-response data and quantitative information on the proportion of virulent strains were identified.

Keywords: Dose-response models; Monte Carlo simulation; Listeriosis; Risk analysis

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1. Introduction

Quantitative risk assessment, (QRA), applied to microbial food safety is a scientifically based methodology to estimate the probability and severity of a health disturbance as a consequence of consumption of food (Whiting and Buchanan, 1997; Cassin et al., 1998; Marks et al., 1998). The terminology and methodology are not yet definitive, but the process involves four main steps: (1) hazard identification, (2) exposure assessment, (3) hazard characterisation and (4) risk characterisation (WHO/FAO, 1995; European Commission, EC, 1997; Buchanan, 1998; Codex Alimentarius Commission, CAC, 1998). A formal risk assessment requires a structured and scientific approach, and should be transparent with the assumptions clearly stated. The result of a risk assessment is a risk estimate, but perhaps equally important is that this approach makes a comparison between different risk management options possible and the gaps in knowledge obvious.

Commonly, many parameters influence the outcome of a single risk assessment. One approach is to use a single point estimate for each of these parameters or, alternatively, to use an estimate that will give a worst case scenario. However, for several reasons these approaches may not be satisfactory. An alternative approach is to perform a probabilistic risk assessment, incorporating Monte Carlo simulations as a tool to evaluate uncertainty and variability by use of special computer software (Burmaster and Anderson, 1994; Vose, 1996, 1998). In this approach, a parameter is represented by a probability distribution, which describes the lack of precise knowledge (uncertainty) about the parameter, and/or the natural variation (variability) in the parameter (Vose, 1998). Thus, for each value of the parameter there is an associated probability that the parameter will assume this value. When performing the Monte Carlo simulation the risk estimate is calculated repeatedly until the result does not change appreciably or until a predetermined number of iterations have been reached. At each iteration, a value is drawn for each parameter from the defined distribution, i.e. values of high probability are drawn more often than those with low probability. Consequently, the result of the simulation is also a probability distribution.

Gravad or smoked (hot- or cold-smoked) salmon and rainbow trout are popular ready-to-eat foods in Sweden. After processing, the fish is packaged and stored for varying times at refrigerator temperatures before consumption without prior heat treatment. Packaged products made from both types of fish have been identified as potential sources of human listeriosis (Ericsson et al., 1997; Loncarevic et al., 1998). The objective of the present work was to develop a quantitative risk assessment model in which the exposure and risk of acquiring listeriosis from consumption of these ready-to-eat foods were estimated. At the present stage with the limited amount of data available it was decided to consider packaged salmon and rainbow trout as one hazard whether gravad, cold- or hot-smoked.

A Monte Carlo risk model was developed in which variables were represented by distributions based on surveys of *L. monocytogenes* in the relevant foods and on demographic and consumption data. The risk per serving and the annual cumulative individual risk based on the number of exposures were calculated using two different dose-response relationships. Using these relationships the estimated number of cases in the population was calculated and compared to the annually reported number of cases of listeriosis in Sweden. Survey data reflected the prevalence and level of *L. monocytogenes* at the last recommended day of consumption so growth or inactivation was not specifically addressed in the risk assessment model. A schematic diagram of the structure of the model used in the risk assessment is shown in Fig. 1. The spreadsheet model and input data are presented in detail in the figures and tables.

2. Hazard identification

L. monocytogenes is a bacterial pathogen causing listeriosis in humans with a variety of symptoms including mild diarrhea, meningitis and septicaemia (Farber and Peterkin, 1991). *L. monocytogenes* is found in many foods and evidence suggests that most exposure is foodborne. Cold-smoked or gravad rainbow trout have been implicated as causing a listeriosis outbreak in Sweden (Ericsson et al., 1997). Based on a characterisation of *L. monocytogenes* strains isolated from humans and food in Sweden, Loncarevic et al. (1998) suggested an association between human listeriosis and consumption of salmon and rainbow trout. Sliced cooked medwurst has

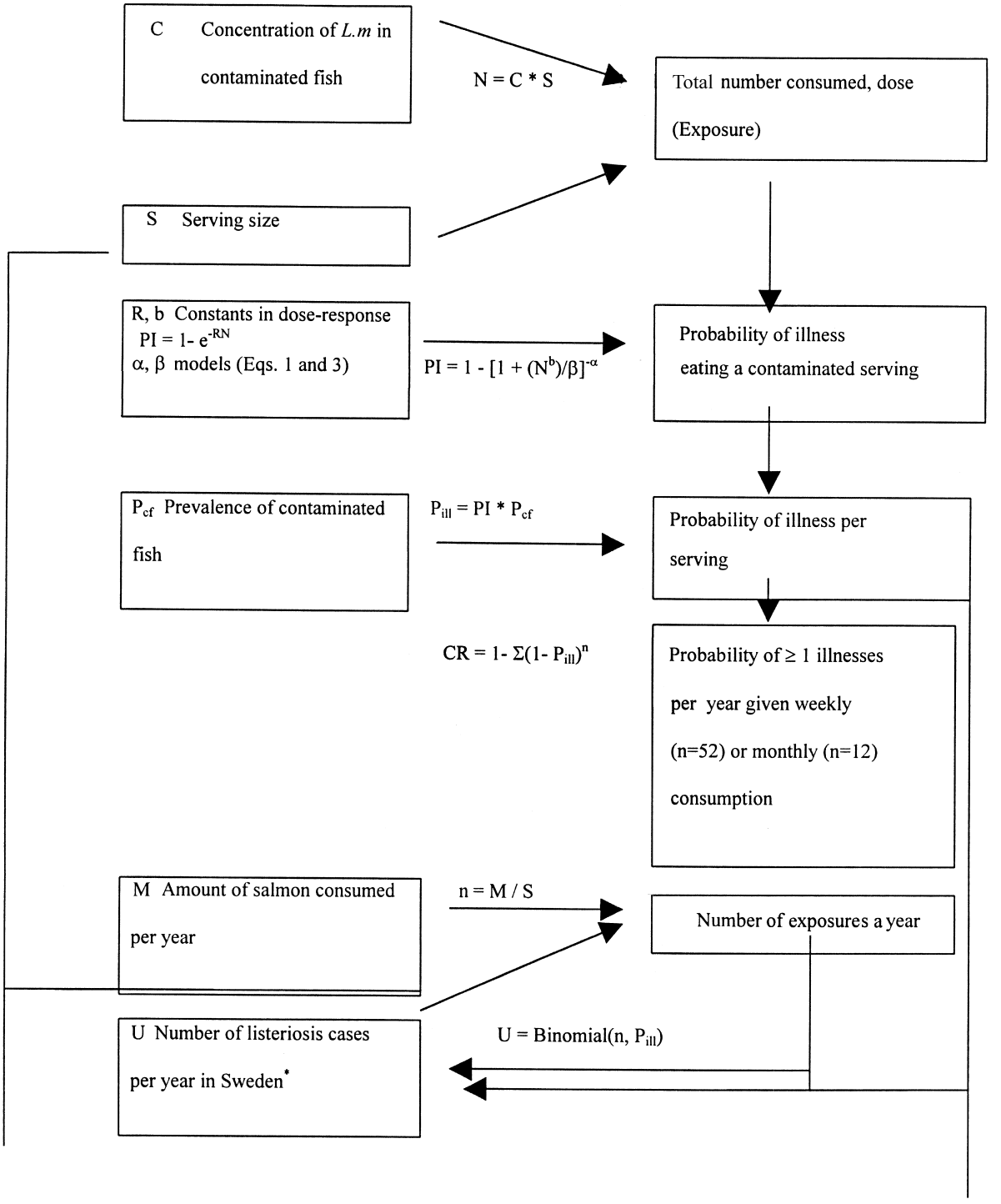


Fig. 1. Schematic diagram of the structure of the model used in the risk assessment. *Since *n* was very large, *U* was approximated by a normal distribution (Fig. 10).

also been implicated as a source of listeriosis in Sweden (Loncarevic et al., 1997). Major risk factors for acquiring listeriosis are immuno-suppression, old age and pregnancy. Initially, all strains of *L. monocytogenes* were considered pathogenic to humans but this assumption is also compared to a scenario where the proportion of pathogenic strains varies uniformly between 1 and 10%, a range suggested by Farber et al. (1996).

3. Exposure assessment

3.1. Prevalence

The distribution for the prevalence of *L. monocytogenes* in packaged smoked or gravad salmon/rainbow trout was estimated from a number of surveys carried out in Sweden between 1993 and 1996 (Table 1 and Fig. 2). The prevalence was described by a cumulative distribution assuming a minimum and maximum prevalence of 1 and 25%, respectively.

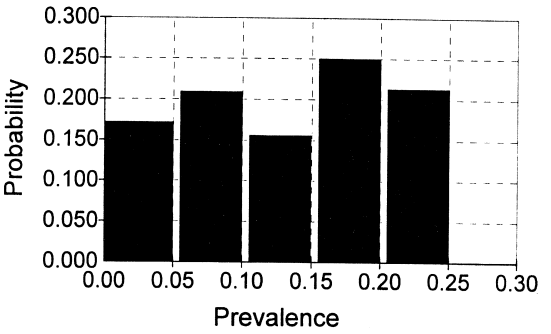


Fig. 2. The distribution for prevalence of *L. monocytogenes* in packaged smoked or gravad salmon/rainbow trout. From this distribution the prevalence of contaminated fish was sampled during the Monte Carlo simulations. The distribution was generated by a cumulative distribution using data from six different surveys (Table 1).

3.2. Concentration

The distribution for the level of *L. monocytogenes* in contaminated packaged smoked and gravad salmon/rainbow trout was estimated from data in SLV (1995) and Loncarevic et al. (1996) (Table 2). In those surveys, samples were stored at 4°C after purchase and analysed on the last recommended day of consumption. The detection limits of the quantitative methods used in the surveys were different. Because of this, the pathogen level in samples contaminated with too few *L. monocytogenes* to be quantified were reported as either <10 or <100 cfu g^{-1}. In this work, the level in those samples was assumed to be 1 and 10 cfu g^{-1}, respectively. The data was described by a cumulative distribution assuming a minimum and maximum level of 1 and 10^6 cfu g^{-1}, respectively (Fig. 3).

3.3. Consumption

The distribution for serving sizes was described by a modified triangular distribution (Fig. 4). This distribution is determined by parameters for the minimum, maximum, and most likely serving sizes and by estimates of the percentages of servings below the min. and max. serving sizes. The parameter values used were derived from information

Table 1
Detection rate for *Listeria monocytogenes* is packaged gravad or smoked salmon/rainbow trout[a]

Source	No. of samples	No. of positives	Prevalence	$F(x)$[b]
Liva-Lab Stockholm (1993)	51	2	0.039	0.143
Röhl (1996)	32	3	0.094	0.286
Röhl (1995)	29	3	0.103	0.428
SLV (1995)	103	17	0.165	0.571
Loncarevic et al. (1996)	94	16	0.170	0.714
Detmer and Blomgren (1995)	35	8	0.229	0.857

[a] The distribution for prevalence was described by the following cumulative distribution RiskCumul(0.01;0.25;{0.039;0.094;0.103;0.165;0.17;0.229};{0.143;0.143;0.286;0.428;0.571;0.714;0.857}).

[b] $F(x)$ is the cumulative probability (Vose, 1996), i.e. $F(x) = i/n + 1$, where i is the rank of the observed data point (prevalence) and n is the number of data points (number of surveys).

Table 2
Level of *Listeria monocytogenes* is packaged smoked or gravad salmon/rainbow trout (cfu g^{-1})[a]

Survey	Salmon/ rainbow trout	Level in positive samples[b] (cfu g^{-1})
SLV (1995)[c]	Gravad	<10,<10,<10,<10,<10, 120, 780, 980, 15 000
	Smoked	<10,<10,<10,<10,<10,<10,<10, 410
Loncarevic et al. (1996)[d]	Gravad	<100,<100,<100, 200, 500, 700, 900, 900, 1300, 3400
	Smoked	<100,<100,<100, 400, 25 400, 132 000
Summary		17% (33/197) positive samples, 1.5 (3/197) of all samples $>10^4$ cfu g^{-1}

[a] The distribution was described by RiskCumul(0;6;{2.08;2.3;2.6;2.61;2.7;2.84;2.89;2.95;2.99;3.11;3.53;4.18;4.4;5.12};{0.35;0.53;0.56;0.59;0.62;0.65;0.68;0.71;0.74;0.79;0.82;-0.85;0.88;0.91;0.94;0.97})
[b] These samples were all positive in a qualitative analysis.
[c] Samples analysed, 103.
[d] Samples analysed, 94.

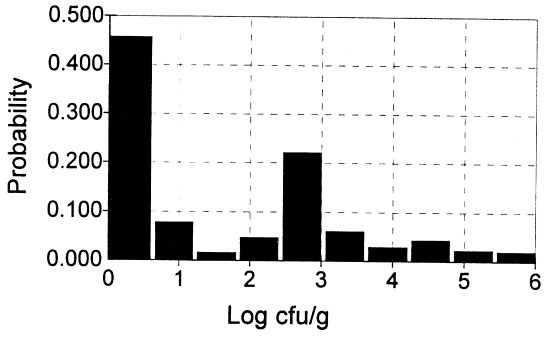


Fig. 3. The probability of different levels of *L. monocytogenes* in contaminated fish. The distribution was generated by a cumulative distribution using data from Loncarevic et al. (1996), and the Swedish National Food Administration (SLV, 1995). A minimum level of 1 cfu g^{-1} and a maximum level of 10^6 cfu g^{-1} was assumed.

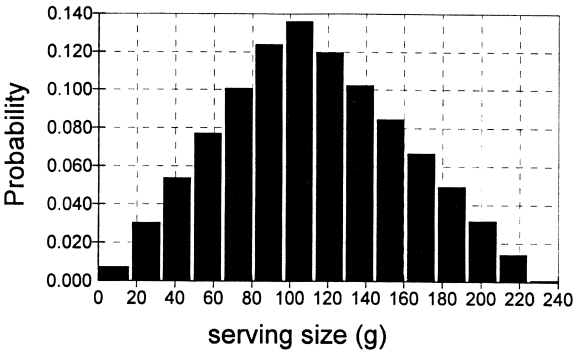


Fig. 4. The distribution for serving sizes. The distribution was generated by a Trigen distribution and the parameters estimated from SLV (1988) and Nordisk Ministerråd København (1998).

reported by SLV (1988) and Nordisk Ministerråd København (1998) (Table 3).

4. Hazard characterisation

Two different dose-response models were used to estimate the risk based on exposure. The first is an exponential dose-response model, which relates *P*, the probability of an adverse effect, with the number of *L. monocytogenes* bacteria consumed, *N*, according to Eq. (1):

$$P = 1 - e^{-RN} \quad (1)$$

R is a constant, specific for each pathogen that helps define the shape of the dose-response curve. The constant, *R*, has been estimated by Buchanan et al. (1997) for *L. monocytogenes* using data on the levels in smoked fish and epidemiological data in Germany. Their approach reportedly yielded a conservative estimate of *R*, i.e. it is likely to overestimate the probability of infection (Buchanan et al., 1997). The authors made the assumption that foods with a concentration over 10^4 *L. monocytogenes* cfu g^{-1} cause illness, and that only members of the high-risk population become ill. Based on German data, Buchanan et al. (1997) estimated *R* to 1.18×10^{-10}. Using the same approach, *R* was estimated to $5.6\times$

Table 3
Surveys on which the estimation of serving sizes were based[a]

Nordisk Ministerråd København (1998)	Cold cuts (meat); 10th percentile: 50 g; 50th percentile: 100 g; 90th percentile: 175 g These values are based on a weighted record method used on 771 individuals from Denmark, Norway and Sweden. (No data available on smoked or gravad salmon).
SLV (1988)	Normal serving size for gravad salmon: 100 g; Normal serving size for cold smoked salmon: 75 g (These data are under revision)

[a] The distribution of serving sizes was described by the following modified triangular distribution RiskTrigen(50;100;175;10;90).

10^{-10} in the present work based on Swedish data. This number was arrived at by assuming that 1.5% of the servings have a concentration of more than 10^4 cfu g^{-1}, since this was the proportion of positive samples with that concentration when data in Loncarevic et al. (1996) and SLV (1995) was summarised (Table 2). The total number of consumers in the age group $\geq$15 years was calculated to be 1.5×10^6 persons (Table 4). People younger than 15 years were not considered since practically all cases of listeriosis in this group recorded during 1992 and 1996 were $<$1 year-old (Arneborn, 1997), and probably pregnancy related. The high-risk part of the general population was approximated to 20% based on data in Table 5. If we assume that the proportion of high-risk people among salmon consumers is the same as that among the general population, then there is $0.2\times1.5\times10^6=3\times10^5$ high-risk consumers in Sweden. Furthermore, if their consumption pattern is the same as that of the non-susceptible salmon consumers, the high-risk population consumes $0.2\times2.2\times10^6=4.4\times10^5$ kg per year (Table 4). Assuming an average serving size of 100 g, then the average number of portions consumed by these persons are 14.7 a year. The yearly number of contaminated portions with more than 10^4 cfu g^{-1} eaten by the high-risk population becomes $3\times10^5\times14.7\times0.015=66\ 150$ portions. The average number

Table 5
Susceptible groups in the Swedish population at higher risk of acquiring listeriosis

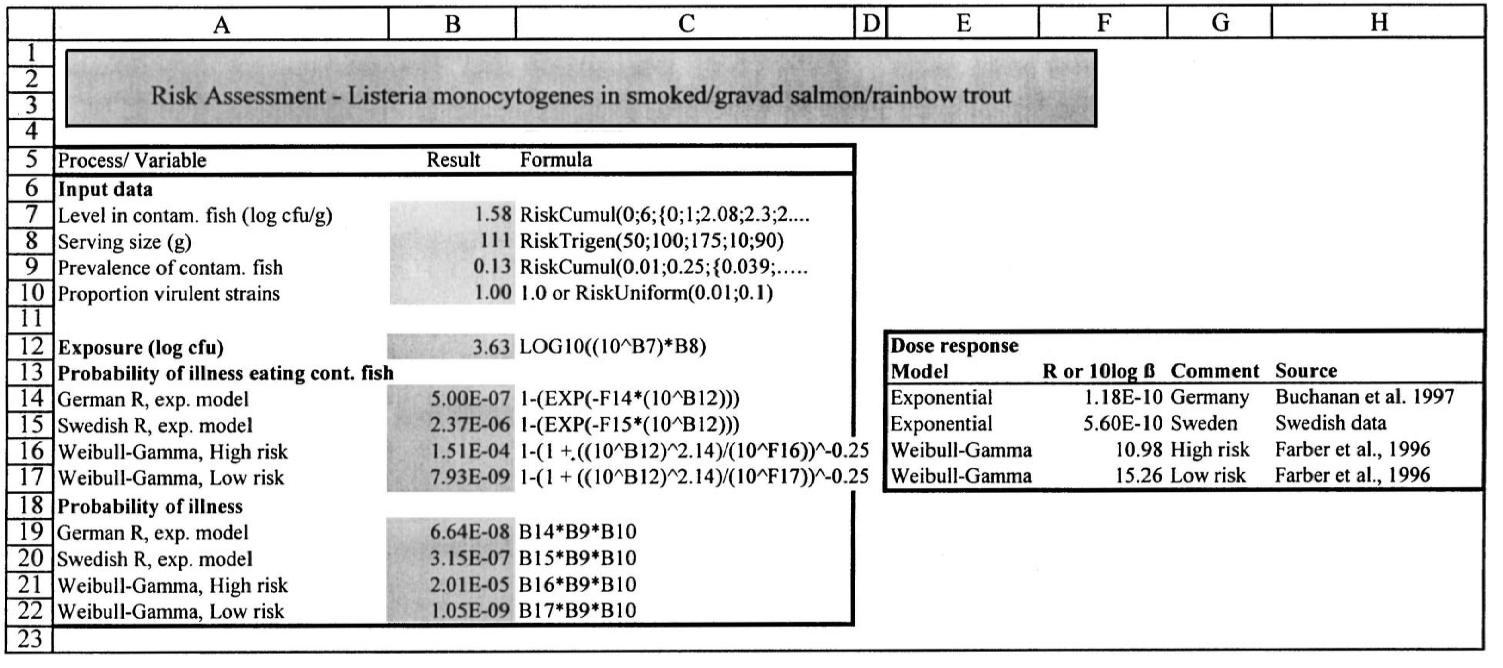
Risk category	% of total population[a]
Pregnant women	1.41
Children $<$1 year	1.13
Elderly $\geq$65 years	17.5
Elderly $\geq$70 years	12.9
HIV/AIDS	0.05[b]
Sum	20.1[c]

[a] These numbers were calculated from data in Statistisk Årsbok (1995, 1996).
[b] Anonymous (1996).
[c] Using $\geq$70 years as the limit for elderly at increased risk decreases the sum to 15.5% of the population. Both figures exclude immuno-suppressed persons that do not belong to any of the above risk groups.

Table 4
Consumption by age group[a]

Age group (years)	No. of people (millions)	% consumers	No. of consumers (millions)	Salmon consumed (10^6 kg/year)
0–1	0.1	0	0	0
1–14	1.66	7[b]	0.12	0.18
15–74	6.32	22	1.40	2.10
75–79	0.34	8	0.03	0.05
$>$79	0.42	8[c]	0.03	0.05
Total/sum	8.84		1.6	2.4

[a] Consumption and population data was compiled from SLV (1989) and Statistisk Årsbok (1996) In the risk assessment, only age groups $\geq$15 years were considered and this segment of the population consists of 1.5 million consumers who eat 2.2 million kg packaged smoked or gravad salmon/rainbow trout per year.
[b] Average of consumers in age groups 1–6 and 7–14 years.
[c] Assumed to be the same as for age group 75–79.



Risk Assessment - Listeria monocytogenes in smoked/gravad salmon/rainbow trout

Process/ Variable	Result	Formula
Input data		
Level in contam. fish (log cfu/g)	1.58	RiskCumul(0;6;{0;1;2.08;2.3;2....
Serving size (g)	111	RiskTrigen(50;100;175;10;90)
Prevalence of contam. fish	0.13	RiskCumul(0.01;0.25;{0.039;.....
Proportion virulent strains	1.00	1.0 or RiskUniform(0.01;0.1)
Exposure (log cfu)	3.63	LOG10((10^B7)*B8)
Probability of illness eating cont. fish		
German R, exp. model	5.00E-07	1-(EXP(-F14*(10^B12)))
Swedish R, exp. model	2.37E-06	1-(EXP(-F15*(10^B12)))
Weibull-Gamma, High risk	1.51E-04	1-(1 +,((10^B12)^2.14)/(10^F16))^-0.25
Weibull-Gamma, Low risk	7.93E-09	1-(1 + ((10^B12)^2.14)/(10^F17))^-0.25
Probability of illness		
German R, exp. model	6.64E-08	B14*B9*B10
Swedish R, exp. model	3.15E-07	B15*B9*B10
Weibull-Gamma, High risk	2.01E-05	B16*B9*B10
Weibull-Gamma, Low risk	1.05E-09	B17*B9*B10

Dose response

Model	R or 10log ß	Comment	Source
Exponential	1.18E-10	Germany	Buchanan et al. 1997
Exponential	5.60E-10	Sweden	Swedish data
Weibull-Gamma	10.98	High risk	Farber et al., 1996
Weibull-Gamma	15.26	Low risk	Farber et al., 1996

Fig. 5. The Excel spreadsheet model used to calculate the probability of illness per serving.

of reported listeriosis cases per year is 37 (Arneborn, 1997). Assuming that all these cases are caused by consumption of smoked or gravad salmon/rainbow trout the probability of illness for high-risk consumers becomes $37/66\,150 = 5.6 \times 10^{-4}$ cases per contaminated serving. The ingested dose in these servings was assumed to be 100 g $\times 10^4$ cfu g^{-1}, i.e. 10^6 cfu, which is a conservative assumption since the level in some of the portions may be larger than 10^4 cfu g^{-1}. R was then calculated by rearranging Eq. (1):

$$R = -[\ln(1-P)]/N$$
$$= -[\ln(1 - 5.6 \times 10^{-4})]/10^6 = 5.6 \times 10^{-10} \quad (2)$$

We assume that the same value for R can be used under Swedish and German conditions and therefore the German R was used in most of the calculations since we wanted an independent estimate of R, not based on data we would later use in the risk assessment.

The second dose-response model is the flexible Weibull–Gamma model suggested by Farber et al. (1996). This model was used by Bemrah et al. (1998) to estimate the risk of listeriosis from consumption of soft cheese made from raw milk. The probability of illness is described by the following equation:

$$P = 1 - [1 + (N^b)/\beta]^{-\alpha} \quad (3)$$

Where P is the probability of illness for an individual exposed to a dose, N. α, β and b are model parameters. For both sub-populations, $\alpha = 0.25$, $b = 2.14$, whereas β is $10^{10.98}$ for the high-risk population and $10^{15.26}$ for the low-risk population (Ross, W.H., Health Canada, personal communication; Bemrah et al., 1998).

5. Risk characterisation

The probability of illness per serving was estimated by running the Excel spreadsheet model (Fig. 5). This estimate was used to calculate the annual cumulative risk based on weekly or monthly exposures, and the annual number of predicted listeriosis cases in Sweden. First, the cumulative individual risk for high-risk groups, CR, was calculated using the following equation:

$$CR = 1 - (1 - P_{ill})^n \quad (4)$$

where n is the number of exposures. However, with this equation an iteration of the model uses a constant value of P_{ill} for each exposure. This is computationally simple but unrealistic and results in an unnecessary large uncertainty in the estimated CR. A second approach was to construct an Excel spreadsheet model (Fig. 6), which for each exposure, n, samples a value for P_{ill} from the previously generated distribution of P_{ill}. This approach was termed the sampling approach. According to the central limit theorem, sampling from any parent distribution yields a new distribution, which approaches normality as the number of samples, i.e. exposures, increases. Accordingly, in the final approach P_{ill} was approximated by a normal distribution with the same mean as the parent distribution of P_{ill} and with the standard deviation divided by the square root of the number of exposures (Vose, 1996).

The annual number of predicted listeriosis cases was estimated based on the number of servings in Sweden per year and the probability of illness per serving, P_{ill}. The latter was assumed to follow a normal distribution and was described according to the central limit theorem (Fig. 7). The number of listeriosis cases was then estimated using the binomial distribution. However, since the @Risk software cannot handle more than 32 000 servings the normal approximation of the binomial distribution was used (Fig. 7).

The Excel spreadsheet models (Figs. 5–7) were simulated, using Latin Hypercube sampling, until the convergence criteria was met ($<1.5\%$ change or 10 000 iterations) in the @RISK 3.5.1 Software (Palisade Corporation, Devon, UK).

6. Results

The predicted mean dose of *L. monocytogenes* ingested by people eating a contaminated fish was 3800 cfu (range 4 to 2×10^8 cfu, Fig. 8). The dose was less than 10^4 cfu in 56% of the iterations and less than 10^6 in 90% of the iterations (Fig. 8).

Based on the ingested dose, the prevalence of contaminated fish and the two dose-response models, the probability of illness per serving, P_{ill}, was estimated (Table 6). The data for the exponential

	A	B	C	D	E	F	G	H	I
1	Estimation of cumulative individual risk - sampling approach								
2	**Exposure no.**	**Sample row no.**	**Address**	**Probability not ill**					
3	1	INTEGER((RAND()*10006-7)	ADDRESS(B3;7)	1-INDIRECT(C3)					
4	2	INTEGER((RAND()*10006-7)	ADDRESS(B4;7)	1-INDIRECT(C4)					
5	3	INTEGER((RAND()*10006-7)	ADDRESSB5;7)	1-INDIRECT(C5)					
6	4	INTEGER((RAND()*10006-7)	ADDRESS(B6;7)	1-INDIRECT(C6)					
7	5	INTEGER((RAND()*10006-7)	ADDRESS(B7;7)	1-INDIRECT(C7)					
8	6	INTEGER((RAND()*10006-7)	ADDRESSB8;7)	1-INDIRECT(C8)					
9	7	INTEGER((RAND()*10006-7)	ADDRESS(B9;7)	1-INDIRECT(C9)					
10	8	INTEGER((RAND()*10006-7)	ADDRESS(B10;7)	1-INDIRECT(C10)					
11	9	INTEGER((RAND()*10006-7)	ADDRESSB11;7)	1-INDIRECT(C11)					
12	10	INTEGER((RAND()*10006-7)	ADDRESS(B12;7)	1-INDIRECT(C12)					
13	11	INTEGER((RAND()*10006-7)	ADDRESS(B13;7)	1-INDIRECT(C13)					
14	12	INTEGER((RAND()*10006-7)	ADDRESSB14;7)	1-INDIRECT(C14)					
15									
16	Probability not ill (12 exp.)			PRODUCT(D3:D14)					
17	Cumulative risk of illness (12 exp.)			1-D16					
18									
10004						9998	1.02E-04		
10005						9999	1.55E-05		
10006						10000	2.96E-06		
10007									

Table from which probability values were sampled. Data generated by simulating the spreadsheet model (GR, Table 6).

Iteration	Prob. of illness
1	3.81E-09
2	1.22E-09
3	5.82E-04
4	3.32E-09
5	1.80E-09
6	1.76E-07
7	2.00E-06
8	1.94E-09

Fig. 6. The Excel spreadsheet model for the sampling approach used to calculate the annual cumulative individual risk of listeriosis based on a monthly exposure, i.e. 12 exposures.

	A	B	C
1			
2	Estimation of annual number of listerosis cases in Sweden		
3			
4	Process/Variable	Result	Formula
5	**Input data**		
6	Consumption per year, (kg)	2.20E+06	From Table 4
7	Serving size (g)	1.11E+02	RiskTrigen(50;100;175;10;90)
8	No of servings	1.99E+07	(B6 * 1000)/B7
9	Fraction consumed by risk groups	0.20	From Table 5
10	Servings consumed by risk groups	3973222	B8*B9
11			
12	**Probability of illness**		(Mean and SD from table 6)
13	German R, exp. model, risk groups	2.80E-05	RiskTnormal(2.8E-5;(1.9E-4/(B10^0.5));0;1)
14	Weibull-Gamma, High risk	1.60E-02	RiskTnormal(0.016;(0.041/(B10^0.5));0;1)
15	Weibull-Gamma, Low risk	2.00E-03	RiskTnormal(0.0020;(0.012/((0.8*B8)^0.5));0;1)
16			
17	**Output - No of cases**		
18	Listeriosis cases, German model	111	RiskNormal(B25;B26)
19	Listeriosis cases - risk groups, W-G model	63572	RiskNormal(B27;B28)
20	Listeriosis cases - low risk, W-G	31786	RiskNormal(B29;B30)
21	Total Listeriosis cases - W-G model	95357	B19+B20
22			
23	Approximation of binomial with normal		
24		Result	Formula
25	Mean prob of illness (German R)	111	B9*B8*B13
26	SD of prob of illness (German R)	11	(B9*B8*B13*(1-B13))^0.5
27	Mean prob of illness (WG high risk)	63572	B9*B8*B14
28	SD of prob of illness (WG high risk)	250	(B9*B8*B14*(1-B14))^0.5
29	Mean prob of illness (WG low risk)	31786	(1-B9)*B8*B15
30	SD of prob of illness (WG risk)	178	((1-B9)*B8*B15*(1-B15))^0.5
31	SD = Standard deviation		
32			

Fig. 7. The Excel spreadsheet model used to estimate the annual number of listeriosis cases caused by consumption of smoked or gravad salmon/rainbow trout.

dose-model using the German *R*, (GR), is illustrated in Fig. 9. The probability of illness per serving estimated by this model was considerably lower than that estimated by the Weibull–Gamma model (Table 6). The mean P_{ill} ranged from 2.8×10^{-5} (GR, high-risk population) to 1.6×10^{-2} (WG, high-risk population). These probabilities translate to 1 case per 36 000 (German *R*), 7700 (Swedish *R*), and 62 (WG) servings, respectively.

Using the exponential dose response model with the German *R*, the annual cumulative risk in high-risk groups based on a weekly or monthly consumption was estimated by three computationally different approaches. All three approaches resulted in very similar mean probabilities, and were estimated to be around 10^{-4} for a monthly consumer and increased to around 10^{-3} for a weekly consumer (Table 7). In the sampling approach, which probably mimics reality the best, the annual cumulative individual risk was estimated to 4.0×10^{-4} (range 8.0×10^{-8} to 5.4×10^{-3}) based on a monthly exposure. This risk increased to 1.5×10^{-3} (range 1.7×10^{-5} to $9.2\times$

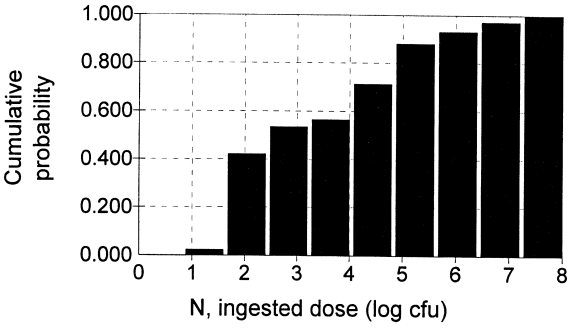


Fig. 8. A cumulative frequency distribution, indicating the exposure to *L. monocytogenes* by eating a contaminated serving.

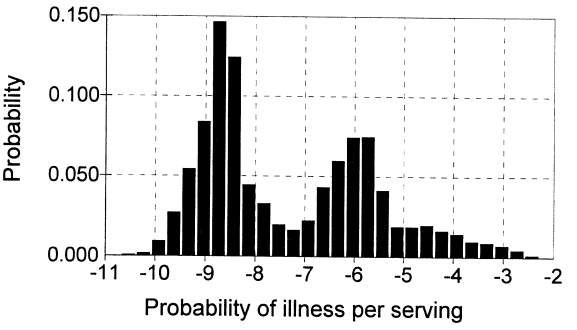


Fig. 9. The distribution for probability of illness per serving, P_{ill}, of packaged smoked or gravad salmon/rainbow trout for a high-risk population. The probability distribution was calculated using the risk assessment model and the exponential dose-response model with the German *R*.

10^{-3}) based on a weekly exposure (Table 7). The mean risks correspond to one per 2500 (monthly) or per 700 (weekly) consumers becoming ill at least once per year. In contrast to the estimated mean, the differences were greater between the median, minimum and maximum probabilities estimated by the three approaches, especially those of the direct approach (Table 7). The sampling approach is, with the present software, more computationally difficult.

Table 6
The probability of illness per serving estimated by two dose-response models

Model	Mean	S.D.	Minimum	Maximum	Median
German R[a]	2.8×10^{-5}	1.9×10^{-4}	2.6×10^{-11}	4.4×10^{-3}	1.1×10^{-8}
WG[b] (high-risk)	1.6×10^{-2}	4.1×10^{-2}	5.6×10^{-12}	0.24	4.0×10^{-7}
WG[b] (low-risk)	2.0×10^{-3}	1.2×10^{-2}	3.0×10^{-16}	0.18	2.1×10^{-11}
Swedish R[c]	1.3×10^{-4}	8.8×10^{-4}	1.2×10^{-10}	2.0×10^{-2}	5.2×10^{-8}

[a] Exponential dose-response models with *R* estimated by Buchanan et al. (1997). Assumes that only the high-risk population is affected.
[b] Weibull–Gamma model suggested by Farber et al. (1996).
[c] *R* estimated from Swedish data in this work. Assumes that only the high-risk population is affected.

Table 7
A comparison between three techniques for computing the estimated cumulative annual risk based on a monthly or a weekly consumption, i.e 12 or 52 exposures per year. The exponential dose-response model with the German *R* was used.

Consumption	Technique	Mean	S.D.	Minimum	Maximum	Median
Monthly	Direct[a]	3.0×10^{-4}	2.0×10^{-3}	2.3×10^{-10}	4.6×10^{-2}	1.4×10^{-7}
	Sampling[b]	4.0×10^{-4}	7.2×10^{-4}	8.0×10^{-8}	5.4×10^{-3}	8.1×10^{-5}
	CLT[c]	6.8×10^{-4}	4.6×10^{-4}	4.1×10^{-7}	2.3×10^{-3}	6.1×10^{-4}
Weekly	Direct	1.3×10^{-3}	8.2×10^{-3}	1.0×10^{-9}	0.19	5.9×10^{-7}
	Sampling	1.5×10^{-3}	1.4×10^{-3}	1.7×10^{-5}	9.2×10^{-3}	1.0×10^{-3}
	CLT	1.8×10^{-3}	1.9×10^{-2}	3.4×10^{-6}	6.0×10^{-3}	1.7×10^{-3}

[a] Eq. (4) was used and the same probability of illness per serving was used for each exposure each iteration.
[b] For each exposure a probability of illness per serving was sampled from a population of 10 000 probabilities generated by a previous simulation of the spreadsheet model.
[c] CLT, Central Limit Theorem. The distribution for the probability of illness per serving was approximated by a normal distribution with the same mean as the probability distribution simulated by the spreadsheet model and used in the sampling technique. According to the CLT the standard deviation was calculated by dividing the standard deviation by the square root of the number of exposures, i.e 12 or 52.

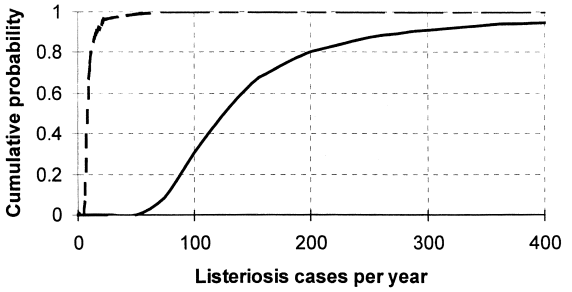


Fig. 10. A cumulative frequency distribution showing the influence of the assumption of the proportion of virulent strains on the estimated number of listeriosis cases per year in Sweden. The graph shows the data based on the exponential dose response model with the German *R* in Table 3. Solid line = all strains virulent. Dashed line = 1 to 10% of strains are virulent. The graph also indicates the uncertainty of the predicted number of listeriosis cases and from the graph the probability of being less than or equal to the *x*-axis value can be read off (Vose, 1996).

In comparison, the central limit theorem approach is more convenient to use.

The annual number of servings in Sweden was calculated based on the total consumption and the distribution of serving sizes (Tables 3 and 4). This information was used together with the distribution for probability of illness per serving (Table 6), to predict the number of listeriosis cases per year in Sweden. The exponential dose-response model predicted 168 cases per year (range 47 to 2779 cases), which is in reasonable agreement with 37 listeriosis cases reported on average per year (Arneborn, 1997). The Weibull–Gamma model predicted 95 000 listeriosis cases per year (range 34 000 to 1.6×10^{6}, Table 8). In comparison, if only a fraction, between 1 and 10%, of the strains are virulent the predicted mean number of annual listeriosis cases decreases to nine (GR) and 5202 (WG, high-risk group), respectively (Table 8). The effect of the assumption of the proportion of virulent strains on the number of predicted listeriosis cases is illustrated in Fig. 10. The cumulative frequency distribution graph also indicates the uncertainty about the predicted number of listeriosis cases estimated by the exponential dose-response model (German *R*, Fig. 10). For instance, under the assumption that all strains are virulent there is 80% probability that the number of annual cases is less than 200 (solid line, Fig. 10).

For a comparison, P_{ill} and the annual number of expected listeriosis cases were also calculated using point estimates of the parameters in the model in Fig. 1. The point estimates chosen were either the mean

Table 8
The number of listeriosis cases per year in Sweden caused by consumption of smoked or gravad salmon/rainbow trout as estimated with the risk assessment model and two different dose response models, the influence of the proportion of virulent strains is also shown

	All strains virulent			1 to 10% of strains virulent[a]		
Model	Mean	Minimum	Maximum	Mean	Minimum	Maximum
German *R*	168	47	2779	9	<1	152
WG (high-risk)	95 000	34 000	1.6×10^{6}	5202	1884	86 000
WG (low-risk)	48 000	17 000	781 000	2619	911	43 000

[a] The proportion of virulent strains was assumed to vary uniformly between 1 and 10%.

Table 9
A comparison between the estimated probability of illness per serving, P_{ill}, and the number of expected listeriosis cases calculated by describing the parameters of the model in Fig. 1 either by distributions (Monte Carlo simulation) or by point estimates

Model	P_{ill}			Listeriosis cases per year		
	MC	Point estimate		MC	Point estimate	
	Mean	Mean	Worst case	Mean	Mean	Worst case
German *R*	2.8×10^{-5}	6.4×10^{-8}	5.1×10^{-3}	168	<1	22 000
WG-high	1.6×10^{-2}	1.9×10^{-5}	0.24	95 000	83	1×10^{6}
WG-low	2.0×10^{-3}	1.0×10^{-9}	0.18	48 000	<1	3×10^{6}

of each parameter or the value giving the worst case scenario, i.e. the maximum value (Table 9).

7. Discussion

It is the frequency of the high probability events more than the mean or the median that determines the acceptance of a process or a product and these events are often the consequence of several parameters being on the margins (Whiting and Buchanan, 1997).

Therefore, the use of point estimates, e.g. the mean or median, of parameters determining the probability of an adverse effect can not give a complete assessment of the risk (Whiting and Buchanan, 1997). Further, the use of the mean, median or worst case, to calculate a point estimate of the probability of different scenarios, illustrated in Table 9, has been criticised for leading to large errors (Cassin et al., 1996). Consequently, in this work an approach in which parameters were described as distributions was chosen and Monte Carlo simulations of the risk assessment model were performed. This made the determination of the frequency of different probability events feasible in contrast to the use of point estimates (Table 9).

The results of any risk assessment should be interpreted carefully and are valid only as much as the data and assumptions made in developing the model are valid. With the limited amount of information available on the prevalence and levels of *L. monocytogenes*, it was decided to treat packaged salmon/rainbow trout as one hazard whether gravad, cold- or hot-smoked. Admittedly, the possibility for *L. monocytogenes* survival during processing is better in gravad and cold-smoked fish, but on the other hand the potential for growth in the case of recontamination may be better in hot-smoked fish. Loncarevic et al. (1996) suggested that this product could be of public health concern since in their survey of these types of fish the highest concentration of *L. monocytogenes* was found in hot-smoked salmon. The distribution for prevalence and concentrations were based on the analysis of samples stored at 4°C after purchase. This is not representative of the variable storage temperatures that these products can be expected to experience in the homes of consumers, and this could possibly lead to an underestimation of both the prevalence and the levels of *L. monocytogenes*. The distributions of these parameters were described by empirical distributions, the cumulative distribution, since the use of a theoretic or parametric model would be hard to justify with our limited knowledge on the prevalence and levels of *L. monocytogenes* (Vose, 1996).

Dose-response relationships are lacking for *L. monocytogenes*. In the present study, two previously published models were compared (Farber et al., 1996; Buchanan et al., 1997; Bemrah et al., 1998). The approach using the exponential dose-response relationship which resulted in numbers that are in reasonable agreement with the number of reported listeriosis cases has been characterised as giving a conservative estimate, i.e. overestimating risks (Buchanan et al., 1997). In comparison, the Weibull–Gamma model (Farber et al., 1996), appeared to be even more conservative and predicted many more listeriosis cases than reported in Sweden (Table 8). Thus, it is possible that the use of the conservative dose-response models, lead to an overestimation of the risks and of the contribution of smoked and gravad salmon/rainbow trout as a source of listeriosis cases in Sweden. However, it should also be kept in mind that the extent of underreporting of listeriosis in Sweden is unknown and that the reported cases in Sweden all represent cases with serious symptoms (Arneborn, 1997). In the WG-model the dose causing illness in 10 and 90% of a low-risk and high-risk human population, respectively, is 10^7 cfu (Farber et al., 1996). In mice, a similar dose causes infection, not necessarily illness, in approximately 90% of those exposed to *L. monocytogenes* but no infection at all in mice with a history of previous exposure (Notermans et al., 1998). It is interesting to note the great effect of previous exposure in light of the probable frequent exposure to *L. monocytogenes* through food (Hitchins, 1996; Notermans et al., 1998). However, caution must be exercised when extrapolating dose data obtained in animal studies to humans, especially when the selected endpoints, e.g. infection and illness, are different.

A sensitivity analysis indicated that P_{ill} was most sensitive to the input distribution describing the level

of contamination, and to a lesser degree to the distributions of prevalence, serving size and the proportion of virulent strains. Thus, focus of future studies should be to collect data on these variables to reduce the uncertainty in the estimation of the variability of the levels of *L. monocytogenes*, and the uncertainty in the estimation of the prevalence or the proportion of virulent strains. It should be noted that the parameters in the dose-response relationships were not included in the sensitivity analysis since they were considered as constants, not variables. However, when more data becomes available it should be possible to represent the uncertainty and variability in these parameters using a Monte Carlo approach (Buchanan et al., 1997).

It is a matter of discussion what constitutes a high probability event. Whiting and Buchanan (1997) stated that outbreaks appear to occur when probabilities exceed 10^{-8} to 10^{-4}. Using the data estimated by the German *R* model the probability of illness per serving exceeded 10^{-8} in 51% and 10^{-4} in 4% of the iterations (Fig. 9). The maximum tolerable concentration of *L. monocytogenes* in some ready-to-eat foods intended for healthy persons have been suggested as 100 cfu g^{-1} (Anonymous, 1994; Qvist, 1996). Assuming a serving size of 100 g, this corresponds to a dose of 10^4 cfu. Using the exponential dose-response relationship and the German *R* the probability of illness per contaminated serving becomes $PI = 1 - e^{-1.1E-10*1E4} = 1.1 \times 10^{-6}$, or stated differently one case per approximately 900 000 contaminated servings. That probability is approximately one order of magnitude smaller than the mean probability of illness per serving, P_{ill} (Table 6), and two orders of magnitude smaller than the probability per contaminated serving, *PI*, estimated in this work (data not shown).

The development of a quantitative risk assessment model, although simplified and partially incomplete, can be a helpful tool to evaluate the relationship between risk and factors which may be used to mitigate risk (Whiting and Buchanan, 1997; Cassin et al., 1998). For instance, the sensitivity analysis indicated the importance of the level of *L. monocytogenes* in determining the probability of illness. Assume that we want to reduce the probability of illness per serving to less than 10^{-7} or 10^{-8}, based on the results shown in Fig. 9. What levels of *L. monocytogenes* may give rise to risks of this order of magnitude? A scenario analysis indicated that the minimum level of *L. monocytogenes* within iterations resulting in a probability of illness greater than 10^{-7} or 10^{-8} was 25 or 2 cfu g^{-1}, respectively. Under the condition that the model is valid, these would be the maximum acceptable contamination levels at the time of consumption considering these tolerable risk levels.

The input parameters to the risk assessment model were described as distributions and, consequently, the output of the model was also a distribution of values. Burmaster and Anderson (1994) suggested that each output distribution should be accompanied by a graph indicating the 10^{-4}, 10^{-6} and point estimates of the output variable, as well as a table of the mean, the standard deviation, the minimum, the 5th percentile, the 95th percentile, and its maximum value. However, the interpretation and understanding of a distribution of probabilities may not be straightforward and it can be a matter of argument how to best convey the results of a risk assessment without losing information. One way to communicate the significance of the estimated probability distribution is to estimate the cumulative risk based on a typical frequency of exposure or the expected number of annual listeriosis cases based on total consumption (Tables 7 and 8). In accordance with the central limit theorem, the so derived risk estimates are approximately normally distributed with the mean largely determined by the mean of the parent probability distribution. For instance, using the mean or the median of the probability of illness (German *R*, Table 6), respectively, as a point estimate of risk, the cumulative risk for 12 exposures in 1 year becomes 1.3×10^{-7} (median) or 3.4×10^{-4} (mean). The latter is much closer to the estimated mean cumulative risk than that based on the median (Table 7). Since in many cases the predicted probability ranges over several orders of magnitudes, the mean may be heavily influenced by a few iterations resulting in high probability of illness. Therefore, it may be preferable to report the result as a cumulative frequency distribution (Fig. 10), from which the probability of different scenarios can be read directly (Vose, 1996), rather than reporting only the mean or the median and perhaps the range of the derived estimates.

8. Conclusion

The choice of dose-response model had a significant impact on the magnitude of the risk estimates. The Weibull–Gamma model predicted greater risks than the exponential model, in which *R*, the constant specific for *L. monocytogenes*, was estimated from German consumption and epidemiological data. Thus, a lack of dose-response data was apparent. The most important parameter determining the risk was the concentration of *L. monocytogenes* at the time of consumption. More quantitative data on the presence and levels of *L. monocytogenes* as well as on the proportion of virulent strains in these fish products is needed to improve the current risk assessment.

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