# *Listeria monocytogenes* on smoked salmon: a case study to evaluate the suitability of available Belgian data for exposure assessment

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## 1. Localisation of the hazard in the food chain

*Listeria monocytogenes* is a Gram positive bacterium that is ubiquitous in the environment and may cause human listeriosis. Consumption of contaminated food is the most important cause of listeriosis. *L. monocytogenes* is one of the leading microbiological causes of food recalls as reported to the EU Rapid Alert System for Food and Feed, mainly associated with animal products[1]. The foods that are most often involved with listeriosis are soft cheeses (based on raw milk), milk products, deli meats, smoked fish (salmon, halibut), salads and in a more general way refrigerated ready-to-eat products with an extended shelf life that are consumed without prior heat treatment[2].

The current case study focuses on smoked salmon produced in Belgium. The results of the Belgian FASFC (Federal Agency for the Safety of the Food Chain) control programme (period 2002-2006) showed that the prevalence of *L. monocytogenes* on smoked salmon is rather high (19.4 %). It is reported in the literature that the levels of *L. monocytogenes* in the smoked salmon are generally low[3].

Smoked salmon is made from raw fillets that are brined or injected with salt. Different routes for contamination of the smoked salmon are possible: it can already be contaminated during catch or aquaculture, but can also be contaminated during production steps like salting, slicing and packaging[4]. Due to product characteristics, smoked salmon can support the growth of *L. monocytogenes*[5].

## 2. Reference terms

In the framework of the FASFC control programme, yearly a large number of analyses are performed. This concerns biological parameters like *Salmonella spp., L. monocytogenes, Staphylococcus aureus, Bacillus cereus* but also chemical parameters like dioxins, PCB's, heavy metals, mycotoxins and animal diseases (e.g. tuberculosis). In 2004 a Belgian survey on the consumption habits of the Belgian population (age 15-99 years), was performed[6].

The current case study focuses on the microbiological hazard *L. monocytogenes* on smoked salmon. The motivation for the choice of the case study '*L. monocytogenes* on smoked salmon' was that several studies identified cold-smoked fish as a high risk product for listeriosis[7,8] and also because a set of data was available from the FASFC control programme.

Data were used from the FASFC database containing information on the prevalence and contamination level of *L. monocytogenes* on smoked salmon, and from the Belgian National Food Consumption Survey with information on the consumption frequency and the serving size. The case study deals with the following key questions: 'How suitable are the above mentioned data to perform a probabilistic exposure assessment ?' and 'What recommendations can be made in order to make similar (future) data more appropriate for future exposure assessments?' (Figure 1).

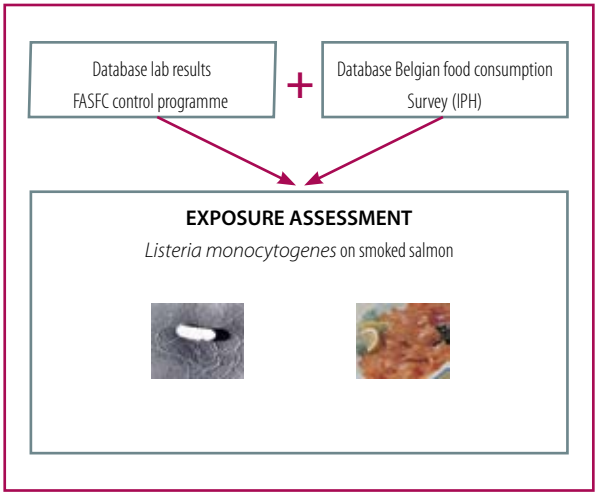


***Figure 1.*** *Schematic representation of the use of the databases in the case study.*

# 3. Risk assessment

## Hazard identification

*Listeria monocytogenes* is potentially pathogenic for humans and may cause listeriosis. This infection can be life-threatening for at risk populations like immuno-compromised persons, the elderly people and pregnant women[9]. Most European countries have an annual incidence of human listeriosis between one and ten reported cases per million inhabitants[10]. The number of reported listeriosis cases in Belgium (ca. 10 million inhabitants) during the years 2002 to 2006 was respectively 44, 76, 89, 62 and 67[11]. Although the incidence of listeriosis in comparison with campylobacteriosis and salmonellosis (respectively 5571 and 3693 cases in 2006[11]) is low, listeriosis is, seen its high mortality rate (20-30 %), an important zoonotic pathogen. Several scientific publications suggest that the principal contamination in cases of listeriosis is foodborne[12,2].

The above mentioned information in combination with the ability of *L. monocytogenes* to multiply at refrigerator temperatures in certain foods like smoked salmon or cheese and its high persistence in food-processing environments necessitates a special attention for this pathogen.

## Hazard characterisation

According to the Codex Alimentarius a hazard characterisation is "the qualitative and/or quantitative evaluation of the negative consequences that can be associated with the hazard".

Several research groups developed dose/response models for establishing the relationship between the ingested dose of *L. monocytogenes* and the probability of illness. The probability of illness from consuming a specified number of *L. monocytogenes* depends mainly on the virulence of the strain and the susceptibility of the consumer.

The Joint Expert Committee on Microbiological Risk Assessments (JEMRA) compared a number of published dose/response models and concluded that the predictions in the dose region corresponding to levels commonly found in food differ widely[13].

Three of these models were used in the case study to estimate the number of Belgian listeriosis cases due to consumption of smoked salmon (see part risk characterisation).

## Exposure assessment

The purpose of the exposure assessment is to make a quantitative estimation of the probable intake of *L. monocytogenes* per serving of smoked salmon by a Belgian consumer. An exposure assessment can be performed by a deterministic approach using point estimations of the input parameters e.g. the average or the worst case but also by a probabilistic approach, using distributions of the input parameters.

In the presented case study, a probabilistic approach for estimation of the exposure of *L. monocytogenes* per serving of smoked salmon was used by means of a model that was run with the software @RISK (version 4.5.5., Palissade Corp.) As input for the model, two probability distributions were required:

i) A probability distribution of the contamination level of *L. monocytogenes* on smoked salmon (number of colony forming units (cfu) per gram of smoked salmon) ;

ii) A probability distribution of the weight per serving (g smoked salmon/serving).

The output of the simulation was also a probability distribution representing the exposure of the consumer to *L. monocytogenes* per serving of smoked salmon.

### *Elaboration of the probability distribution of the contamination level of L. monocytogenes on the smoked salmon*

To elaborate this distribution, the following test results from the database of the FASFC were used:

1. 576 results (2002-2006) of the test "detection of *L. monocytogenes* in 25 g smoked salmon". For this qualitative test, the test result is either 'absent' (≈ '<0.04 cfu/g') or 'present' (≈ '≥0.04 cfu/g'). The smoked salmon was sampled at the production sites and the analyses were performed immediately after sampling. The prevalence was 19.4 %.

2. 209 results (2002-2005) of the test "detection of *L. monocytogenes* in 0.01 g smoked salmon". For this qualitative test, the test result is either 'absent' (≈ '<100 cfu/g') or 'present' (≈ '≥100 cfu/g'). The smoked salmon was sampled at the production site and the tests were performed at the end of the shelf life of the smoked salmon (after cooled storage at 4 °C/7 °C ). The prevalence was 4.8 % (≈ 4.8 % ≥100 cfu/g). On these 209 smoked salmon samples, the analysis 'detection in 25 g' was also performed immediately after sampling, representing 209 results of

the group of 576 results mentioned above. Thirty-seven of these 209 analyses (17.8 %) were positive (≈ 17.8 % >0.04 cfu/g).

The above mentioned data contain some shortcomings as input data for elaboration of a probability distribution describing the contamination level of *L. monocytogenes* on the smoked salmon:

i) It concerns qualitative data that, when combined (209 samples), allow to classify the samples in three categories corresponding with a different contamination level range : 82.2 % <0.04 cfu/g , 13.0 % between 0.04 and 100 cfu/g and 4.8 % ≥100 cfu/g. However for a quantitative exposure assessment this approach is not sufficient. To complete the assessment, it was assumed that the growth of *L. monocytogenes* on smoked salmon was limited to maximal 105 cfu/g smoked salmon. Further a quantitative distribution for the contaminated samples (≥0.04 cfu/g) was obtained after fitting the (three cumulative) data (points (0 %, 1 cfu/g; 73.0 %, 100 cfu/g; 100 %, 105 cfu/g) to an exponential curve. This choice was based on the following expert opinion (assumption) of the FASFC Scientific Committee Microbiology working group: the smoked salmon that have ≥0.04 cfu *L. monocytogenes*/g are more probably contaminated at a low level than at a high level and the curve of this probability (in function of the contamination level) follows an exponential decreasing shape.

ii) Another shortcoming of the data concerns the combining of qualitative data ('detection in 25 g' and 'detection in 0.01 g') obtained at two different points in time, respectively at the 'end of production' and at the 'end of the shelf life'. Ideally only qualitative results obtained at one moment in time should be combined. For this, it was assumed that the results of the analyses 'detection in 25 g' determined at the point in time 'end of production' are equal to the results that hypothetically would have been obtained for the same analyses performed at the point in time 'end of the shelf life'. It was also assumed that 'the end of shelf life' equaled the moment of consumption for the consumption of all the smoked salmons by the consumer.

This implicates that the potential growth of *L. monocytogenes* on the smoked salmon during refrigerated storage (doubling time between 40 and 49 h at 5 °C[14,13]) for one part of the data, (the analysis results 'detection in 25 g') was not taken into account. Also the growth due to possible temperature abuse during storage in the distribution or by the consumer was not taken into account in the case study. This can possibly lead to an underestimation of the risk. On the other hand, the assumption that 'the end of shelf life' equaled the moment of consumption for all smoked salmons may imply an overestimation of the risk. Indeed most foods are consumed on the day of purchase or shortly after it whereas likely only a limited part remain in the home refrigerator till the end of the shelf life before consumption.

Figure 2 presents the probability distribution of the contamination level of *L. monocytogenes* on smoked salmon after application of the above-mentioned assumptions on the FASFC data. It should be stressed that the application of these assumptions has a considerable impact on the uncertainty of the distribution[15].

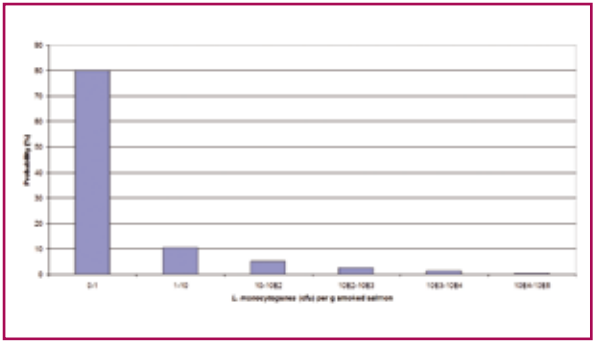

***Figure 2.*** *Probability distribution for the contamination level of L. monocytogenes on smoked salmon.*

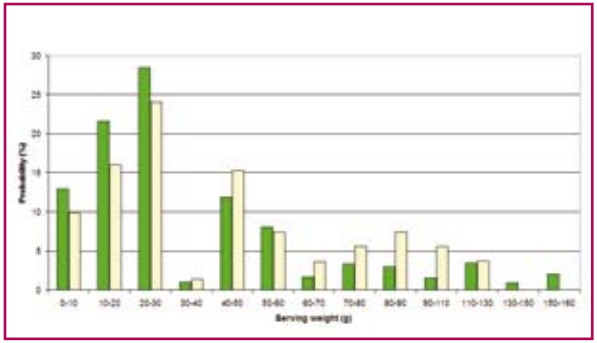

***Figure 3.*** *Probability distribution of the serving size of smoked salmon for the age groups 15-59 years (green) and 60-99 years (yellow).*

### *Elaboration of the probability distribution of the weight per serving of smoked salmon*

For the elaboration of the probability distribution of the weight of the servings of the smoked salmon, data were used from the Belgian National Food Consumption Survey executed in 2004 by the Scientific Institute for Public Health[6]. The survey concerned 3245 consumers older than 15 years, who were interviewed twice (two nonconsecutive days) to determine what they consumed during the last 24 hours. The database contains information on 114 consumed servings of smoked salmon and also on the age of the consumers.

Quantitative data for a total of 114 servings of smoked salmon were available. A division was made representing two age groups: 15-59 years and 60-99 years. The average serving size for these two groups is respectively 38 g (n= 61) and 44 g (n= 53). The data are quantitative and it was evaluated that, although rather limited in number, these data were suitable as input for the elaboration of a quantitative distribution (Figure 3).

After performing Monte Carlo Simulations (100000 iterations), using the two above mentioned distributions for the contamination level and consumption size (Figure 2 and Figure 3) as input for the exposure model, a probability distribution for the exposure of *L. monocytogenes* per serving size of smoked salmon was obtained (Figure 4).

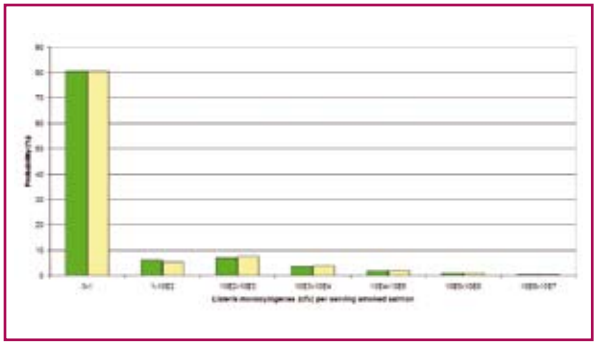

***Figure 4.*** *Probability distribution for the exposure of L. monocytogenes per serving of smoked salmon for the age groups 15-59 (green) and the age groups 60-99 years (yellow).*

Table 1 represents the percentiles for this distribution for the age groups 15-59 years, 60-99 years and 15-99 years. Taking into account the uncertainty linked to the input data and the small difference between the exposures per serving for the different age groups, it can be concluded that the differences in exposure between the age groups are not significant.
For the population 15-99 years, the output of the simulations shows that for 85 % of the servings the exposure is less than about 75 cells of *L. monocytogenes*. Five percent of the servings implicate an exposure per serving more than 2500 cells of *L. monocytogenes* and 1 % of the servings implicate an exposure of more than 170000 cells of *L. monocytogenes*.

***Table 1.*** *Percentiles for the exposure assessment: number of L. monocytogenes per serving of smoked salmon.*

| Percentiles (%) | 15-59 years | 60-99 years | 15-99 years |
|---|---|---|---|
| 10 | 0 | 0 | 0 |
| 80 | 0 | 0 | 0 |
| 85 | 68 | 83 | 75 |
| 90 | $2.7 \times 10^2$ | $3.2 \times 10^2$ | $3.0 \times 10^2$ |
| 95 | $2.3 \times 10^3$ | $2.7 \times 10^3$ | $2.5 \times 10^3$ |
| 97.5 | $1.7 \times 10^4$ | $1.9 \times 10^4$ | $1.8 \times 10^4$ |
| 99 | $1.6 \times 10^5$ | $1.8 \times 10^5$ | $1.7 \times 10^5$ |

## Risk characterisation

In order to estimate the number of Belgian listeriosis cases due to the consumption of smoked salmon, the output from the exposure assessment describing the exposure per serving of smoked salmon (population 15-99 years), was used as an input for 3 different published exponential dose/response models : Lindqvist & Westöö[12] Buchanan et al.[16] and WHO[13].

The three models are single hit exponential models with the underlying assumption that one single *L. monocytogenes* can cause disease. The three models differ in the value for the parameter R (the probability that a single cell will cause invasive listeriosis), reflecting different assumptions for the virulence and host characteristics. It concerns three models for the high risk population. It was estimated that about 20 % of the Belgian population (2 million persons) belongs to the high risk population for listeriosis[12].

The number of yearly consumed smoked salmon servings was estimated from the Belgian Consumption Survey : 6.6 million consumed servings per million persons (age group 15-99 years). For this, the assumption was made that the numbers of servings of smoked salmon measured on two days could be extrapolated to the whole year and that the eaters of the smoked salmon on these two days represent the eaters of the smoked salmon over 365 days.

Monte Carlo Simulations with 100 000 iterations on the three models were applied. The output of the simulation indicated an estimated yearly number of listeriosis cases for the immuno-compromised persons (2 million), due to the consumption of smoked salmon, of 86, 13 and 1, using respectively the models of Lindqvist & Westöö[12], Buchanan et al.[16] and WHO[13].

As mentioned above, smoked salmon is one of the ready-to-eat products in Belgium for which the prevalence of *L. monocytogenes* is high (19.4 %). The output of the risk characterisation, based on Belgian data with the contamination level of *L. monocytogenes* and consumption data, suggests that it is likely that consumption of smoked salmon contributes to the disease burden caused by *L. monocytogenes*.

However, it should be mentioned that smoked salmon is only one of the foods where *L. monocytogenes* can occur in a high prevalence: the prevalence in other foods such as minced meat (42 %), smoked halibut (33 %) and tuna salad can also be quite high[17].

The estimated number of listeriosis cases varied about 100-fold according to the used dose/response model. This reflects the large uncertainty linked to these models. Despite this difference, and also taken into account the assumptions, it can be observed that the estimated numbers due to the consumption of smoked salmon are in the same order of magnitude as the total number of reported cases of listeriosis in Belgium: 67 reported cases of listeriosis in Belgium in 2006.

## 4. Obtained targets and recommendations

In the case study, data from the FASFC database and data from the Belgian National Food Consumption Survey were used and evaluated for their suitability as input data for the probabilistic exposure assessment "*Listeria monocytogenes* on smoked salmon". Subsequently to this analysis also a risk characterisation was performed in order to estimate the number of listeriosis cases due to consumption of smoked salmon.

It was shown that after the application of a number of assumptions, data from the FASFC and data from the IPH Belgian National Food Consumption Survey can be used as input data for performing a probabilistic exposure assessment. No specific shortcomings of the consumption data of smoked salmon from the IPH consumption survey were identified for elaboration of a probability distribution of the serving size. For the contamination data corresponding with the level of *L. monocytogenes* on smoked salmon, it is recommended to dispose of quantitative data (enumerations) instead of qualitative data (presence/absence testing), obtained at one time moment e.g. production or retail.

In the framework of a food control agency, analyses are performed in order to check compliance with the legislation. The test results that were used in this case study were therefore not specifically obtained with the aim of performing a quantitative risk assessment. As mentioned above the shortcomings of the data concern the disposal of qualitative data instead of quantitative data and the different points in time for the analyses.

From 2006 on, the European Community Regulation (EC) No 2073/2005 on microbiological criteria came into force. This regulation requires for *L. monocytogenes* in ready-to-eat foods compliance with two food safety criteria: absence in 25 g at "the end of production" and contamination level <100 cfu/g "during the shelf life". In the FASFC control programme for 2007, analyses were performed to check compliance with this regulation and this for different ready-to-eat foods like smoked salmon, sliced meat and cheese products.

Although still not ideal, these results are more suitable as input data for risk assessment than the data used in this case study. The analyses planned by FASFC for checking compliance with the criterion "<100 cfu/g" at the distribution concern enumerations and will thus result in quantitative results. The results of the analyses required to check compliance with the criterion "absence in 25 g" remain qualititative.

Also the disadvantage of disposing of data at two different points in time ('end of production' and 'during shelf life') remains.

For a food control agency, it would be useful to rank the different food products according to the risk (exposure) for the consumer[7]. This information can then subsequently be used for adoption of the FASFC control programme, aiming to protect the consumer's health in a maximal way. The study presented here forms the methodological basis for this more extended ranking.

## 5. Samenvatting

In het kader van het controleprogramma van het Federaal Agentschap voor de Veiligheid van de Voedselketen (FAVV), worden jaarlijks een groot aantal analyses uitgevoerd. Het betreft biologische parameters zoals *Salmonella spp., Listeria monocytogenes* en *Bacillus cereus* maar ook chemische parameters zoals dioxines, PCB's, zware metalen, mycotoxines en dierenziekten (bv. tuberculose). In 2004 werd een voedselconsumptiepeiling uitgevoerd voor de Belgische populatie (leeftijd 15-99 jaar) door het Wetenschappelijk Instituut voor Volksgezondheid (WIV).

De hierboven beschreven gevalsstudie focust op het microbiologische gevaar *L. monocytogenes* op gerookte zalm. Data werden gebruikt van de FAVV-databank die informatie bevat over de prevalentie en het contaminatieniveau van *L. monocytogenes* op gerookte zalm en van de databank voor de Belgische voedselconsumptie.

Er werd onderzocht hoe geschikt deze data zijn om een probabilistische blootstellingsschatting uit te voeren alsook welke aanbevelingen er kunnen gemaakt worden om gelijkaardige (toekomstige) data beter geschikt te maken voor toekomstige blootstellingsschattingen.

Geen specifieke tekortkomingen voor de consumptiedata werden geïdentificeerd voor het opstellen van een waarschijnlijkheidsdistributie van de portiegrootte. Voor het opstellen van een waarschijnlijkheidsdistributie overeenkomend met het niveau van *L. monocytogenes* op gerookte zalm, is de grootste tekortkoming dat er geen kwantitatieve data (tellingen) beschikbaar zijn. Enkel kwalitatieve data (aanwezigheid/afwezigheid) zijn momenteel beschikbaar. De nodige veronderstellingen die met deze tekortkoming gepaard gaan, brengen een aanzienlijke onzekerheid mee voor de blootstellingsschatting. Er wordt aangeraden om, indien het de bedoeling is de analyseresultaten te gebruiken voor een probabilistische blootstellingsschatting, kwantitatieve bepalingen uit te voeren (tellingen) i.p.v. kwalitatieve bepalingen (aanwezigheid/afwezigheid).

Sedert 2006 worden in het kader van het FAVV-controleprogramma volgens Verordening (EG) nr. 2073/2005 voor *L. monocytogenes* in bepaalde kant-en-klaar levensmiddelen twee types analyses uitgevoerd : i) tijdstip productie, bepaling in 25 g (voedselveiligheidcriterium: afwezig), en ii) tijdstip distributie, telling (voedselveiligheidscriterium : <100 kve/g). De gevalsstudie toonde aan dat, mits bepaalde veronderstellingen, deze data, samen met de Belgische consumptiedata kunnen gebruikt worden voor een blootstellingsschatting aan *L. monocytogenes* en wanneer dit voor verschillende levensmiddelen wordt uitgevoerd, laat dit toe

deze levensmiddelen te rangschikken volgens het risico. Deze informatie kan dan vervolgens gebruikt worden voor de eventuele bijsturing van het controleprogramma teneinde de gezondheid van de consument maximaal te beschermen.

## 6. Résumé

Dans le cadre du programme de contrôle de l'Agence Fédérale pour la Sécurité de la Chaîne Alimentaire (AFSCA), un grand nombre d'analyses sont réalisées annuellement. Cela concerne des paramètres biologiques tels que *Salmonella spp., Listeria monocytogenes* et *Bacillus cereus* mais aussi des paramètres chimiques tels que les dioxines, les PCB's, les métaux lourds ou les mycotoxines, et les maladies animales (par exemple, la tuberculose). En 2004, une enquête de consommation alimentaire fut réalisée pour la population belge (âges de 15 à 99 ans) par l'Institut scientifique de Santé Publique (ISP).

L'étude de cas décrite ci-dessus s'intéresse au danger microbiologique *L. monocytogenes* dans le saumon fumé. Des données issues de la banque de données de l'AFSCA, qui contient des informations sur la prévalence et le niveau de contamination de *L. monocytogenes* dans le saumon fumé, et de la banque de données pour la consommation alimentaire belge ont été utilisées.

Il a été examiné dans quelle mesure ces données étaient appropriées pour réaliser une estimation probabiliste de l'exposition et aussi quelles étaient les recommandations qui pouvaient être faites afin de rendre des données similaires (futures) encore plus appropriées pour des estimations futures de l'exposition.

Aucun lacune spécifique au niveau des données de consommation n'a été identifiée pour l'élaboration d'une distribution de la probabilité des grandeurs de portion. Pour l'élaboration d'une distribution de la probabilité correspondant au niveau de *L. monocytogenes* dans le saumon fumé, la lacune la plus importante est qu'aucune donnée quantitative (dénombrement) n'est disponible. Seules des données qualitatives (présence/absence) sont actuellement disponibles. Les hypothèses qui vont de pair avec cette lacune entraînent une incertitude considérable pour l'estimation de l'exposition. Il est conseillé, si l'objectif est d'utiliser les résultats d'analyse pour une estimation probabiliste de l'exposition, de réaliser des déterminations quantitatives (dénombrements) au lieu de déterminations qualitatives (présence/absence).

Depuis 2006, dans le cadre du programme de contrôle de l'AFSCA, deux types d'analyses sont réalisées pour *L. monocytogenes* dans certaines denrées alimentaires prêtes à être consommées suivant le Règlement (CE) n° 2073/2005 : i) au moment de la production, une détermination dans 25 g (critère de sécurité alimentaire : absence) et ii) au moment de la distribution, un dénombrement (critère de sécurité alimentaire : <100 ufc/g). L'étude de cas a démontré, moyennant certaines hypothèses, que ces données peuvent être utilisées ensemble avec les données belges de consommation pour une estimation de l'exposition à *L. monocytogenes* et que, lorsque ceci est réalisé pour plusieurs denrées alimentaires, cela permet de classer ces denrées alimentaires selon le risque. Ces informations peuvent ensuite être utilisées pour l'adaptation éventuelle du programme de contrôle afin de protéger au maximum la santé du consommateur.

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