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Additional Information

Safety assessment of smoked fish related to *L. monocytogenes* prevalence using risk management metrics

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One of the fundamental objectives of European food law is the protection of human health. In this framework, the administration has to ensure that there are control measures from "farm to fork", which maintain product safety in each stage of the food chain. With this in mind, the objective of this paper was to assess the level of safety of smoked fish in relation to *L. monocytogenes* in the early stages of the chain. This was carried out by evaluating the results obtained by the official control of the Valencian region related to the level of implementation of pre-requisites and HACCP. The prevalence of this organism in the industry and the retail stage was also measured. In order to discern whether these values were within the international consumer protection objectives a practical case focusing on smoked salmon was studied. The results showed that the management system in the industry is effective. However, there is a real increase in the prevalence in the samples taken in the supermarket. The ALOP values estimated for smoked salmon indicated that the level of safety achieved is good in a very high percentage of cases, though governments and the different agents in the food chain must continue working to improve and attain new safety goals.

1   Introduction

The quest for a high level of protection for human life and health is one of the fundamental objectives of European food law (Regulation (EC) No 178/2002). An integrated approach is necessary to ensure food safety "from farm to fork". Consumers, primary producers, the agro-food Industry and the administration are the agents involved in the definition of food quality and safety requirements (Doménech et al., 2007).

The administration is in charge of ensuring the citizens' well-being. In this case, governmental controls are one of the main tools available to control risks, implementing quality systems and meeting minimum requirements that ensure consumers' health. Food safety management systems such as HACCP or pre-requisites like: good hygiene and manufacturing practices, appropriate cleaning, sanitation programs, are required by the governmental authorities for the prevention or inhibition of the growth of pathogens (van Schothorst et al, 2009; Gorris, 2005). However, pre-requisites and HACCP are specific to each factory and do not directly link the effectiveness of control measures that are critical for safety with an expected level of health protection. Also "traditional metrics" such as microbiological criteria (MC) are used in order to provide information about the level of stringency expected in

a food safety control system and verify that this level of control is being achieved. However, they are not enough to define the level of control that industry should to achieve.

During the past decade, there was an increasing interest in developing tools to link the requirements of food safety programs with their expected public health impact (Codex, 2007). To advance in risk management, new food safety risk management metrics such as Appropriate Level of Protection (ALOP), the Food Safety Objective (FSO) and Performance Objective (PO), have emerged (Buchanan & Appel, 2010). One difficulty when implementing the ALOP concept is that the ALOP may not be described in terms that can be used by the food industry or government regulatory agencies to set a target for food safety systems, for example, the ALOP may be described as a reduction in illnesses, whereas industry or government need a target based on the number of microorganisms in a food. The International Commission on Microbiological Specifications for Foods (ICMSF, 2002) has proposed the establishment of FSO to provide a link between the ALOP and target points in the supply chain. The FSO defined as ''the maximum frequency and/or concentration of a microbial hazard in a food considered tolerable for consumer protection at the time of consumption'' converts the ALOP into parameters that can be controlled by food producers and monitored by government agencies, (CAC, 2004). FSO can be used by Government regulatory agencies to communicate public health goals to the industry and other stakeholders in a form that can provide a measurable target (Walls & Buchanan, 2005). If the growth of a microorganism is possible/likely during storage and distribution, the FSO must be translated to a PO to compensate for the amount of growth expected between sampling and consumption. The PO is the maximum level (frequency and/ or concentration) of a hazard in a food at a specified point in the food chain. It verifies whether food control measures are effective, and safety is maintained in every stage of the food chain (CAC, 2004). A PO may be the same as the FSO if the frequency/concentration of the hazard stays at the same level between the point at which the PO is established and consumption; otherwise, Codex indicates that the PO can be more or less stringent than the FSO according to the likelihood of the hazard to increase or decrease between the PO and consumption.

The objective of this paper was to assess the level of safety of smoked fish in relation to *L. monocytogenes* in the early stages of the chain i.e. industry and retail. With this aim in mind, the results obtained by the official control of the Valencian region related to the level of implementation of pre-requisites and HACCP were evaluated. Moreover, the effectiveness of these management systems were measured taking into account the level of prevalence of this organism in the industry. In addition, this prevalence was also measured in the retail stage, since this is one of the main points inspected by the administration. Finally, in order to discern whether the prevalence values obtained in both stages of the food chain were within the consumer protection objectives proposed by international reference, a practical case focusing on smoked salmon was studied.

2   Materials and methods

2.1.   Items checked
The geographic scope of this research is limited to health department-14 Xativa/Ontinyente, which covers 64 municipalities in the Valencian region

(Spain). The analyzed data correspond to official inspections made from 2007 to 2009. The type of inspection carried out by the Administration depends on the size of the company. Food establishments are classified as Small companies (employing less than 3 workers) or Medium companies (with more than 3 and less than 9). In each year, approximately 1350 Small and 66 Medium companies were surveyed, all of them, subject to monitoring and food control by the Public Administration. In all companies the level of compliance of prerequisites and HACCP were checked considering the following items:

- Food handling: Training and personal hygiene practices must be observed, and legal requirements must be fulfilled.
- Hygiene and Cleaning: The conditions of cleanliness and sanitation of the equipment and supplies. Any failure to comply with the Cleaning and Disinfection Plan is considered a non-conformity.
- Pest control: Regulation or management of any animal perceived to be detrimental to food safety. Conditions of application of pest control procedures should be considered by the companies. For example, a non-conformity will arise when there are no effective measures to combat pests, or they are installed in places that could be considered a hazard to food or staff safety.
- Storage: The conditions under which raw materials, ingredients and products are stored as well as everything related to the packing used in the establishment. A non-conformity is considered, for example, when containers are exposed to the elements or they are near sources of pollution.
- Structure and Design: Design of the food industry plant and equipment in

a way that hygienic conditions are safeguarded. A proximity to pollution sources as well as an unsatisfactory maintenance of industrial facilities such as floors, walls, roofs, gutters, doors and windows will be considered non-conformities.
- Traceability: Proper tracking of raw materials and products, both forwards and backwards.
- Waste control: Ability of the company for the storage and management of its industrial waste. A non-conformity will arise when, the storage of waste is done in such a way as to cause: spread of odours, attraction of insects or contamination of other products or surfaces that come into contact with food. Deviations of the Waste Plan or ineffectiveness and incompleteness of the former are considered non-conformities as well.
- Water supply: Quality of water must be ensured, above all if it comes into contact with the food processing.
- Labelling: Correct labelling of the product in accordance with general and specific legal requirements.
- Processing: Control of the whole production process: Reception of raw materials, processing transactions and handling practices (with emphasis on heat treatment and cooling), wrapping and packaging, including the supervision of each control parameter and its respective measure.

2.2. Non-conformities

The non-conformities or deficiencies observed were classified into three types according to their severity:

- Type I. Deficiencies which involve a minor failure to comply with the rules, but that do not affect the safety of the product.

- Type II. Deficiencies which involve the failure to comply with the rules, and could affect the safety of the product.
- Type III. Deficiencies which involve the failure to comply with the rules, and definitely affect the safety of the product.

2.3. Samples collection

A total of 509 samples of packaged smoked fish were analyzed in two stages of the food chain: a) Fishing industry (258 samples) and retail (251 samples). As shown in Table 1, Smoked salmon (188 samples), smoked cod (27 samples) and other smoked fish such as tuna, anchovy and swordfish (43 samples) were analysed in the industry. As shown in Table 2: Smoked salmon (176 samples), smoked cod (32 samples) and other fish (43 samples) were taken from supermarkets. Samples were collected by the Official Food Control Services of the Department of Health of the Valencian administration between 2002 and 2010. The number and type of samples analyzed in this study were determined by the Valencian health administration according to Regulation (EC) 882/2004 and other information such as consumption data, the risk related to the product, the information from the Rapid Alert System for Food and Feed, and data collected in previous years.

2.4. Sample examination

Samples were examined by official control laboratories, which are accredited by ENAC (the body designated by the Spanish Government to assess technical competence in accordance with international standards) following the standard ISO/IEC 17025: 2005 which describes the general requirements for the competence of testing and calibration laboratories.

The detection of pathogens was performed following NF EN ISO 11290-1 and was counted using NF EN ISO 11290-2. This method involves two selective enrichments in Fraser half and Fraser broth (Biomerieux, Marcy L'Etoile, France). Presence/absence testing of L. monocytogenes in 25 g was performed using the AFNOR validated VIDAS LMO2 method (LMO2; bio-Merieux, Inc., Durham, NC), an enzyme linked fluorescent assay (ELFA) (Biomérieux, Marcy-l'Etoile, France). A positive result must be confirmed following the standard plating procedures using the remaining broth stored at 2–8ºC.

If results were positive an isolate from Fraser broth and ALOA agar was made, and then the confirmation was made with the ADN AccuProbe L. monocytogenes culture identification test (bioMérieux ref. 39500/Gen-Probe Cat. No. 2920).

Microbiological results were interpreted in accordance with microbiological criteria of the official control according to the indications of the Commission Regulation (EC) No 2073/2005). These criteria use the level of bacterial contamination as an indicator of food safety, and classify foods with a L. monocytogenes count of 100CFU/g or more for ready-to-eat food placed on the market during their shelf life as legally unsatisfactory.

2.5. Statistical analysis.

Descriptive analyses of the data were undertaken using Statgraphics 5.0. Relative proportions were compared using the Chi-squared test (X^2) and Fisher's exact test. Also, comparisons of means were made. A probability value of less than 5% was deemed to be significant.

2.6. Suitability of results calculated for cold smoked salmon

To determine if the microbiological values obtained in the industry and at retail complied with the recommended levels of protection, this paper has taken the results of the Joint FAO/WHO expert consultation on development of practical risk management strategies based on microbiological risk assessment outputs (FAO/WHO, 2006), table 1 as reference. Where PO in the industry, PO at retail, FSO and ALOP were calculated based on the following assumptions:

- Mean serving size is 57 g (FDA/FSIS, 2003).
- 95% of the product will be sold within 14 days of production.
- When handled appropriately, the product is maintained at ≤3ºC between the point of manufacture and the time of sale and that 95% of the product will be sold with 14 days of production 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 3ºC, 5ºC and 7ºC, 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).
- The duration of the maximum storage time within the home is affected by the temperature of the home refrigerator. It is assumed that in 95% of the cases storage time is ≤ 14 days in a home refrigerator at 5ºC, whereas this value drops to ≤ 7 days in a home refrigerator at 7ºC.
- The ALOP is calculated by substituting the FSO-serving value in the exponential model according to the formula: $P=1-e^{-r10^{\wedge}(FSO-Serving)}$

3 Results and discussion

3.1. Non conformities

Fig. 1 shows the percentage of type I, II and III non-conformities that were found in the smoked fish industry. Taking into account the severities of the non-conformities, it is important to emphasize that type I non-conformities, which do not involve a hazard to consumer safety, are common to the whole fish industry: small companies had the highest percentage (94%), followed by large (92%) and medium (80%). Type II are much less frequent (6, 8 and 19% respectively), and type III non-conformities, very serious faults, were not found in large companies and are practically nonexistent, not exceeding 1% in medium and small companies. The statistic study showed that these little differences between the size of companies were not significant (p-value=0.9031) in relation to the type of non conformity, nor the item analysed.

Fig. 2 shows the level of compliance of the different items checked in the companies inspected by the administration, specifying in each case if the non conformity is type I, II or III. The item with the highest number of Type I non-conformities is "structure & design" followed by "hygiene & cleaning". However, type II non-conformities were found in the same items but in the opposite order. The items: traceability, food handler, hygiene, labelling, pest control and waste control all had one type III non-conformity each.

3.2. L. monocytogenes prevalence in the smoked fish industry

L. monocytogenes was present in 7 of the 258 samples of smoked fish analysed in the industry, which

represents 2.71% prevalence. Specifically, 1 "Salmon" sample (760 CFU/g), 1 "Cod" sample (96 CFU/g), and 5 samples in the group "Other" (98, 180, 320, 1400, 2300 CFU/g), i.e. the prevalence for the different types of fish was 0.53%, 3.71% and 11.63%, respectively (Table 2). The Chi-squared test showed that these differences in types of smoked fish in relation to prevalence of *L. monocytogenes* were significant (p-value= 0.0005).

Taking into account the load, 2 of the 7 samples contaminated with *L. monocytogenes* were less than 100CFU/g. However, 5 samples, which represent 1.93% of the total number of samples, were higher and consequently exceed the FSO at the time of consumption and involve a real risk to consumers.

3.3. *L. monocytogenes prevalence at retail*

Table 3 shows the prevalence of *L. monocytogenes* in 251 samples of smoked fish analyzed at retail. This microorganism was present in around 25% of the samples: "Salmon" (28.16%), "Cod" (25%) and "Other" smoked fish (24.32%). However, the differences between the three groups were not significant (p-value= 0.8546).

Microbiological quality criteria were acceptable in all cases of cod and the group "Other" (≤100CFU/g). However, 4 samples of salmon, which represent approximately 2% of the samples targeted, were unacceptable (i.e. 500, 7400, 7500 and 15000 CFU/g).

3.4. *Application example of the suitability of results in smoked salmon*

The U.S. Healthy People 2020 initiative, in relation to the level of protection, aimed to reduce the rates of listeriosis by 50 percent, to 2 cases per

million people per year for a base population, for all foods, and all contamination levels (USHP, 2011). Taking into account that smoked salmon consumption in the Valencian region is 140 g per person per year (MAPA, 2009) and considering a serving size of 57g (FAO/WHO, 2006), 2.45 servings per person per year are consumed. That means a probability of listeriosis per serving of $8.16*10^{-7}$. Comparing this value with the assumptions made by the FAO/WHO (Table 1), it can be seen that to achieve this safety objective the PO in the industry has to be 1.01Log(CFU/g), and the PO at retail 1.99Log(CFU/g).

Fig.3 shows the probability that the prevalence of *L. monocytogenes* exceeds the dose at both stages of the food chain. The POs needed to reach the international safety objectives in *L. monocytogenes* are indicated, and the areas representing the values that exceed the POs are highlighted.

The results obtained for *L. monocytogenes* in the final smoked salmon product sampled in the industry showed that 99.47% achieve a PO of 0Log(CFU/g) and 0.53% exceed a PO of 1Log(CFU/g). Moreover, data from the retail stage show a clear increase from 0.53% to 27.84% in the prevalence of *L. monocytogenes* obtained in the industry. Taking into account the level of contamination, the main increase occurred in the group that exceed 1Log(CFU/g) (25.57%). The group with a dose higher than 2Log(CFU/g) was associated with inappropriate practices that could favour the growth of *L. monocytogenes* in samples in which microorganisms had not originally been detected in the company. Finally, a dose of 3Log (CFU/g) or higher was associated with inadequate conditions between the two points of the food chain, which means an increase of the

load in samples already contaminated in the company.

4   Discussion

The authorities should ensure that the industry applies the appropriate GHP and HACCP in order to guarantee food safety. The findings of this paper assessed the level of implementation and effectiveness of these systems. In general, the smoked fish industry has a good level of self-control. Most serious non-conformities are practically non-existent, only in small and medium companies were values of less than 1% found; the items, "structure & design" "hygiene & cleaning" and "food handler" being the source of these non-conformities. These results coincide with those given by Autio et al. (1999) and Dauphin et al, 2001 who concluded that in the processing of smoked salmon the machines are particularly susceptible to contamination, because of the difficulties in efficient cleaning and disinfecting. Rorvik (2000) highlighted that smoked salmon processing involves a lot of handling by workers, as well as the use of technically complex equipment, which makes a systematic implementation of hygienic precautions and the HACCP plan necessary. Also, Di Pinto et al, 2010, showed that the significant presence of *L. monocytogenes* detected in smoked salmon samples may be largely attributed to raw materials and post-processing contamination.

The prevalence of *L. monocytogenes* found in the industry by official controls in the Valencian area was different depending on the species of fish; the average value for all smoked fish being 5.3% (n=258). These results are slightly lower than those found by Dauphin et al 2001 who found 9.53% of *L. monocytogenes* in smoked salmon (n=141). This is a little higher than the 4% found by Vaz-Velho et al., 2001 in the production line of cold-smoked fish.

Recent US data have shown that 4–5% of smoked fish samples were positive for *L. monocytogenes* (n=2800) (Gombas et al. 2003). A Danish study (Jørgensen, Huss, 1998) concluded that there is a large plant-to-plant variation in contamination rate: in some plants all product samples were positive whereas other plants produced products where *L. monocytogenes* was not detected. In relation to the dose, *L. monocytogenes* typically occurs at levels of <10 CFU/g, but is sporadically isolated at higher levels between 10^4 and 10^6 CFU/g.

Prevalence of *L. monocytogenes* found at retail by official control was around 26% (n=251). This finding is similar to other authors such as Uytendaele et al., 2009 who detected a prevalence of 28.8% (n=90). Lower values in smoked salmon were obtained by Van Coillie et al., 2004 with 21% (n=81) positive cases and Dass et al., 2011 with 21.6% (n=120) in cold smoked salmon. Several studies revealed a relatively higher prevalence of 34.15% (n=132) in smoked salmon Di Pinto et al, 2010 and 33% (n=18) in smoked halibut Van Coillie et al., 2004. The wide range of results may possibly be due to the smoking process, the type of fish or storage conditions (Rovik, 2000). Despite the high prevalence of *L. monocytogenes* in smoked fish, the contamination levels were generally below 100 CFU/g. Nevertheless, in the present study 4 samples out of 243 exceeded that limit, all of them being smoked salmon. Similar results were obtained by Uytendaele et al., 2009 (2 halibut and 2 eel out of 90 samples) and Van Coillie et al., 2004 (2 halibut and 1 salmon out of 81 samples). Higher values were found by Gombas et al, 2003 (9 out of 114 samples) and Dominguez et al 2001 (20 out of 170 samples of smoked fish).

The *L. monocytogenes* prevalence found in the final product sampled in the industry and retail reveals a clear

increase of contamination between both stages of the food chain. This growth could be due to the fact that *L. monocytogenes* can multiply considerably in smoked fish during storage at refrigerated temperatures (Rovik, 2000). Faults in transportation (loading and unloading times, hygiene, temperature, etc.) or inadequate conditions of temperature and hygiene at the supermarket are other possible sources of contamination.

The results found by official control in the industry in the Valencian region comply 100% with the international rate of listeriosis recommended by the U.S. Healthy People 2020 initiative (USHP, 2011). Moreover, the increase in the prevalence of *L. monocytogenes* observed at retail showed that 98.31% of the cases remained at the same safety level but 1.69% exceeded recommended levels.

In conclusion, management measures allow us to set specific criteria and see if they are achieved. Their application in this article has shown that the effectiveness of the management system in the industry is correct and that small deviations detected could account for the 0.53% prevalence of *L. monocytogenes* detected in the industry.

Moreover, it has been verified that there is a real increase in the prevalence of *L. monocytogenes* in the stages from the company warehouse until the sampling carried out in the supermarket. This fact highlights the importance of studying what happens, and taking action in the intermediate stages such as transport, platform and cold room in order to maintain food safety.

Finally, although the ALOP values estimated indicated that the level of safety achieved is good in a very high percentage of cases, governments and the different agents in the food chain must continue working together in order to improve and attain new safety goals.

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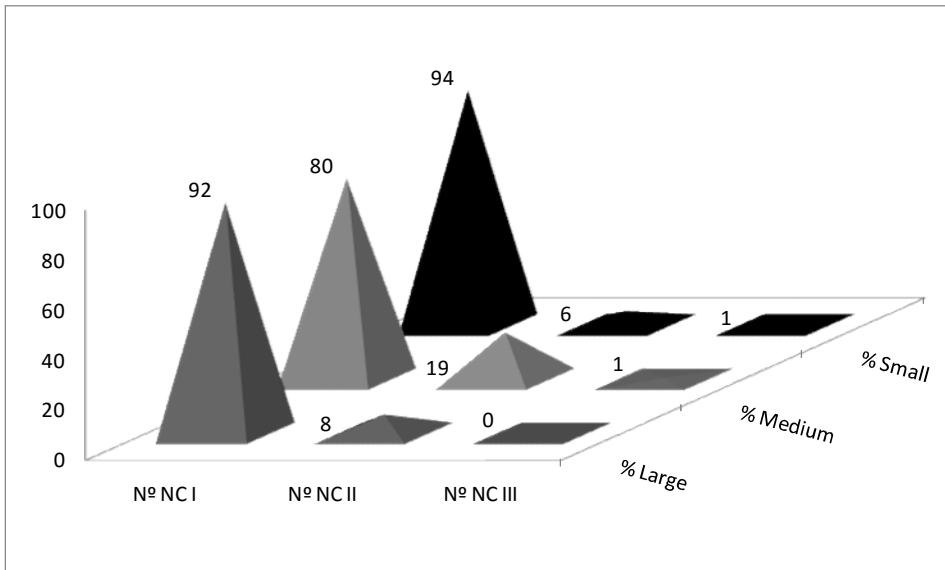
Fig. 1. Percentage of non-conformities of type I, II and III that were found in large, medium and small smoked fish companies
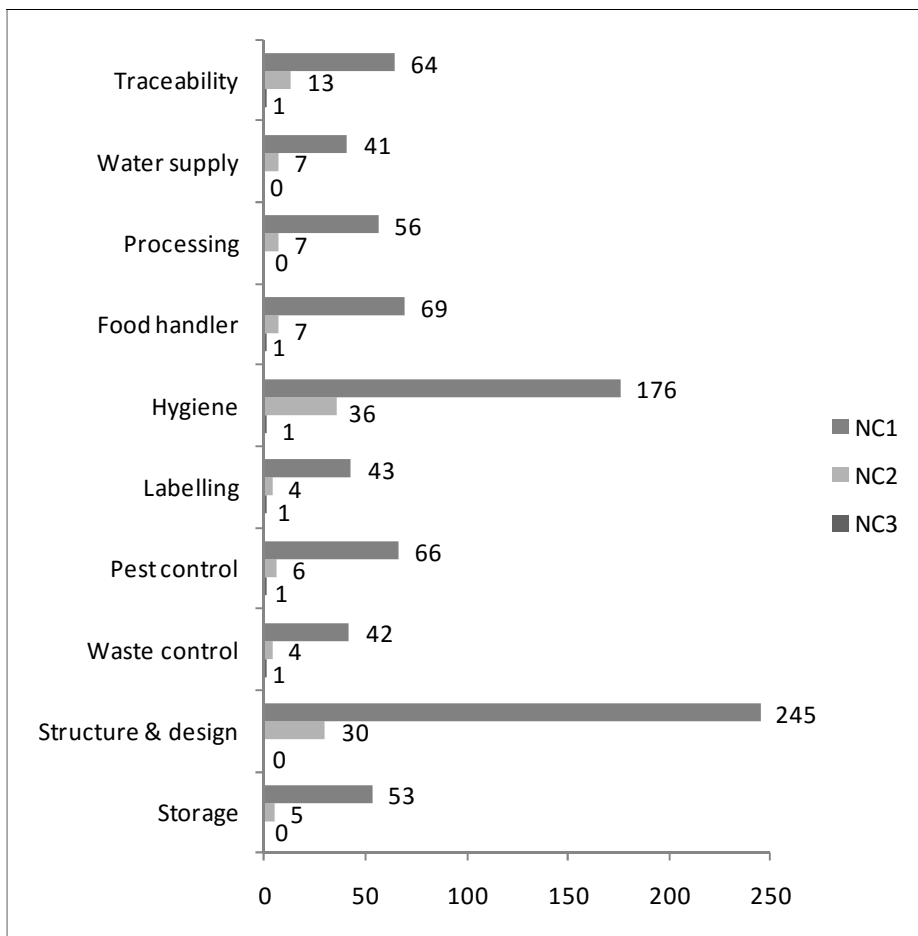

Fig. 2. Number of non-conformities detected in the smoked fish industry in relation to the inspected item
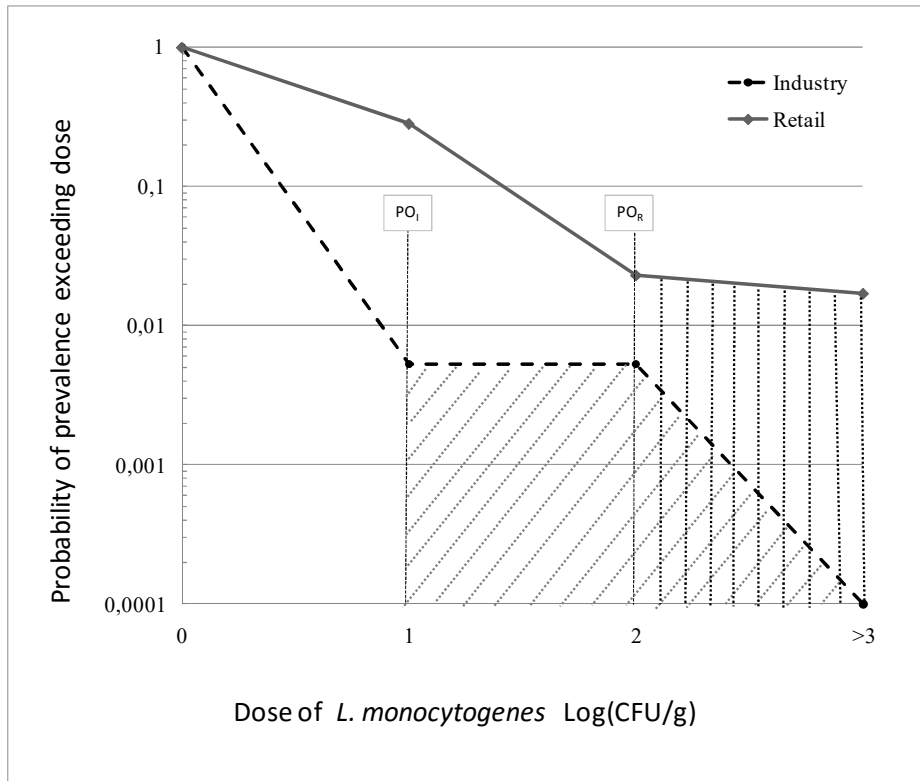
Fig. 3. Probability of prevalence exceeding a dose of *L. monocytogenes*

Table 1. Management metrics applied in smoked cold fish chain (FAO/WHO, 2006)

PO industry Log(CFU/g)	PO retail Log(CFU/g)	FSO Log(CFU/g)	FSO Serving Log(CFU/serving)	ALOP Cases/serving
3.00	3.98	6.11	7.86	7.7×10^{-5}
2.00	2.98	5.11	6.86	7.7×10^{-6}
1.00	1.98	4.11	5.86	7.7×10^{-7}
0.00	0.98	3.11	4.86	7.7×10^{-8}

Table 2. Prevalence of *L. monocytogenes* in the industry with respect to the type of fish

Smoked fish	No. samples	Absence in 25g No. (*)	Presence in 25g No. (%) of samples	No. samples 10-99 CFU/g	No. samples 100-999 CFU/g	No. samples ≥1000 CFU/g
Salmon	188	187(99.5)	1(0.5)	0(0)	1(0.5)	0(0)
Cod	27	26(96.3)	1(3.7)	1(3.7)	0(0)	0(0)
Other	43	38(88.4)	5(11.6)	1(2.3)	2(4.7)	2(4.7)

No. (%) of samples

Table 3. Prevalence of *L. monocytogenes* at retail with respect to the different groups

Smoked fish	No. samples	Absence in 25g No. (*)	Presence in 25g No. (%) of samples	No. samples 10-99 CFU/g	No. samples 100-999 CFU/g	No. samples ≥1000 CFU/g
Salmon	176	127(72.2)	49(27.8)	45(25.6)	1(0.6)	3(1.7)
Cod	32	24(75)	8(25)	8(25)	0(0)	0(0)
Other	37	28(75.7)	9(24.3)	9(24.3)	0(0)	0(0)

No. (%) of samples