Maisons-Alfort, 7 February 2013

The Director General

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

concerning the request to re-assess seafood products posing a risk to pregnant women in the PNNS guide "Nutrition during and after pregnancy"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

The French Agency for Food, Environmental and Occupational Health and Safety (ANSES) received a formal request on 3 April 2012 from the Directorate General for Health (DGS) to re-assess seafood products posing a risk to pregnant women in the National Health and Nutrition Programme (PNNS) guide "Nutrition during and after pregnancy".

1. BACKGROUND AND PURPOSE OF THE REQUEST

Background information provided in the DGS request

The AFSSA Opinion of December 2009 on the increase in the number of cases of listeriosis and the possible link to changes in methods of production, preparation and consumption of food products includes an overview of products identified by various countries as a risk to certain population groups, including pregnant women, with respect to *Listeria monocytogenes.* Among the seafood products of concern, undercooked fish is mentioned along with other products such as taramasalata, tuna salad and peeled shrimps.

In the PNNS guide "Nutrition during and after pregnancy" issued by the French National Institute of Prevention and Health Education (INPES) in September 2007 (section "Prevention of listeriosis and toxoplasmosis), pregnant women are advised not to consume raw shellfish, raw fish (sushi, surimi, taramasalata) and smoked fish (salmon, trout).

According to data from the professional federation, the production process for surimi complies with an AFNOR standard requiring a cooking step followed by a pasteurisation step in the final packaging that destroy heat-sensitive pathogenic bacteria including *L. monocytogenes*. In its request, the DGS mentions a risk of post-pasteurisation contamination.

ANSES was therefore asked to:

- assess the risks to pregnant women related to consumption of surimi;
- determine whether the degree of risk requires a special warning specific to pregnant women concerning this product in the PNNS guide "Nutrition during and after pregnancy";
- determine whether the other foodstuffs cited in the AFSSA opinion on the increase in cases of listeriosis (undercooked fish, taramasalata, tuna salad and peeled shrimps) also require a special warning.

Questions addressed

Pregnant women are potentially susceptible to any infection caused by a foodborne pathogenic microorganism.

The Agency's expert appraisal covers the products mentioned in the formal request regarding the risk related to *L. monocytogenes*.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in compliance with Standard NF X 50-110 "Quality in expertise activities - General requirements of competence for an expertise activity (May 2003)".

The collective expert appraisal was carried out by the Expert Committee (CES) on Food-related biological risk assessment (BIORISK) on the basis of an initial report issued by a group of rapporteurs.

The appraisal took into account the referenced scientific articles and information provided as part of a hearing by representatives of the relevant industry, on the self-monitoring data, the production processes and the physico-chemical properties of the products under assessment.

3. ANALYSIS AND CONCLUSIONS OF THE EXPERT COMMITTEE

I. European regulations on microbiological criteria for *L. monocytogenes* in foodstuffs (Commission Regulation (EC) No 2073/2005, as amended)

The microbiological food safety criteria for *L. monocytogenes* are based on a risk assessment approach. Categories of food with different levels of risk for listeriosis have been defined on the basis of the following criteria: production process, characteristics of use by consumers, types of consumers, and intrinsic properties of foodstuffs concerning possible multiplication of *L. monocytogenes*. This approach results in the establishment of four categories of foodstuffs, in increasing order of risk:

- Non ready-to-eat and ready-to-eat foods for which the probability of contamination is very low: specifically, products that undergo heat treatment in the final packaging or, for the fishery sector, live bivalve molluscs. For this category, the risk of listeriosis is very low and it was not considered necessary to define criteria for *L. monocytogenes.*
- Ready-to-eat foods that do not support the growth of *L. monocytogenes*, other than those intended for infants or for special medical purposes: the European Regulation indicates that products with pH ≤ 4.4 or a_w ≤ 0.92, products with pH ≤ 5.0 and a_w ≤ 0.94 and products with a shelf life of less than 5 days, automatically belong to this category. The risk is higher for this category and the Regulation stipulates a maximum level of contamination of 100 CFU/g.
- Ready-to-eat foods that support the growth of *L. monocytogenes*, other than those intended for infants or for special medical purposes: for these foods that have a higher risk level, the regulatory limit is also 100 CFU/g maximum, until the end of shelf life. However, since these foods enable multiplication of *Listeria*, it may be simpler to demonstrate absence in 25 g at the end of production.
- Ready-to-eat foods intended for infants and ready-to-eat foods intended for special medical purposes: this category is considered to pose the highest risk, and the Regulation requires absence of the pathogen in 25 g for the entire shelf life of the product.

II. Epidemiological data concerning consumption of seafood products and occurrence of cases of listeriosis

Worldwide, nine outbreaks with a confirmed or suspected link to the consumption of seafood have been reported. Most of these outbreaks were small scale. Two of them involved gastro-enteritis. The most recent reported outbreak took place in 2004. Four outbreaks were reported from Northern Europe (three in Finland, one in Sweden) and were related to consumption of gravad, smoked, or cold salted fish, most often sold in vacuum packs.

The other outbreaks that occurred in New Zealand, Australia, the United States and Canada were attributed to the consumption of smoked mussels in two instances, non-specified shellfish in one case, shrimps in one case, and imitation crab meat (surimi) in one case (Table 1).

Table 1: Main characteristics of published listeriosis outbreaks in which seafood was a suspected cause

Country	Suspected products	Level of evidence*	Year	Number of cases, clinical forms	Serotype of strains isolated in patients and food	References
New Zealand	Shellfish – raw fish	DE	1980	22, neonatal forms	1b	(Lennon, *et al.*, 1984)
USA	Shrimps	AE	1989	2 maternal-neonatal forms + 10 non-invasive forms among the 36 partygoers	4b	(Riedo, *et al.*, 1994)
Australia	Smoked mussels	DE, M	1991	3, gastro-enteritis	1/2b	(Misrachi, *et al.*, 1991)
New Zealand	Smoked mussels	DE + M	1991-1992	4, invasive forms	1/2	(Brett, *et al.*, 1998)
Sweden	Vacuum-packed gravad rainbow trout Cold-smoked trout	DE, M	August 1994-June 1995	9, invasive forms	4b	(Ericsson, *et al.*, 1997) (Tham, *et al.*, 2000)
Canada	Imitation crab meat	DE, M	1996	2, gastro-enteritis	1/2b	(Farber, *et al.*, 2000)
Finland	Vacuum packed cold-smoked trout	DE, M	Not indicated Article submitted in 1998	5, gastro-enteritis	1/2a	(Miettinen, *et al.*, 1999)
Finland	Vacuum packed fish	DE, M	1999-2000	10, invasive forms	1/2	(Hatakka, *et al.*, 2000)
Finland	Cold-salted fish products	DE	Nov 2003-Dec 2004	7, invasive forms	1/2a	(Lyytikäinen, *et al.*, 2006)

** DE descriptive epidemiology, AE analytical epidemiology, M microbiology: detection of an indistinguishable L monocytogenes strain in the patients and in the suspected food.*

In France, seafood has not been incriminated in any listeriosis outbreaks to date. A case-control study on the risk factors for sporadic cases of listeriosis carried out in 1997 did not identify the consumption of seafood as a risk factor for this infection.

A relationship with consumption of smoked salmon was strongly suspected in an episode of clustered cases in 2004. The episode involved six cases caused by rare strains of *L. monocytogenes* with the same serotype (1/2a) and the same *Asc*I/*Apa*I pulsed-field gel electrophoresis (PFGE) pulsotypes that occurred between 17 December 2003 and 21 January 2004. The available epidemiological information suggests that the six cases had a common origin (cases grouped in time and caused by a rare strain). The most plausible cause was consumption of smoked salmon served during end of year parties due to the high proportion of consumption by the patients (100%) and a statistically significant relationship between the occurrence of the cases and consumption of salmon shown by a case-control analysis. Moreover, this episode was caused by a strain of serotype 1/2a, rarely related to human cases but often found in smoked salmon. The available information did not however enable identification of the origin of the salmon consumed by the patients. The occurrence of the cases over a short period of time suggests that distribution of the possibly contaminated batches was limited in time, over the month of December.

Consumption of smoked salmon has been also been suspected in two other episodes.

An episode that occurred in 2006 involved five cases caused by strains of *L. monocytogenes* with the same serotype and the same pulsotype, between 26 December 2006 and 12 February 2007. All

five patients had consumed smoked salmon. The consumption period covered the end of year celebrations, when salmon consumption is common. However, the observed proportion of consumption was higher than that found over the same period among 20 cases of listeriosis with a different strain (50%). Information on purchase locations and brands was not suggestive of salmon from the same source.

The other episode, which occurred in 2011, involved five cases caused by strains of *L. monocytogenes* with the same serotype 4b and the same *Asc*I/*Apa*I PFGE pulsotypes (frequent) between 18 June 2011 and 27 August 2011. All five patients had consumed smoked salmon. The proportion of smoked salmon consumers (100%) was very unusual for this time of year (29% of consumers over the same period in the *Listeria* database of the French Institute for Public Health Surveillance (InVS)). The epidemiological data available did not make it possible to identify the source of the salmon consumed by the patients. As the causative strain was very common, it could have been five unrelated sporadic cases.

III. French data comparing the microbiological characteristics of human *L. monocytogenes* strains with strains isolated from seafood products

Hong*, et al.* (2007) compared genetic diversity using PFGE and DNA microarrays among French strains of *L. monocytogenes* isolated from patients (n=179) and those from dairy products (n=21), pork meat products (n=126), and seafood (n=79) isolated by the laboratories of the General Directorate for Competition Policy, Consumer Affairs and Fraud Control (DGCCRF) between 2000 and 2001. They concluded that the foodborne serotypes were primarily of genoserogroup IIa (serovars 1/2a or 3a). Strains in genoserogroup IVb (serovars 4b, 4d or 4e) were more frequently isolated in human cases than in food. The distribution of strains isolated from fish or seafood appear to be very different from those found in pork products. Strains in fish or seafood were mainly of genoserogroup IIa and only 5% were of genoserogroup IVb. This may explain, in part, why outbreaks related to contaminated fish or seafood have not been observed in France (Rocourt*, et al.*, 2000).

The National Reference Centre (NRC) for *Listeria*, the National Reference Laboratory (NRL) for *L. monocytogenes* and the ANSES Laboratory for Food Safety in Boulogne-sur-Mer have pooled their molecular typing results (PFGE with *Asc*I/*Apa*I restriction enzymes) for strains isolated from patients and those from seafood (taramasalata, surimi, shrimps and raw fish) collected since 2005. These results were obtained from typing French strains from self-monitoring tests of producers, Directorate General for Food (DGAL) product alerts[1] and from official monitoring and control plans. Similar patterns showing fewer than two bands difference between profiles were found for the strains isolated from seafood products and from humans (preliminary results). Additional analyses are on-going.

IV. Impact of production processes and possible growth of *L. monocytogenes* in the products of interest

- **<u>Surimi</u>**

Standard NF V 45-068 (2002) describes the production conditions for products derived from fish meat or surimi, commonly known as “surimi”. These products, when sold refrigerated in stick form, grated, etc., are cooked in their final hermetically sealed packaging at a temperature of at least 70°C for 100 minutes, or using equivalent treatment ($F_{70}^{1} \geq 100$). A survey carried out during the Quant’HACCP research programme for microbiological quantitative risk assessment confirmed application of these limits by producers. This pasteurisation eliminates any *L. monocytogenes* present in the raw material, and the hermetically sealed packaging protects the products from recontamination. Discontinuous pasteurisation takes place in autoclaves and continuous pasteurisation in tunnel pasteurisers. In the case of tunnel pasteurisation, monitoring measures must be implemented due to the risk of uneven heat treatment through a bulk effect.

When these products are sold frozen, they do not necessarily undergo cooking in the final packaging but the standard requires that a microbiological control test be performed to verify the

[1] Definition of a “product” alert: non-compliance with the safety criterion defined in Regulation (EC) No 2073/2005 amended <u>or</u> product meeting the definition of an unsafe foodstuff under Article 14 of Regulation (EC) No 178/2002 (situations described in detail in the guide on management of alerts intended for producers in the food chain)

absence of *L. monocytogenes* in five 25 g samples of product. Surimi produced in accordance with the recommendations of this standard carries the labelling “compliant with Standard NF V 45-068” on its packaging. According to information provided by the professional federation, frozen surimi is not intended for direct sale to consumers (inter-company sale) and accounts for a negligible proportion of French production (<1%).

There are few data concerning the prevalence of *L. monocytogenes* in these products. Between 2006 and 2012, no test for *L. monocytogenes* was found to be positive among 65 samples analysed by the Joint Laboratories Service Unit of the DGCCRF and the General Directorate for Customs and Indirect Taxation (DGDDI).

In the event of contamination after opening of the packaging or during preparation of ready-to-eat products containing surimi (cold delicatessen products, salads, sandwiches), the concentration in *L. monocytogenes* may increase during storage of these products, which have intrinsic characteristics that support proliferation of the bacterium. Studies on surimi salad have shown that the pH of this type of product is about 6.3 and that its water activity is slightly above 0.98 (Augustin*, et al.*, 2011). Surimi sampled from a refrigerator of a Canadian consumer affected with listeriosis showed contamination levels of 10^9 CFU/g (Farber*, et al.*, 2000). Studies carried out on the incriminated surimi showed that in the event of high levels of initial contamination (about 10 000 CFU/g), the *L. monocytogenes* concentration multiplied by 100 after 17 days of storage at 4°C. In the event of low initial contamination levels (about 1 cell/g), results were more nuanced and no growth was observed for 28 days of storage at 4 and 10°C (Farber*, et al.*, 2000). Another study showed a 4-log increase in levels of *L. monocytogenes* in several batches of surimi after 6 to 10 days of storage at 8°C (Augustin*, et al.*, 2011).

Since 2006 in France, only one product alert has concerned surimi contaminated with *L. monocytogenes* genoserogroup IIa (<10 CFU/g).

- **Taramasalata**

Taramasalata is an emulsion containing vegetable oil and salted and/or smoked cod roe with a composition indicated in Standard NF V45-072 “Taramasalata and derived products". The production process for this product does not include a pasteurisation step. A code of practice is currently being drafted by industry and recommends systematic pasteurisation of the raw material (fish roe). Pasteurisation appears to be necessary since literature data indicate frequent contamination of fish roe with *L. monocytogenes.* Prevalence observed in two Finnish and Japanese studies on roe from several species of fish sold refrigerated were 18% (Miettinen, 2006) and 10% (Handa*, et al.*, 2005), respectively. pH was in the 4.2-5.1 range (due to the addition of preservatives: sodium benzoate, sodium acetate, potassium sorbate, lactic and citric acid) making this product unsuitable for growth of *L. monocytogenes*. pH testing in the mass of the foodstuff is essential for this type of product.

Analyses carried out by the Joint Laboratories Service Unit of the DGCCRF and the DGDDI in France for the period 2006-2012 indicate that *L. monocytogenes* is frequently found in taramasalata with 8.4% of tests showing positive results in 25 g (for 431 analyses). The observed concentrations nonetheless remain low since only one sample showed a level > 100 CFU/g (990 CFU/g) in 541 counts. Self-monitoring tests carried out by French manufacturers confirm these findings since they demonstrate for the period 2009-2012 a level of 12.5% positive results in 25 g (for 401 analyses), with no result higher than 10 CFU/g. Since 2006 in France, 23 product alerts have concerned taramasalata with different compositions contaminated with *L. monocytogenes* genoserogroup IIa (<10 CFU/g to 2 100 CFU/g).

- **Peeled cooked shrimps**

Peeled cooked shrimps and more generally, cooked crustaceans and molluscs (including crab, spiny lobster and scampi, lobster, common whelk, periwinkle) may be contaminated with *L. monocytogene*s during handling after the cooking step.

Ben Embarek (1994) reported a prevalence of 10% for *L. monocytogenes* in cooked prawns. Self-monitoring data from operators in this sector in France indicate a prevalence of about 4% for refrigerated cooked shrimps at the end of shelf life for the period 2005-2006 (estimate for about

2000 analyses) and a prevalence of about 1% in finished products for the period 2011-2012 (estimate for about 8000 analyses).

pH of shrimp meat is between 7.2 and 8 and provides a favourable environment for proliferation of *L. monocytogenes*. In naturally contaminated shrimps, 10 to 1000-fold increases in *L. monocytogenes* counts were observed during storage at 4°C for 2 weeks (Farber, 1991). Increases in counts for *L. monocytogenes* of 4 log in 4 days at 4°C were reported in cooked crayfish stored under food-quality film or vacuum-packaged. This increase was 4 log in 8 days when the product was stored in a protective atmosphere composed of 75% CO_2 (Pothuri*, et al.*, 1996). In crab meat, increases were 4 log in 12 days at 5°C or in 8 days at 10°C (Brackett & Beuchat, 1990). Generally, Ben Embarek (1994) estimates that *L. monocytogenes* populations multiply by 100 to 1000 in 1 to 2 weeks at 4-5°C.

Since 2006 in France, 51 product alerts have been recorded for cooked shrimps contaminated with *L. monocytogenes* primarily of genoserogroup IIa (<10 CFU/g to 1700 CFU/g), then IIb (serovars 1/2b, 3b or 7) and IVb.

- **Raw fish**

L. monocytogenes is relatively common in raw fish fillets. Undercooked fish may therefore also have residual contamination with *L. monocytogenes*.

A Finnish study (Miettinen, 2006) showed that *L. monocytogenes* was present in 9% of trout and that this contamination mainly affected the gills (8%) and exceptionally the skin and viscera (<1%). The prevalence of the pathogen in raw fillets was between 0% and 10% depending on the study (Ben Embarek, 1994). For example, a survey carried out in Denmark in 1994-1995 on 232 samples of raw fish showed *L. monocytogenes* in 14% of samples (presence in 25 g): 2.6% of samples contained between 10 and 100 CFU/g, and 0.5% (1 sample) was contaminated with more than 100 CFU/g (Nørrung*, et al.*, 1999).

Analyses carried out by the Joint Laboratories Service Unit of the DGCCRF and the DGDDI in France for the period 2006-2012 indicate that *L. monocytogenes* is often present in raw fish or in products containing raw fish undergoing various processes (sushi, sashimi, carpaccio, tartare) with 4.7% positive results in 25 g for 320 analyses. However, these products contain low concentrations and no counts > 100 CFU/g were observed in 455 tests. For raw fish that undergoes little preparation (fillets, steaks), none of the 48 tests carried out were found to be positive and the 503 counts performed over this period were all below the quantification threshold.

Growth of *L. monocytogenes* is generally slow in these matrices. Macrae and Gibson (1990) observed no significant growth of *L. monocytogenes* in raw salmon stored at 4°C. Likewise, Leung *et al.* (1992) found no growth of *Listeria* in catfish fillets during 16 days of storage at 4°C. Wang and Shelef (1992) observed some growth of *L. monocytogenes* in cod fillets after an interval of 10 days at 5°C. Although growth is possible in certain cases, it does not pose a risk to the consumer since raw fish spoils before high *Listeria* concentrations can be reached. Cod stored at 5°C spoiled after 8 days, before *Listeria* growth could begin. Wang and Shelef (1992) and other authors report spoiling of salmon fillets after 6 days at 4°C (Rasmussen*, et al.*, 2002).

Since 2006 in France, three product alerts have been issued for raw fish (Pangasius [15 000 CFU/g], halibut [10 CFU/g], hake [3300 CFU/g]) and two for sushi contaminated with *L. monocytogenes* of genoserogroup IIa (4 product alerts) and IIb (1 product alert).

V. Conclusion of the "Biorisk" Expert Committee (CES)

The rationale is summarised in the table below:

Product	Heat treatment	Possibility of contamination or recontamination during process	Prevalence	Possibility of growth	Number of product alerts in France since 2006	Epidemiological link (outbreaks or clustered cases described in the literature)
Surimi	yes	no	-	++	1	+
Taramasalata	no	yes	++	+/-	23 (<10 CFU/g to 2100 CFU/g)	-
Peeled crustaceans sold cooked	yes	yes	++	+++	51 (<10 CFU/g to 1700 CFU/g) (cooked shrimps)	+
Raw fish	no	yes	+	+/-	5	+

In response to the questions posed in the formal request:

- The production process for refrigerated surimi now includes a pasteurisation step in the packaging (Standard NF V 45-068 [2002]) enabling elimination of possible *L. monocytogenes* present in the raw material. A specific warning for this product does not appear to be justified.
- Given prevalence levels and/or the possibility of growth of *L. monocytogenes*, the following products potentially pose a risk for pregnant women: taramasalata, peeled crustaceans sold cooked, and raw fish having undergone preparation such as sushi, sashimi, carpaccio, and tartare.

These foodstuffs must comply with the microbiological safety criteria stipulated in Commission Regulation (EC) No 2073/2005, as amended, and require a use-by date when they are pre-packaged.

Ready-to-eat foodstuffs in which *L. monocytogenes* may develop pose a potential risk when recommended storage conditions (temperature/time) or preparation practices are not followed. As such, compliance with domestic hygiene measures remains essential for the prevention of listeriosis and in particular: compliance with the cold chain, temperature (4°C) and shelf life for refrigerated products, and prevention of cross-contamination (refrigerator cleanliness, hand washing, cleaning of utensils and surfaces, etc.).

The "Biorisk" Expert Committee (CES) reiterates that *L. monocytogenes* is not the only microbiological hazard that may affect the safety of food consumed by pregnant women. Moreover, foodstuffs other than seafood should also be taken into account. Therefore, it would be beneficial to extend revision of the guide "Nutrition during and after pregnancy" to other microbiological hazard /foodstuff combinations to provide a wider update of recommendations, taking into account epidemiological data and data on the prevalence of hazards and changes in technological processes.

4. CONCLUSIONS AND RECOMMENDATIONS OF THE AGENCY

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions of the Expert Committee (CES) on Food-related biological risk assessment.

The Director General

Marc Mortureux

KEY WORDS

Listeria monocytogenes; Seafood; Pregnant women; National Health and Nutrition Programme; PNNS.

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ANNEX: Results of seafood product analyses carried out by the Joint Laboratories Service Unit of the DGCCRF and the DGDDI for 2006-2012

Type of product		Test in 25 g	Counts (CFU/g)		
		Number of positives/number of analyses	<10	<100	Others
Surimi	sticks	0/49	110	6	/
	flakes	0/10	27	2	/
	products derived from surimi (salads, scallop dishes, pasta)	0/6	94	22	/
Taramasalata		36/431	505	28	7 (between 10 and 70 CFU/g) 1 (at 990 CFU/g)
Shrimps	cooked peeled	0/4	30	1	
	cooked (peeled?)	3/9	92	19	1 (at 10 CFU/g)
	cooked (?)	0/6	77	4	1 (between 1 and 40 CFU/g)
	products derived from shrimps	0/4	48	9	/
Raw fish	sushi, sashimi, carpaccio, tartare	15/320	420	35	1 (at 50 CFU/g)
	fillets, steaks	0/48	475	28	