Results of **histamine monitoring in refrigerated fish** with high histidine concentrations in France (2010-2012 and 2015)

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Abstract
Fresh fish with high concentrations of histidine are the main contributors to histamine risk. From 2010 to 2012, a monitoring plan for fresh fish with high concentrations of histidine was carried out. Sampling was established according to consumption data. It took into account both seasonal and regional distribution, in order to be representative of consumer exposure. Mean histamine concentrations showed little differences between sampled fresh fish. Probabilities of exceeding the regulatory limits or concentrations that have a known impact on consumer health appeared to be better indicators of food safety and quality. The species that most contributed to consumer exposure, with high concentrations of histamine, was chilled tuna. In addition, the 2015 results, obtained from a smaller sample, show there is greater uncertainty regarding the indicators, and possible changes in consumer exposure can thus no longer be estimated.

Keywords
Biogenic amines, Histamine, Surveillance, Fish

Résumé
Surveillance de l'histamine dans les poissons réfrigérés à forte teneur en histidine en France (2010 à 2012 et 2015)
Les poissons frais à forte concentration en histidine sont les plus forts contributeurs au risque histaminique. Une surveillance de l'histamine dans les produits de la mer est organisée chaque année depuis 2005 par la direction générale de l'Alimentation. De 2010 à 2012, l'échantillonnage pour les poissons frais à forte concentration en histidine, établi à partir des données de consommation (notamment de la répartition saisonnière et régionale des consommations), a permis d'obtenir des résultats représentatifs de l'exposition des consommateurs. Les contaminations moyennes en histamine présentent peu de différences entre les différents poissons frais suivis. Les probabilités de dépasser les seuils réglementaires ou les concentrations qui ont un impact connu sur la santé des consommateurs apparaissent comme un meilleur indicateur de la qualité sanitaire des aliments. L'espèce qui contribue le plus à l'exposition des consommateurs, avec des concentrations élevées en histamine, est le thon réfrigéré. En outre, les résultats de 2015 établis à partir d'un échantillonnage réduit par catégorie de poissons frais montrent que l'incertitude sur les indicateurs devient plus importante et ne permet plus d'estimer d'éventuelles évolutions de l'exposition des consommateurs.

Mots-clés
Amines biogènes, histamine, surveillance, poisson

Health context

Histamine

Histamine belongs to the class of biogenic amines, which are involved in metabolism in humans, animals and plants. With regard to food, these substances are non-volatile amines formed by the decarboxylation of amino acids by microbial and tissue enzymes. More than 200 bacterial species are capable of producing histidine decarboxylase, and can produce histamine depending on the environmental conditions.

Histamine is an essential physiological compound for humans. However, food can supply too much of it; it then disrupts the body and induces poisoning in the form of a "pseudo-allergic" reaction.

In France, there is no specific mandatory reporting for histamine-related poisoning, which is monitored through the reporting of food-borne outbreaks. The number of these outbreaks in which histamine's role was confirmed rose from nine in the early 2000s (Delmas *et al.*, 2005) to more than 27 in 2006 (InVS, 2007). Several unverified assumptions were put forward to explain this increase: changes in the affected products (fish species consumed, geographical fishing areas, etc.), changes in consumer practices, and improved operation of the reporting system (AFSSA, 2009).

The most recent data show a smaller number of food-borne outbreaks. In 2014 in France, histamine's involvement was confirmed or strongly suspected in respectively seven and 25 food-borne outbreaks, affecting 36 and 115 people (InVS, 2014). Histamine accounted for 3% of outbreaks whose agent was confirmed (InVS, 2014). In 2014, at the European level, 74 food-borne outbreaks involving histamine were reported (EFSA, ECDC, 2015).

Background of histamine surveillance in foods in France

Regulations

Histamine is regulated for fishery products only. The safety criteria are defined in Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. This regulation applies to the industry own control programmes undertaken by operators to verify the safety of the product batches they place on the market. In this context, they are required to collect nine samples per batch (n=9); the mean concentration for these nine samples must be less than or equal to 100 mg.kg-1 (m); no more than two samples (c=2) can have a concentration between 100 (m) and 200 mg/kg (M) but none can exceed 200 mg/kg (a factor of two is authorised for these values for products that have undergone enzyme maturation, such as anchovies). Regulation (EU) No 1019/2013 of 23 October 2013 provides some clarifications regarding the ability to consider only one sample (n=1) for the verification of foods at retail level; the concentration of histamine must not exceed 200 mg/kg. It adds a safety criterion for fish sauce produced by fermentation of fishery products (n=1; m=M=400 mg/kg) (EU, 2013).

Main foods containing histamine

Histamine can be found in fermented food products such as wine, beer, sauerkraut, cheese (Roquefort, Gruyère, Cheddar, Gouda, Edam, Emmental, Gorgonzola), delicatessen meat (salami, chorizo, dried sausage), chocolate, and hung game, as well as in non-fermented products such as spinach and especially certain fish (Suzzi and Gardini, 2003; Lavizzari *et al.*, 2007).

However, the large majority of food-borne outbreaks involving histamine (over 70%) are associated with fish and fishery products (FAO/WHO, 2013). Only some fish species can contain a large quantity of histamine, due to their high histidine levels. The fish most commonly involved in cases of poisoning belong to the Scombridae family. In fact, the term "Scombroid fish poisoning" is used to describe poisoning due to histamine in fishery products. Other classes of fish are recognised as presenting a risk (Table 1, Guillier *et al.*, 2011).

Surveillance plans

Given the increase in the number of food-borne outbreaks between 2000 and 2006, the Directorate General for Food (DGAL) submitted a request to AFSSA in 2008 to improve the surveillance plan organised every year since 2005. An Opinion was issued (AFSSA, 2009) and the DGAL surveillance plan for histamine was revised. The proposed plan (which was implemented for the 2010-2012 period) directly assessed consumer exposure to histamine. This plan relied on the risk levels of the various categories of seafood products associated with species with high histidine concentrations. The overall approach is described in detail in the AFSSA Opinion of 2009 and a scientific article (Guillier *et al.*, 2011). The plan focused on high-risk product categories (fresh fish). For each category, the sampling plan was then defined based on consumption data, in order to ensure spatial and seasonal representativeness. The samples were divided up proportionally for these two criteria between eight major regions (North, East, Paris region, West, Centre-West, South-West, Centre-East, South-East) and six periods of the year (January-February, March-April, etc.). Samples were taken in the distribution (supermarkets, fishmongers) and catering stages, respecting a distribution in proportion to the relative quantities of fish associated with places of consumption (at home and outside the home).

The surveillance plan undertaken in 2015 had the same objective, but did not adhere to the same constraints regarding the spatial and temporal distribution of the samples. The 212 samples of fresh fish were planned in the stage of direct delivery to consumers. The number of samples to be taken by region was established in proportion to the size of the human population. Samples were collected from various batches to ensure the representativeness of results. The variability of contamination levels could not be estimated for three categories (herrings, sardines and fresh salmon) in 2015 since the contamination levels were below the limit of quantification.

Data used and methods for characterising data on histamine contamination in products

Source of the data

Data on histamine contamination in fishery products provided by the DGAL were extracted from the Access database pooling public surveillance data developed during the prototyping of the proposed health section of the Food Observatory (OSSA).

These data come from the DGAL's surveillance plans covering 2010-2012 and 2015. In order to comply with the format and nomenclature requirements of the European database, the data have been recoded by ANSES according to "Standard Sample Description ver.2.0" (SSD2) and the FoodEx2 food description and classification system. Figure 1 shows the breakdown of the 1686 histamine concentration data between the four years (2010, 2011, 2012 and 2015) and the various categories of fresh fish. Only data related to tuna (yellowfin and

Table 1. All of the fish species potentially at risk for the histamine hazard (according to AFSSA (2009) and Guillier *et al.* (2011)). The fish categories analysed in the surveillance plan appear in dark red

Class	Species	English name
Arripidae	*Arripis trutta*	Australian salmon
Amodytidae	*Ammodytes tobianus*	Lesser Eel or Small Sandeel
Belonidae	*Belone belone*	Garfish
Carangidae	*Seriola dumerili (Risso)*	Greater amberjack
	Seriola lalandii	Yellowtail amberjack
	Caranx spp.	Jack or Blue Runner
	Trachurus spp.	Horse mackerel
Coryphaenidae	*Coryphaena hippirus*	Mahi-mahi
Clupeidae	*Sardinella sirm*	Sprat
	Amblygaster sirm	Spotted sardinella
	Sardinops sp.	Sardinella, Madeiran
	Sardina pilchardus	Sardine
	Clupea harengus	Herring
	Sprattus spp.	Sprat
	Harengula spp.	Herring, Pacific Thread
	Alosa pseudoharengus	Alewife or River Herring
	Spratelloides gracilis	Herring, Silver-stripe Round
Engraulidae	*Anchoa* spp.	Anchovy
	Anchoviella spp.	
	Engraulis spp.	
	Cetengraulis mysticetus	
	Stolephorus spp.	
Gempylidae	*Lepidocybium flavobrunneum*	Escolar
	Rivetus pretiosus	
Istiophoridae	*Makaira (Tetrapterus) audax (poey)*	Marlin
	Istiophorus spp.	Sailfish
Lutjanidae	*Aphareus* spp.	Snapper
	Aprium virescens	
	Pristipomoides spp.	
Pomatomidae	*Pomatomus saltatrix*	Bluefish
Sciaenidae	*Seriphus politus*	Queenfish
Scomberesocidae	*Cololabis saira*	Pacific saury
Scombridae	*Auxis thazard*	Bonito tuna
	Acanthocybium solandri	Wahoo
	Euthynnus alleratur	Little Tuna or Kawakawa
	Katsowonus pelamis	Skipjack tuna
	Sarda sarda	Atlantic bonito
	Scomber japonicus	Pacific mackerel
	Scomber scombrus	Atlantic mackerel
	Scomberomorus cavalla	King mackerel
	Scomberomorus maculatus	Atlantic Spanish mackerel
	Scomberomorus regalis	Cero
	Scomberomorus brasiliensis	Serra Spanish mackerel
	Thunnus alalunga	Albacore
	Thunnus albacares	Yellowfin tuna
	Thunnus obesus	Bigeye tuna
	Thunnus thynnus	Atlantic bluefin tuna
	Thunnus atlanticus	Blackfin tuna
Salmonidae	*Salmo salar, Oncorhyncus* sp.	Salmon
Serranidae	*Epinephelus* sp.	Grouper
Xiphiidae	*Xiphias gladius*	Swordfish

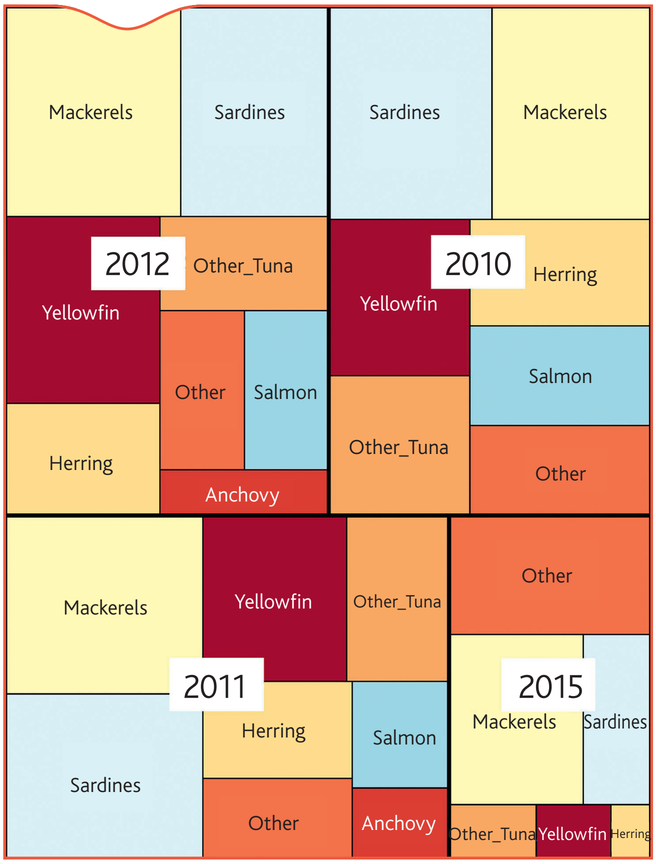

Figure 1. Hierarchical display of the breakdown of the 1686 data from the surveillance plan for refrigerated fresh fish in 2010, 2011, 2012 and 2015. The areas are proportional to the sampling distribution

other species of the *Thunnus* genus), mackerels, sardines, herrings and salmon are analysed here. The "Other" category contains data for various other fish species (e.g. horse mackerel, grouper, swordfish) for which the sample populations do not enable an analysis with sufficient statistical power. This option to monitor species other than those most commonly consumed had been proposed in the AFSSA Opinion of 2009, in order to provide the opportunity, as part of the surveillance plan, to study species and origins of seafood products subject to outbreak surveillance.

Statistical methods

Since most of the results of the histamine surveillance plans are below the limit of quantification, the use of descriptive statistics (mean, median, etc.) is of limited interest and would even lead to biases if data below this threshold were randomly set at the value of the limit of quantification (LOQ) for the method. In this context, data modelling is a genuine advantage to improve the overall description process for an empirical distribution. The methodology applied here for histamine is directly inspired by methodologies applied in microbiology (Busschaert *et al.*, 2010; Pouillot and Delignette-Muller, 2010).

The other methodological objective was to characterise uncertainty for the distributions used to improve knowledge of variability in product contamination. There are several available methods for assessing uncertainty, including bootstrapping (re-sampling technique) and the Bayesian approach (Commeau *et al.*, 2012). Bootstrapping has been used to characterise uncertainty for descriptive statistics based on distributions.

The statistical functions used to adjust the log-normal distribution for censored data and to characterise uncertainty for the quantiles of interest are those of the R package "fitdistrplus" (Delignette-Muller *et al.*, 2015). Figure 2 shows the values estimated from the surveillance data. Mean

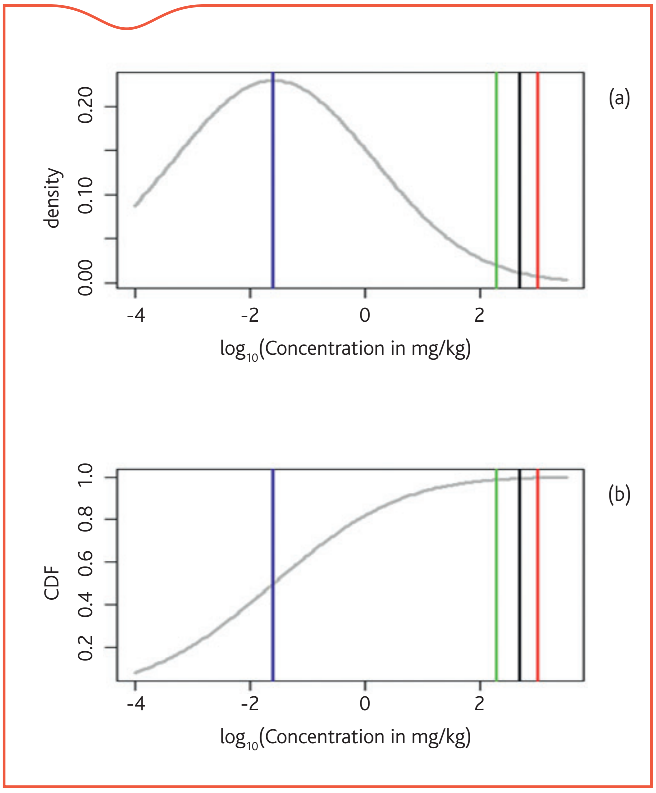

Figure 2. Illustration of a log-normal distribution used to characterise the histamine concentration data of the surveillance plan. (a) Density, (b) Cumulative distribution (CDF). Legend for the quantiles used to characterise the distribution: blue=median/mean, green=200 mg/kg, black=500 mg/kg, red=1000 mg/kg

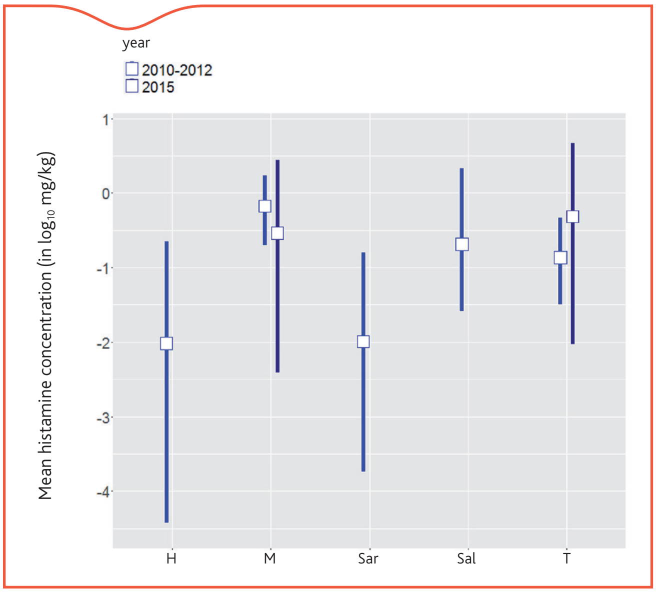

Figure 3. Mean concentrations in $\log_{10}$ (mg/kg) of histamine in the various seafood products monitored in the surveillance plans (H=Herrings, M=Mackerels, Sar=Sardines, Sal=Salmon, T=Tuna). The most probable values (dots), 95% credible intervals for the mean concentrations (error bars)

concentrations and probabilities of exceeding the respective thresholds of 200, 500 and 1000 mg/kg have been estimated.

Surveillance plan results and discussion

Since contamination levels did not differ significantly between the four years, they will be presented as a whole. Figure 3 gives mean contamination levels for the various categories of fresh fish. For

2010-2012, mean contamination levels were respectively 0.01, 0.76, 0.01, 0.18 and 0.15 mg/kg for herrings, mackerels, sardines, salmon and tuna. The uncertainty associated with these estimates confirms that the differences between fish in terms of mean contamination were low and generally insignificant (only the mean contamination for mackerels was significantly higher than that for sardines). There were fewer data for 2015, resulting in greater uncertainty regarding the results; this means that changes in contamination between 2012 and 2015 could not be estimated. Figure 4 shows the probability of reaching high levels (in relation to the regulatory threshold and those associated with a high probability of inducing poisoning) for each fresh fish. The data analysis shows that the probability of reaching high contamination levels is higher for tuna than for the other categories of fish with high histidine concentrations. As for the mean contamination levels, potential changes in probabilities of high contamination could not be estimated due to the small number of samples for 2015.

The contamination levels observed in fresh fish in the consumption stage in France were of the same order of magnitude as those provided in an international summary presented in a FAO/WHO report (2013). For example, in the Netherlands, the mean concentration of histamine in fresh tuna was 14 mg/kg in 2010 and the probability of exceeding 200 mg/kg was 2.9%. Other more recent publications report probabilities of exceeding the 200 mg/kg threshold of below 3.3% (Michalski, 2016; Petrovic *et al.*, 2016). However, the median contamination values and probabilities of exceeding the limit values are only representative of the foods analysed. As it is almost impossible from the survey reports to know whether sampling was representative of the country's consumption profile, the results cannot be compared among countries (FAO/WHO, 2013).

The decision to devote a certain number of samples to fresh salmon had been proposed in the AFSSA Opinion of 2010. There were still doubts regarding the potential involvement of this fish in cases of histamine poisoning (Emborg *et al.*, 2002). The analysis of the data from the 2010-2012 plans shows that histamine levels can be high in this type of fish. Surveillance plan data obtained for salmon confirm current knowledge on the possible contamination of this fish by histamine (Løvdal, 2015). The median contamination levels estimated for salmon under these surveillance plans are robust. The probability of exceeding higher concentration levels is much more uncertain. Unlike for other fish with high concentrations of histidine, it is not certain for salmon that microbial growth and/or the initial histidine concentration enable high contamination levels to be reached. In other words, the distribution used suggests high levels whereas actual histamine contamination might not exceed a certain level. The maximum concentrations observed for fish with high histidine levels exceed 2000 mg/kg. To our knowledge, this level of contamination has never been observed for salmon.

With a sampling plan that is not representative of consumption, the data must be adjusted to assess exposure. Statistical adjustment consists in taking the sampling plan's data into account to assign a particular "weight" to each sample based on its category. Weighting depends on the consumption of each fish; the weight is greater than 1 if its category is not sufficiently represented in relation to its share of consumption, and is less than 1 if it is overrepresented. However, it is difficult to adjust data if the plan includes several that are below the limit of quantification (Williams and Ebel, 2014). In this case, the data were not adjusted because for each product category, the samples they came from were directly representative of consumption data (Guillier *et al.*, 2011). Since a very high percentage of the analysed data are below the limit of quantification, it will be necessary to continue using sampling representative of exposure for future surveillance plans. The 2010-2012 plans used sampling representative of the seasonality and regional distribution of consumption (AFSSA, 2010). The data analysis shows that some factors have little influence on contamination levels. It therefore does not appear necessary to strictly index the distribution of samples to the seasonality of consumption or to all French regions.

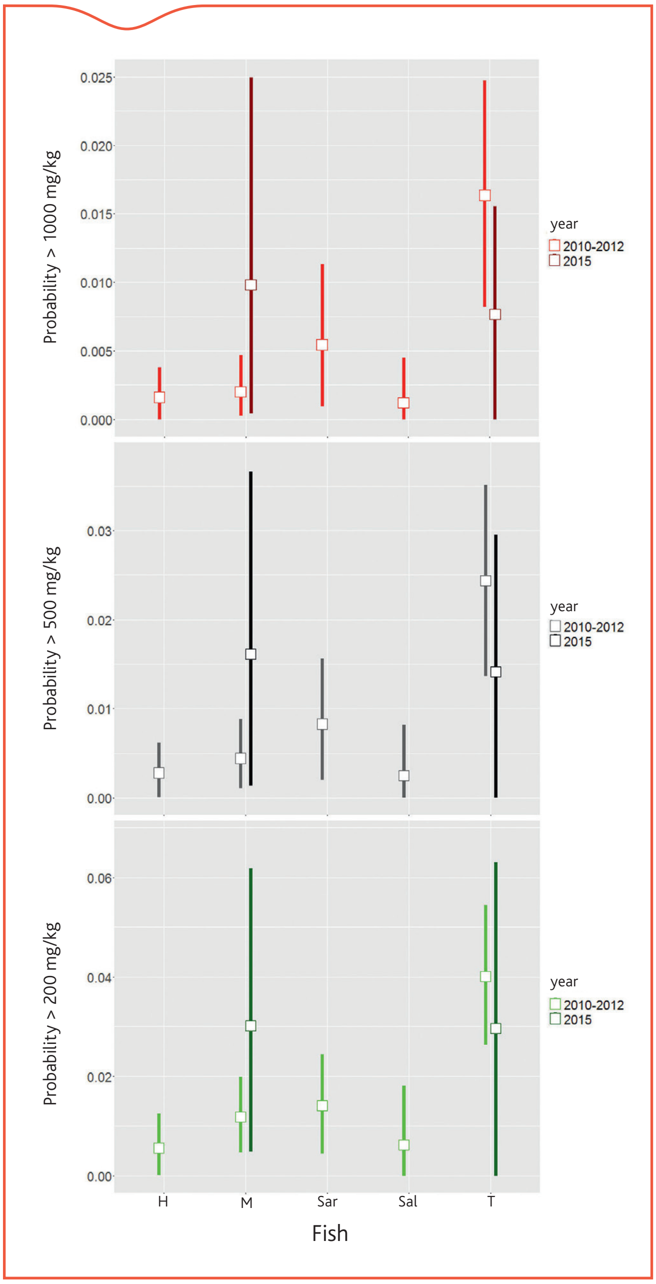

Figure 4. Probabilities of exceeding histamine concentrations in various seafood products of (a) 1000 mg/kg, (b) 500 mg/kg, and (c) 200 mg/kg (H=Herrings, M=Mackerels, Sar=Sardines, Sal=Salmon, T=Tuna). The most probable values (dots), 95% credible intervals for the mean concentrations (error bars)

The data analysis provides a classification of the fish that contribute most to histamine exposure. Tuna appears to be the most contributing species in terms of contamination levels. The assessment of histamine exposure undertaken through surveillance plans is paving the way for attributing cases of histamine poisoning in France to the various categories of fresh fish. Combining estimates of histamine exposure (concentration data from surveillance plans together with consumption data) with the dose-response relationship (used to calculate the probability of observing an effect in consumers based on the ingested hazard dose), including potential differences due to the specific susceptibility of sub-populations of consumers, would enable risk to be assessed as a relative or absolute number of human cases related to the various sources.

The FAO/WHO report (2013) raised the issue of the role of other biogenic amines (possible "potentiating" effect or not). Data need to be acquired to examine this issue. Thus, the accredited laboratories in the network of the National Reference Laboratory for histamine have been requested to submit not only histamine concentrations but also concentrations of other biogenic amines (putrescine, cadaverine and tyramine). These data are essential to understand potential correlations between these amines and will make it possible to assess consumer exposure.

Conclusion

Future surveillance plans for histamine and biogenic amines will continue to monitor consumer exposure using the methodology proposed in the AFSSA Opinion of 2009. To monitor changes in this exposure, the sampling plan inspired by that used for 2010-2012 will be implemented, keeping the same fish categories. The results obtained in 2015 with a limited surveillance plan compared to 2012 indicate that it is preferable to keep only one category of fresh fish per year. This will provide sufficient statistical power to estimate changes in the exposure of French consumers to histamine in fresh fish.

References

Afssa (2009). Avis sur les propositions d'amélioration du plan de surveillance histamine. https://www.anses.fr/fr/system/files/MIC2008sa0310.pdf.

Busschaert, P., Geeraerd, A., Uyttendaele, M., Van Impe, J. (2010). Estimating distributions out of qualitative and (semi) quantitative microbiological contamination data for use in risk assessment, Int J Food Microbiol, 138 : 260-269.

CE (2005). Règlement (CE) n°2073/2005 de la Commission du 15 novembre 2005 concernant les critères microbiologiques applicables aux denrées alimentaires. JO UE 22/12/05, L 338:1.

CE (2007). Règlement (CE) n°1441/2007 de la Commission du 5 décembre 2007 modifiant le règlement (CE) n°2073/2005 concernant les critères microbiologiques applicables aux denrées alimentaires. JO UE 07/12/2007, L 322:12.

Commeau, N., Parent, E., Delignette-Muller, M.-L., Cornu, M. (2012). Fitting a lognormal distribution to enumeration and absence/presence data, Int J Food Microbioly, 155 : 146-152.

Emborg, J., Laursen, B.G., Rathjen, T., & Dalgaard, P. (2002). Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2°C. J Appl Microbiol, 92: 790-799.

Delignette-Muller, M.L., Dutang, C. (2015). fitdistrplus: An R Package for Fitting Distributions, J Stat Soft, vol. 64 1-34.

Delmas, G., Le Querrec, F., Weill, F. X., Gallay, A., Espié, E., Haeghebaert, S., Vaillant, V. (2005). Les toxi-infections alimentaires collectives en France en 2001-2003. Rapport INVS. http://invs.santepubliquefrance.fr//Dossiers-thematiques/Maladies-infectieuses/Maladies-a-declaration-obligatoire/Toxi-infections-alimentaires-collectives/Publications/Articles.

EFSA, ECDC (2015). The European Union Abstract report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA J 13(12):4329. doi:10.2903/j.efsa.2015.4329.

FAO/OMS (2013). Joint FAO/WHO Expert Meeting on the Public Health Risks of Histamine and Other Biogenic Amines from Fish and Fishery Products. ISBN: 9789240691919. http://apps.who.int/iris/bitstream/10665/89216/1/9789240691919_eng.pdf.

Guillier, L., Thébault, A., Gauchard, F., Pommepuy, M., Guignard, A.& Malle,

P. (2011). A risk-based sampling plan for monitoring of histamine in fish products. J Food Prot, 74(2) : 302-310.

Hungerford, J. M. (2010). Scombroid poisoning: A review. Toxicon, 56(2): 231-243.

InVS (2007). Données relatives aux Toxi-infections alimentaires collectives (Tiac) déclarées en France en 2006 et 2007. Disponible en ligne (http://www.invs.sante.fr/surveillance/tiac/donnees_2007/Tiac_donnes_2006_2007.pdf).

InVS (2014). Surveillance des toxi-infections alimentaires collectives. Données de la déclaration obligatoire, 2012. Disponible en ligne sur: http://www.invs.sante.fr/Dossiers-thematiques/Maladies-infectieuses/Maladies-a-declaration-obligatoire/Toxi-infections-alimentaires-collectives/Donnees-epidemiologiques

Lavizzari, T., Veciana-Nogués, M. T., Weingart, O., Bover-Cid, S., Mariné-Font, A., & Vidal-Carou, M. C. (2007). Occurrence of biogenic amines and polyamines in spinach and changes during storage under refrigeration. Journal of agricultural and food chemistry, 55(23), 9514-9519.Løvdal,

T. (2015). The microbiology of cold smoked salmon. Food Control, 54, 360-373.

Michalski, M. (2016). The Content of Histamine in Fresh and Smoked Fish Commercially Available in Poland. In IAFP's 12th European Symposium on Food Safety.

Petrovic, J., Babic´, J., Jaksic, S., Kartalovic, B., Ljubojevic, D., & Cirkovic, M. (2016). Fish Product–Borne Histamine Intoxication Outbreak and Survey of Imported Fish and Fish Products in Serbia. J Food Prot, 79(1), 90-94

Suzzi, G., Gardini, F. (2003). Biogenic amines in dry fermented sausages: a review. Int J Microbiol, 88(1) : 41-54.

Williams, M.S., Ebel, E.D. (2014). Fitting a distribution to censored contamination data using Markov Chain Monte Carlo methods and samples selected with unequal probabilities. Env Sci Technol, 48: 13316-13322.