BASELINE

SELECTION AND IMPROVING OF FIT-FOR-PURPOSE SAMPLING PROCEDURES FOR SPECIFIC FOODS AND RISKS

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SEVENTH FRAMEWORK PROGRAMME

TABLE OF CONTENTS

BASELINE - www.baselineeurope.eu
Project funded under the Food, Agriculture and Fisheries, and Biotechnology theme (KBBE)
SEVENTH FRAMEWORK PROGRAMME

SUMMARY

Sampling of food is needed to ensure food safety, but sampling is also costly. Therefore, the number of samples needs to be kept low. In some cases, other approaches than sampling can be used to ensure food safety.

The relevance of applying alternative approaches depends partly on the contamination route and partly on the distribution of risk agent in the food. If the risk agent can be related to a specific geographic area, like *Vibrio*, virus or toxins in shellfish, monitoring of the area may be at least as useful as analyzing product samples.

Studies in BASELINE have showed that *L. monocytogenes* cells are unevenly distributed and present in very low levels in fish products immediately after processing. However, positive samples may contain high populations after some days of cold storage even if they contained only traces of the bacterium during processing. Sampling may therefore represent an underestimation of risk if product sampling during processing is the only measure to control the risk. We have suggested three alternatives.

Good design of facility and good processing practices, including good HACCP implementation (already mandatory) and environmental control.

Better risk analyses including

Challenge studies for estimating maximum shelf life at required storage conditions needed to control the Listeria risk in the specific product.

Use of predictive modelling for estimating the effects of abuse conditions.

Better risk communication along the distribution chain including

More traceability and more user-friendly access to the information

Use of time-temperature indicators

Relevant information to the consumer about risks and recommended use, particularly for susceptible consumers. The information can be given directly on the label as text or pictures or as two dimensional barcodes which can be scanned using smart phones.

Mercury compounds accumulate in tuna over time. The body mass can therefore be used to estimate the levels of mercury compounds, but there are variations between species. Based on our studies, three choices are possible to reduce the risk of mercury in tuna:

Rejection of tuna fish over a defined weight limit to ensure that mercury levels are below the regulations for albacore, yellowfin and skipjack tuna.

Accept all tunas because the risk to have a contaminated fish over the mercury threshold is negligible (less than 2% regardless of the weight of each species, except Atlantic bluefin tuna)

To apply the sampling plan for chemical analysis for Atlantic bluefin tuna

INTRODUCTION

Sampling of food is needed to ensure food safety, but sampling is also costly. Therefore, the number of samples needs to be kept low. In some cases, other approaches than sampling can be used to assess the prevalence and levels of food risk agents. Some examples: Sampling of products can be simplified by analyzing pooled samples, production and water environment can be sampled instead of the products, and parameters which correlate with the risk agents can be used as indicators. The best approach depends on the variation of the risk agent in the batch or area to be sampled. In addition to direct and indirect sampling, tools like risk communication and restricted distribution of foods likely to hold high risk levels to certain market segments can be used to ensure the food safety. These approaches are useful when the risk levels vary, possibly in an unpredictable way, presence of the risk agent cannot be avoided, the risk agent may multiply at normal or abuse conditions, or the risk can be easy eliminated by the consumer during preparation of the food.

In BASELINE, we have studied the distribution of selected risk agents in some fish and seafood products and suggested safety criteria and sampling plans (see deliverables 1.2, 1.3 and 1.4). In the present deliverable, we suggest some alternatives to sampling, which can be considered as supplements to sampling. The focus is mainly on *Listeria* in fish products and mercury compounds in tuna. Virus, *Vibrio* and toxins in shellfish are covered in other deliverables, and are only briefly mentioned in the next chapter.

CONTAMINATION ROUTES AND DISTRIBUTION OF RISK AGENTS – RELEVANCE FOR SAMPLING STRATEGY

A risk agent may be related to an area. This is often the case for the human pathogen *Vibrio* spp, viruses and toxins from algae in shellfish. The risk agents are introduced into the shellfish from the surrounding water. If a risk agent is present in the water over time it is likely the levels in shellfish will be high because shellfish filter significant quantities of water and they have limited range. Sampling sea water in the area may be more cost-effective than sampling of the shellfish itself. This approach is used in some countries as a supplement to sampling, for instance, for assessment of toxins in blue mussels in Norway. It should be mentioned that there will be some variations in risk agent levels within a contaminated area. Some of them are systematic, and can be included in the estimation of risk agent content. Further, analyses of pooled samples can be used to estimate variations within an area (see deliverable 1.3 and 1.4).

Wild fish, for instance tuna, have a great range. Risk agents like mercury compounds accumulate in the fish, causing a concentration of chemical contaminant levels over time. From a food safety control point of view, the correlation between mercury compound levels and age or size of the fish is very useful. Different accumulation and range and distribution between tuna species are likely, but this can be included in the models. We report here a study indicating how tuna weight can be used to estimate the level of mercury compounds.

Farmed fish has, like shellfish, limited range, but like tuna, they accumulate chemical compounds from the feed. Farmed species like salmon are bred for quick growth and are fed with analyzed feed. Thus, it is assumed that the levels of contaminants will be lower than in wild species of a comparable weight. Fish weight is therefore a less relevant parameter for farmed fish than for wild fish.

Farmed fish may be cultivated in very pure or in contaminated water areas. Pathogen bacteria like *Listeria monocytogenes* may be present on skin and gills on fish in the sea and transferred to the fillet during slaughter and processing. Pathogens may also be transferred to the fillet directly from process equipment and people, indicating that sampling of water surrounding the fish cage may not give a representative picture of the prevalence.

Our results in BASELINE (deliverable 1.2) illustrates that levels of *L. monocytogenes* in naturally-contaminated fresh and smoked salmon are very low shortly after processing, and that the majority of samples may be negative even when the bacterium has been present as an environmental contaminant in the factory for years. This distribution pattern, i.e. low levels and non-homogeneous distribution within a lot, is very challenging for sampling because of the possibility of selecting only negative samples from a batch when some positive samples are actually present

Responsible FBOs use additional measures to control the *Listeria* risk. They may for instance carry out sampling of the production environment, perform extensive cleaning if *Listeria* is detected, apply hygienic design of the facilities, or sell the products frozen, i.e. under conditions where the bacterium cannot grow to infective levels. FBOs who have *Listeria* in their facilities may continue production provided the levels do not exceed the infective dose, the limit value set by customers or the Food law.

In the present report, we give some examples to how alternatives to sampling can be used to manage the food safety risk.

ALTERNATIVES TO SAMPLING OF *L. MONOCYTOGENES* IN SALMON AND SEA BASS PRODUCTS

The following approaches could be applied by stakeholders to control *L. monocytogenes* in ready-to-eat foods by means of increasing preventive activities and reducing analytical procedures in the end product. These approaches are in accordance with the proposals of Codex Alimentarius Commission (CAC, 2007) and other authors (Luber et al., 2011) to control *L. monocytogenes* throughout the food chain, from primary production through consumption. Some of them are already mandatory, while others may need development before they can be implemented.

Good design of facility and practices

Good HACCP implementation (already mandatory):

Good understanding of GHP and HACCP at the industry level is needed in order to use these risk management tools in an effective way. It is conclusively reported that inadequate training is the major cause of HACCP failure. In this respect, the risk manager (Food Authorities or Industry) should organize specific courses for a clearer understanding of what HACCP guidelines mean and training to implement appropriately the specific program.

Environmental control:

In products with a high growth potential of *L. monocytogenes*, avoidance of recontamination will be more important than final product testing. Testing food-contact surfaces for *L. monocytogenes* is more important and cheaper for detecting and controlling the hazard than food sample testing. This procedure was recently made mandatory in Canada (Food Directorate, 2010). Standard protocols for checking contamination on surfaces have also been developed in Europe (Carpentier et al 2009).

Surimi has a very high growth potential for *Listeria* (see Deliverable 1.2). Taking into account the extended shelf life recorded for this product at retail level (between 30 and 58 days), the only way to assure food safety is to prove that no contamination after heat treatment and/or during handling and packaging of product has occurred. A monitoring program should consider a number of factors to ensure the programme effectiveness such as the sampling locations (surfaces, equipment, etc.), type of samples (food contact and non-food contact), number of samples and frequency of sampling, analytical methods and actions to be carried out in case of positive findings.

Audits to detect poor practices

Sampling can be manipulated, particularly when the risk agent is non-homogenously distributed in a batch. In correspondence with commercial laboratories, we have been informed that there are FBOs (not involved in BASELINE) that send a lot of samples for analysis, but ask the lab to report all the negative samples in one letter, and the positive ones in another letter. We assume that FBOs which misuse sampling in this way are likely to make shortcuts in other parts of their business as well. Audits by food authorities or certification audits following a food safety standard or management standard may be useful for identification of FBOs with poor practices., even though there are many examples of outbreaks related to companies where such audits have been carried out.

Better risk analyses

Challenge studies and estimation of shelf life

An alternative to sampling of *L. monocytogenes* has been already considered for ready-to-eat foods in the Food Law. In the EU-regulation 2073/2005, the limit for *L. monocytogenes* in ready-to-eat products is 100 cfu/g in products that do not support growth, and 100 cfu/g at the last day of shelf life in products that do support growth. The criterion takes into account that contamination of

products with very low levels of *L. monocytogenes* in foods may occur, and that absence of the bacterium cannot be guaranteed. The focus on the last day of shelf life indicates that industry has to *predict* the level in the products, not only analyse product samples when they release them for sale. In other terms, they have to introduce performance objectives for their products and processes. We have used this approach in BASELINE to estimate performance objectives, storage conditions and shelf-life for fresh salmon intended used in raw, ready-to-eat products (Deliverable 1.2). We have based our studies on naturally-contaminated samples, which is one of three possible approaches according to European guidelines about risk assessment of *Listeria monocytogenes* in ready-to-eat foods (Beaufort et al, 2009). The two other approaches are challenge studies carried out with inoculated samples under realistic time-temperature conditions and predictive modelling.

Predictive modelling for estimating effects of abuse conditions

Predictive modelling is a well-established research area from the early 1990s. Predictive growth model programs like the Seafood Safety and Spoilage Predictor have been developed (http://sssp.dtuaqua.dk/). Other models are described in BASELINE in deliverable 6.1. Such models make it possible to estimate the effect of different time-temperature conditions on the growth of *L. monocytogenes* for different products, including abuse temperature conditions. Cold-smoked salmon is a commonly used food for development of predictive models. Even though predictive models may not give accurate results for a specific product, they are well suited to illustrate the consequence of abuse temperatures or deviations in pH and additives during processing, and therefore in risk analyses.

Better risk communication along the distribution chain

Traceability

A documented traceability system (including records of temperature during transport and storage at the point of sale, the day of catch or production) could be used to obtain more useful information along the food chain. For example, a QR code (two-dimensional bar code) can be included for smartphone users, which can read the code and convert it to a URL directing the smartphone's browser to the website of a company, store, or product associated with that code providing specific information.

Time-temperature indicators on the label

It is clear from the BASELINE results that the level of *L. monocytogenes* in contaminated salmon strongly relates to storage time and temperature conditions. Although product expiry dates are usually observed by food operators (involved in distribution and sale), the recommended storage temperatures by producers are not always maintained for different reasons (lack of monitoring of temperatures, non-maintenance of refrigeration equipment, etc.). Therefore, an alternative to laboratory analysis (in the case of Official controls) could be to monitor the real temperatures of smoked salmon packages during distribution (transport) and storage at market (cold cabinets). In the cases that temperature abuse would be observed, then specific analysis could be carried out, or a predictive model be applied to assess the effects of the abuse temperatures. Time-temperature indicators may be useful measures to ensure the food safety of sushi, sashimi and cold-smoked salmon produced from fresh salmon. Thermosensitive labels are easily attached to the packaging of chilled or frozen products for easy monitoring of temperature abused during the product's lifetime (there is a change on label color when the product is over the fixed maximum temperature for a specified time, giving us information regarding the compliance of cold food chain).

Relevant information to the consumer

The manufacturer has a responsibility to provide clear information regarding storage conditions of RTE foods to avoid the risk of infection by *L. monocytogenes*. In addition, food safety messages

should be included in the label of RTE products, with clear recommendations or instructions for consumers and people at risk. Messages should be clear and concise on how to avoid the risk. In addition, new technologies could provide more useful information on the label (see "traceability" above).

Consumers have the responsibility to store and prepare the purchased food in a safe manner. If the manufacturer establishes temperature storage between 0 and 4ºC, they should be aware about the best way to shop (refrigerated products at the end), transport (isothermal bags) and store at home (in the coldest place of domestic refrigerator). Usually this information does not reach consumers in an effective way, and more efforts should be undertaken to reduce the risk of infection by *L. monocytogenes.* For example, consumers do not usually know the temperature of their own refrigerators, and they think that the temperature is the same in the entire refrigerator regardless of shelf or contents).

Susceptible groups such as old people, pregnant women and other immunocompromised patients should be informed about the severe diseases caused by consumption of food contaminated with *L. monocytogenes.* They should know that certain RTE products are more risky products for pathogen transmission than others (for example, there has been reported higher prevalence of *L. monocytogenes* in smoked salmon than in pate; Garrido et al., 2009). Therefore, more educational programmes are needed.

All stakeholders probably agree that risk communication is needed, but the delineation of responsibility of food authorities, FBOs and consumers may not be as clear as we indicate above. In many countries, it is the responsibility of the food authorities to advise susceptible consumers, and the manufacturers' responsibility to design their processes and products to fulfil the criteria. An outcome of BASELINE in this regard, is that Norwegian authorities consider advising pregnant women about the storage conditions for salmon intended used for sushi.

Conclusion

Alternatives or supplements to product sampling are needed to minimize the risk of products with high levels of *L. monocytogenes* on the market. We have suggested three approaches, some are already mandatory, while others need further development before they can be implemented.

- Good design of facility and processing practices, including good HACCP implementation (already mandatory), environmental control and audits from external partners.
- Better risk analyses including:
    - Challenge studies for estimating maximum shelf life under suitable storage conditions needed to control the Listeria risk;
    - Use of predictive modelling for estimating effects of abuse conditions.
- Better risk communication along the distribution chain including:
    - More information related to traceability;
    - Inclusion of time-temperature indicators;
    - Inclusion of relevant information to the consumer about recommended use, particularly for susceptible consumers. The information can be given directly on the label as text or pictures, as two dimensional barcodes which can be scanned using smart phones, or in other ways suitable for the target audience.

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BODY WEIGHT AS INDIRECT MEASURE FOR MERCURY IN TUNA

It is an established opinion that mercury compounds in tuna relate to fish size and tuna species. A selection of tuna by weight may therefore be relevant as an alternative to laboratory analysis as documentation of PO claims. Many publications present a relationship between the weight of tunas and mercury levels, as for the yellowfin tuna in Florida (USA) (Adams, 2004). Indeed, tuna have long lifespans and the longer tunas live, the greater the concentration of mercury, essentially from natural origins, is in the fish. Accidental release of mercury in the sea does not significantly affect the contamination of tuna because it is a pelagic fish. A selection by tunas' weight could rely on this theory: a heavy tuna has a high mercury level. If the relationship is confirmed, producers could have to eliminate tunas having a weight over a given threshold. Moreover, sampling plans for chemical analysis can engender high costs. This alternative method could be cheaper, so more attractive to producers, while ensuring a proper consumer safety.

Raw data collecting: tuna weight and mercury level

For yellowfin tuna, the following publications were used: Aldrin *et al.* (1973), Adams (2004), Kojadinovic *et al.* (2006), Métongo & Kouamenan (1991) and Thibaud (1971). One publication recorded only the length of tunas (Kojadinovic *et al.* (2006).. Tuna weight was calculated by the formula: Weight (kg) = $3.1.10^{-5}$ x Length $^{2.858}$ (cm) (Tantivala, 2000). The natural range of yellowfin tuna are the Atlantic Ocean, the Pacific Ocean and the Indian Ocean. A total of 347 data point for weight and mercury levels were used for yellowfin tunas.

For albacore tuna, the following publications were used: Morrissey *et al.* (2004) and Kumar *et al.* (2003). The fish contaminated with 1.01 µg Hgt/g was eliminated from the data set because it was considered an aberrant point. The origin of tuna is the Pacific Ocean. A total of 106 values for weight and mercury levels were used for albacore tunas.

For bigeye tuna, the publication of Aldrin *et al.* (1973) was used. The origin of bigeye tuna is the Atlantic Ocean. A total of 56 values for weight and mercury levels were used for bigeye tunas.

For Atlantic bluefin tuna, the following publications were used: Licata *et al.* (2005), Morales-Nin & Fortuno (1990), Relini *et al.* (2007) and Storelli *et al.* (2010). The origin of Atlantic Bluefin tuna is the Mediterranean Sea. A total of 89 values of weight and mercury levels were used for Atlantic bluefin tuna.

For skipjack tuna, the following publications were used: Kojadinovic *et al.* (2006) and Kumar *et al.* (2003). For the first publication, only the length of tunas is recorded. So, tuna weight is calculated by using the formula: Weight (kg) = $1.55.10^{-6}$ x Length $^{3.39301}$ (cm) (Silas, 1985). The origins of skipjack tuna are the Pacific Ocean and the Indian Ocean. A total of 30 values of weight and mercury levels were used for skipjack tuna.

Relationship between weight and mercury level for fresh tuna

First, scatter plots present the relationship between mercury levels and weight of each fish for each tuna species (Figure 1).

If a relationship is verified, the selection by tuna weight can be continued.

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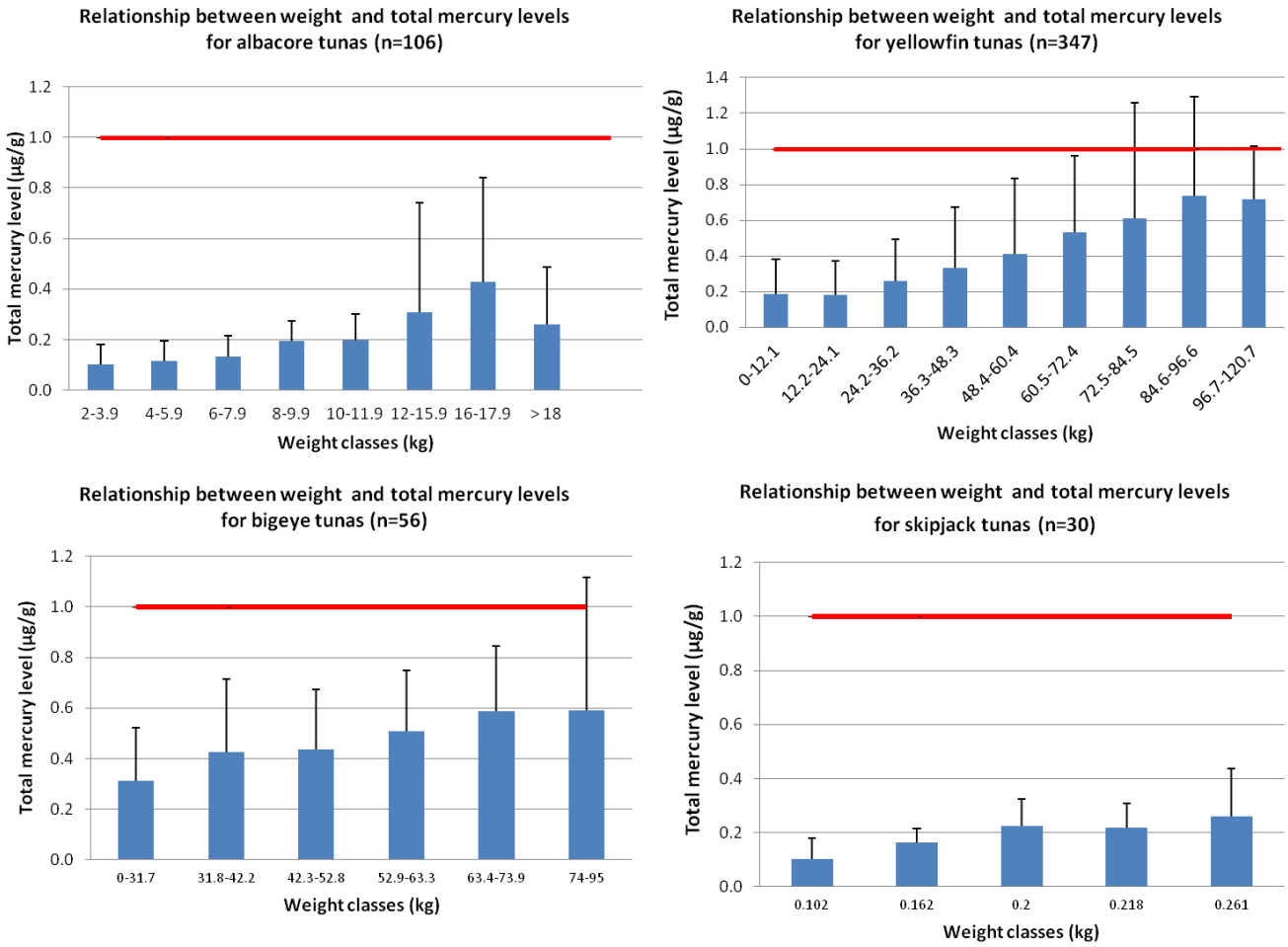

Figure 1. Relationship between weight classes and total mercury levels for four tuna species with error bars symbolizing 2 standard deviations

A relationship between weight and total mercury level was verified for each species except for Atlantic bluefin tuna. In the case of Atlantic bluefin tuna, small tuna could be highly contaminated whereas heavy tuna could be less contaminated. Consequently, this species will not be discussed further in this report.

Overrun of regulation threshold

Threshold of weight

The relationships between weight classes, defined by the Sturges' method, and total mercury levels for five fresh tuna species are presented in the Figure 19. For each class, mean and standard deviation (SD) are calculated. Then, a threshold of weight was set where a risk of exceeding the mercury level set at 1µg/g is possible (using 2SD, 95% of population, reaches mercury level about 0.8 µg Hgt/g ww).

Application of weight thresholds for each species is detailed in Table 1. No weight thresholds are necessary for skipjack tuna because no skipjack tuna exceeded the mercury concentration threshold. The feasibility of this hypothesis will be verified by comparing the weight threshold to the average weight of tuna caught by fishermen.

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Table 1. Threshold of weight and medium weight of caught fish

Specie	Threshold of weight (kg)	Medium weight (kg)
Albacore tuna	12	< 15 (IFREMER, 2001)
Yellowfin tuna	48	< 50 – 70 (ECOTAP, 2003)
Bigeye tuna	63	< 60 (ECOTAP, 2003)
Skipjack tuna	None*	4 – 10 (ECOTAP, 2003)

*: observed skipjack tunas weight ranges from 0 to 6 kg

For each species, the weight threshold approaches the average weight, so this solution is not absurd to implement it.

Percentage of overrun the mercury threshold at 1µg Hgt/g ww

Distribution of mercury levels of these weights were tested by the Excel add-in software: @risk (Palisade) to estimate the percentage of tunas which exceed 1µg Hgt/g ww. The best fitting distribution applied is this one defined in paragraph §3.A. Results are presented in the table 2.

Table 2. Percentage of tunas exceeding the mercury threshold setting at 1µg Hgt/g ww and number of tunas in each class

	% of tunas exceeding the mercury threshold (N)		
Specie	Regardless the weight	Under the threshold of weight	Over the threshold of weight
Albacore tuna	0.1 (106)	0 (79)	1.2 (27)
Yellowfin tuna	1.8 (347)	0 (205)	5.8 (142)
Bigeye tuna	0.6 (56)	0.1 (45)	2.8 (11)
Skipjack tuna	0 (30)	-	-

The risk of accepting tuna contaminated with mercury is negligible when they are under the weight threshold. The exception is skipjack tuna which has no weight restrictions. Even if the risk to exceed mercury standard level is low, the implementation of a weight threshold could be used to avoid contaminated tunas.

Conclusion

To conclude, three choices are possible to avoid the risk from mercury:

To reject tuna exceeding a defined weight to ensure that no fish exceeds the mercury concentration threshold over the mercury threshold for albacore tuna, yellowfin tuna and skipjack tuna

To accept all tunas because the risk of having a contaminated fish over the mercury threshold is negligible (less than 2% regardless of species weight except Atlantic bluefin tuna)

To apply the sampling plan for chemical analysis for Atlantic bluefin tuna

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