Content archived on 2024-06-18

Selection and improving of fit-for-purpose sampling procedures for specific foods and risks

Reporting

Project Information

BASELINE

Grant agreement ID: 222738

Project website

Project closed

Start date
1 August 2009

End date
30 November 2013

Funded under
Specific Programme "Cooperation": Food, Agriculture and Biotechnology

Total cost
€ 6 746 649,49

EU contribution
€ 5 154 885,00

Coordinated by
ALMA MATER STUDIORUM - UNIVERSITA DI BOLOGNA
Italy

Final Report Summary - BASELINE (Selection and improving of fit-for-purpose sampling procedures for specific foods and risks)

Executive Summary:
Food safety is a broader term, which means an assurance that food will not cause harm to the consumers when it is prepared and/or eaten according to its intended use. Food safety is a global issue that affects

the health of populations in both industrialized and developing countries. In 2002, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) held a joint consultation meeting to explore the principles and to establish guidelines for incorporating microbiological risk assessment in the development of food safety standards, guidelines and related texts. In this framework, the overall BASELINE objective was to provide harmonised and validated sampling strategies, structured as International standards, to support the European policies in food safety and to be suitable for food producers, in order to collect comparable data to improve quantitative risk analysis of selected biological and chemical agents.
The main risk based metric improved through the results of the project was that named as Performance Objective (PO), representing the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before consumption that provides or contributes to the achievement of an appropriate level of protection in humans against that hazard. During the project, prevalence and concentration data regarding a selection of biological and chemical hazards in specific food products, along the food chain, were collected and modelled to derive POs. For biological hazards, POs were defined as maximum percentage of samples positive for selected biological risk or as quantitative values, when possible. Sampling plans to verify the compliance to the proposed POs were suggested following the scheme of Regulation 2073 for microbiological criteria.
The results collected in WP1 to 5 show the approach followed for seafood, eggs and egg products, dairy products, meat products and plant products. Such approach include the experimental plans as well as the improvement of new analytical methods for data collection in relation to biological as well as chemical hazards. The modelling and validation work performed in WPs 6 and 7 was suitable for all food products investigated. The main outputs of data modelling were collected in a software tool which will allow users to calculate their own POs but also to collect data using an harmonized and statistically based scheme. The potential users can be food producers but also food authorities. The application of the software tool is supported by a dedicated webinar as well as training sessions dedicated to the different food products.
Even if the POs in the BASELINE project were calculated based on distribution results, without a connection with food safety objectives (FSO) and appropriate level of human protection (ALOP), this link will be immediately possible whenever risk assessment models for the selected food/hazard combinations will be available. Therefore, the BASELINE outputs strongly contribute to the implementation of Regulation (EC) 178 of the European Parliament and of the Council states aimed to base food law on risk analysis in order to achieve the general objective of a high level of protection of human health and life.

Project Context and Objectives:
CONCEPT AND PROJECT OBJECTIVE(S)
Food safety is a broader term, which means an assurance that food will not cause harm to the consumers when it is prepared and/or eaten according to its intended use. Food safety is a global issue that affects the health of populations in both industrialized and developing countries. A high level of protection of public health is one of the fundamental objectives of food legislation, as laid down in Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002. However, with the increasing food international trade and the fact that manufacturing sites in one country may provide raw materials to other manufacturers of finished goods (products) for large numbers of consumers, it is critically important that there be a harmonisation of food safety control procedures.
Safe food is produced by adhering to good hygienic practices (GHP), good manufacturing practices (GMP), good agricultural practices (GAP) and implementation of food safety risk management systems

such as hazard analysis critical control points (HACCP). However, the level of safety that these food safety systems are expected to deliver has seldom been defined in quantitative terms. Therefore, in 2002, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) held a joint consultation meeting to explore the principles and to establish guidelines for incorporating microbiological risk assessment in the development of food safety standards, guidelines and related texts. In this consultation, concepts such as Appropriate Level of Protection (ALOP) and food safety criteria were discussed in detail. In 2003, the CAC adopted the Guidelines for the Judgment of Equivalence of Sanitary Measures Associated with Food Inspection and Certification Systems (CAC, 2003). Afterwards, in 2004 it defined the so-called Food Safety Objective (FSO) and Performance Objective (PO). The FSO articulates the joint target of a food chain, including all relevant links in that chain and is common to all other food chains relevant to a pathogen/commodity combination. Performance objectives and performance criteria are two new concepts recently proposed to complement food safety objectives with respect to food safety control measures and process criteria regarding operational management of food safety. They complete the microbiological criteria, as reported by Regulation (EC) No 1441/2007 and Maximum Residue Limits (MRL) for many chemical contaminants, as reported by Regulation (EC) No 1881/2006.
Governments and food industries are expected to apply these concepts in practice, each within their own remit. To decide on such concepts and to implement them, it is of eminent importance to have information on the statistical and physical distribution of micro-organisms and chemical contaminants in foods but also to harmonise the sampling procedures and isolation methods in order to have comparable data.
It may appear impossible to harmonise the sampling schemes for food risks due to the huge diversity in food production and distribution of food in the different countries in Europe. However, the huge diversity is actually a benefit in the development and harmonisation work, because there is so much experience to learn from the different countries and food segments. To be relevant, the sampling schemes have to be built on the real, not simplified, production and distribution lines should consider the consumer food trends towards ready-to-eat foods, exotic or very local foods, health promoting foods, etc.

Regarding physical distribution, there might be a homogenous or an extremely inhomogeneous distribution of risk agents in the food matrixes. In case of a large, homogeneously distributed microbial contaminant in a batch, the agent will be detected by every sampling procedure, but in case of a very large but local contamination it may not. Also the statistical distribution is of the highest importance: most often, larger contamination levels are assumed to be log-normally distributed, though this is not based on solid information. However, if the number of contaminants per unit approaches one, continuous distributions are not appropriate. In order to determine which statistical distribution describes the reality well, large amounts of data are needed. This applies in particular to the adequate quantification of probabilities in the tails. These tails are important for the total exposure and for selecting the statistical distribution that most appropriately expresses this total exposure. Both aspects of distribution are also very relevant in risk assessments, where in many cases only prevalence data are available, whereas good quality quantitative data on contamination levels are lacking. It is well known that extreme contamination levels will have a large influence on the exposure. For these reasons European cooperation and harmonisation may lead to more awareness of upcoming risks, so they can be rapidly implemented in sampling schemes.
Within the described context, the overall BASELINE objective is to provide harmonised and validated sampling strategies, structured as International standards, to support the European policies in food safety and to be suitable for food producers, in order to collect comparable data to improve quantitative risk analysis of selected biological and chemical agents.

Beside the coordination and supervision of the scientific, technical and administrative issues between partners, the monitoring of the scientific quality of the results, the management of the contacts with EU Commission, and the management of the intellectual property, the specific objectives of the project were:

(1) To review the sampling schemes currently available for food authorities and food producers to collect data suitable for quantitative risk assessment at European level. The sampling protocols which were reviewed were those related to selected food/hazard combinations. Target foods included both products largely distributed in the EU (ex. smoked salmon, whole eggs, raw milk, poultry products with skin, lettuce pre-cut ready to eat salad) and niche products sold locally (ex. long eggs, unpasteurized cheese, Chorizo). The criteria driving the selection of biological and chemical hazards to investigate were their relevance for human health and the availability of data suitable for risk assessment. The review of available sampling schemes applied by both food producers and food authorities aimed to check weakness and strength of such protocols. The review on sampling plans and regulations revealed that there is a need for improvement and definition of microbiological criteria and other food safety criteria as well as corresponding sampling procedures.

(2) To assess the relevance and suitable limit values of POs and FSOs for biological and chemical risks, with special reference to the POs. The suggested POs were derived from the distribution of prevalence and concentration of specific biological hazards as well as chemicals in the investigated food chains. The biological hazards for which POs were formulated include foodborne bacteria (ex. Salmonellaenterica, VTEC, Listeria monocytogens) and viruses (ex. Enteric viruses) which are more frequently involved in human outbreaks as well as for the most relevant food chemical contaminants (ex. mycotoxins, pesticides).

(3) To evaluate the need for new or adapted methods for sampling and testing of the risk factors identified. The selected protocols and methods were those rapid, cost effective, characterised by specificity and sensitivity, able to produce comparable data between EU laboratories. They were presented and discussed with representatives of food authorities and food producers.

(4) To develop predictive mathematical models for biological risks and investigate and model sources and pathways of chemical contaminants to improve sampling schemes. These models were the structure on which to refine the design of food sampling schemes for efficient prediction of contamination and to reduce the number of analysis needed in food sampling schemes, with sufficient accuracy and reliability. The combined uncertainty of measurement results and distribution information were included in the development of the models. The data modelled included those on pathogens growth and/or inactivation during transport and storage at retail and consumer house. Such data were complemented with results concerning the fate of hazards during preparation procedures, like cooking. These data, largely missing in the literature, are needed to quantify the real risk that a hazard pose for human health at the time of consumption.

(5) To validate and harmonise the sampling schemes developed in the project and alternative detection methods. The validation of new methods was performed according to the principles of the international harmonised protocols for proficiency testing and the ISO 16140. The validated methods are often based on molecular techniques which are generally more fast of the cultural methods. Therefore, their application

should represent a huge advantage in terms of time needed to detect a food risk. The sampling schemes developed in the project were structured as those presented in the Commission regulation 2073/2005, last amended by Reg. EC 1441/2007.

(6) To share and disseminate the scientific knowledge deriving from the project to stakeholders. The dissemination of the project results was judge. In fact, an exchange of knowledge and experiences between SMEs and scientists was done along the project and the final results were presented to the SMEs through dedicated training session available on the web. The project results were presented and discussed with food authorities during dedicated workshops. Finally the most significant project results were shared within the scientific community through scientific papers published in International Journals.
Since a possible reason for the PO targets not being used could be that there is a little guidance on how to establish them, the software tool developed in the project show a user friendly approach to calculate these new food safety metrics.
The implementation of risk based metrics, such as POs, in future European Regulations might assist governments in conveying health goals throughout the food chain. An ALOP/FSO/PO based policy requires more than a better understanding of risks assessment or better process management of individual businesses. It requires an integrated approach in risk assessment, in management, and above all in risk communication. This means a new challenge in the way scientists, politicians, policymakers and food operators interact and the results of the BASELINE project aim to address this challenge presenting a possible approach to define POs for specific food/biological hazard combinations.

Project Results:
The work developed during the BASELINE project was articulated according to the main objectives to reach. In relation to the first one, regarding the review of sampling schemes currently available for food authorities and food producers to perform food safety quantitative risk assessment at European level, Table 1 summaries the food/risk combinations, for which sampling schemes were reviewed, when available. Target foods included both products largely distributed in the EU (ex. Smoked salmon, whole eggs, raw milk, poultry products with skin, lettuce pre-cut ready to eat salad) and niche products, sold locally (ex. long eggs, unpasteurized cheese, Chorizo). The criteria driving the selection of biological and chemical risks to check were their relevance for human health and the availability of public available quantitative data.
All countries carry out sampling of L. monocytogenes in fish and ready-to-eat products of fish, but the sampling frequency vary. In case of farmed fish, i.e. salmon and sea bass, most of the sampling is carried out by the food business operators. Some national survey programs are in place, but not every year. For processed products of farmed salmon, like cold smoked salmon and sushi, more surveys are carried out. The largest one is the currently ongoing European survey of L. monocytogenes in ready-to-eat products, which is carried out in all European countries. Samples of smoked fish represent 33 % of the samples in this program. The data represent a useful contribution for the development of relevant POs for L. monocytogenes in fresh fish that is going to be further processed to smoked fish and other ready-to-eat products.
The microbial criteria for L. monocytogenes in regulation 2073/2005 are based on international risk assessments of the bacterium in ready-to-eat products. The outcome of the assessment was that 100 cfu/g of L. monocytogenes in the product at the last day of shelf life was considered to give the appropriate

level of protection (ALOP). The microbial criteria are divided in products that do support growth, and products that do not support growth. For products that do not support growth, the L. monocytogenes criterium is 100 cfu/g at any stage in distribution chain, as the level will be constant. For products that do support growth, on the other hand, the food business operators have to carry out studies to document that the level of L. monocytogenes in their products will not exceed 100 cfu/g at the last day of shelf life, and set up own limit values for the products when they send it to the market. Even though this is a useful approach, it is a challenge to design and evaluate the results of such studies, both for the food business operators and the food safety authorities. Useful guidelines have been developed. However, better knowledge of the variation of commercial products, both in terms of composition, distribution of preservatives, temperature variation by heat treatment, as well as presence and growth of L. monocytogenes in the product is needed, both for correct categorization of the product, and to develop useful performance objectives (POs) and performance criteria (PCs) at various process steps. Further, more knowledge about the contamination level and growth of Listeria in ingredients for ready-to-eat products is needed, as the guidelines are not developed to detect such aspects.
Specific criteria for Vibrio and virus in shellfish has not yet been set up, partly due to lack of risk assessments needed as a basis for the criteria, and partly due to lack of good analytical methods. Instead, the legislation is based on monitoring of the production hygiene. A classification system of production areas based on the level of E. coli in the shellfish is used to decide whether the shellfish can be consumed directly/raw or first be purified, processed or relayed. The focus has until recently been on the harvest step only, but growth of Vibrio spp in shellfish during the distribution and processing line has been taken into account in Codex during the last years, and PO criteria can be developed. Sampling based on indicators is a cost effective solution, but the weak point is that E. coli is not necessarily a good indicator for Vibrio. Thus, sampling based on E. coli may underestimate the risks. See attached Annex1 - Table 1 – Food/risk combinations for which sampling protocols have been reviewed.

Regarding Biotoxins in shellfish, Regulation (EC) No 854/2004 requires that production areas must be periodically monitored to check for, among other things, the presence of toxin-producing plankton as well as biotoxins in live bivalve molluscums. Testing is required to be carried out for the toxins belonging to the following toxin groups: Diarrhetic Shellfish Poisoning (DSP); Paralytic Shellfish Poisoning (PSP); Azaspiracid Shellfish Poisoning (AZP) Amnesic Shellfish Poisoning (ASP). Sampling to assess the toxin levels in shellfish is generally based on two approaches; sampling of the shellfish itself, and sampling of the surrounding water in order to detect algal blooms that in the next step may lead to high levels of toxins in shellfish. The Commission is concerned of the periodicity of water sampling. In some European areas the sampling periods have now been reduced from 15 days to 7 days during the summer period, since 15 day sample periods have been found in some conditions to miss the entire growth and decline cycle. The sampling schemes are different in commercial zones and non-commercial zones. The sampling frequency varies for different toxins, type of shellfish, as well as with season. This involve that the sampling is risk based, as the sampling frequency is higher for toxins, areas and seasons when the probability of positive detection is high, and in areas where more people are likely to become ill in case of an outbreak. It seems that increased sampling frequency will increase the likelihood of detection of contaminated shellfish, as algal blooms may come rapidly, but, analyses are expensive. Ways to reduce the analysis costs will therefore improve the cost/benefit and risk/benefit of sampling.
Regarding mercury and Metmercury in tuna, two strategies can be used for the sampling zone of tuna, either the disembarkation/handling place or the fishing zone. Sampling in a given fishing zone is interesting

as it enables a better understanding of the contamination mechanisms, that is to say the origins of the contamination. But, as tuna is a highly migratory fish, the differences between the fishing zones may not be very important. On the other hand, if the sampling zone chosen is the disembarkation/handling place, an assessment of the population's mercury intake via tuna meat can be made. Detailed sampling plans of mercury in tuna are present in many countries. In general, it is found that large fish holds higher levels of mercury compounds than small fish. Food authorities in many countries have therefore chosen to give like pregnant women the advice to avoid tuna and other “big fishes” over a certain size. Further analyses of literature data and surveys already carried out by others may make it possible to improve the sampling schemes by taking specie and other parameters into account.
Regarding mycotoxins in feed, there is so far little knowledge about the correlation of mycotoxins in the feed and fish, but research projects to investigate this aspect has started in parallel with the BASELINE project. Indicative results from these are that the growth and quality of fish get poor when the feed contain mycotoxins.

For whole eggs and selected pasteurized egg products, sampling is generally done according to Reg. [EC] 853/2004 in connection with 2073/2005, amended in some cases by national law. Sample sizes for industrial products (pasteurized and dried eggs) comprise often n = 5 (acc. To Reg. (EC) 2073/2005). The location is often after the heating process. The methods applied for official control are mostly ISO cultural methods, but for the producers also rapid methods like PCR. Official control is risk oriented and is auditing the self-controls of the producers, which investigate larger sample numbers. There is no European legislation for official sampling of whole eggs (only broad sampling in breeding herds and laying herds during raising and laying period acc. to Reg. EC 2160/2003); however, there are some voluntary sampling plans done by official authorities. Mostly there is no differentiation between products from whole egg, egg yolk or egg white.

In all partner countries official food control is performed by federal state authorities for pasteurized milk at retailers based on the Reg EC 2073/2005 (1441/2007), and national surveys. Samples are tested for Salmonella spp. and L. monocytogenes and various pathogen microorganisms. Official sampling schemes for pathogens in cheeses are available for L. monocytogenes but not in the case of VTEC. Also, products collected at retail may undergo tests for many different microorganisms/pathogens. Some producers have their own sampling plans and they tested more microorganisms that are in regulations (they implement their hygienic standards). Few data have been collected by milk producers but they seem to follow official sampling protocols when they are available. The collection data performed on sampling protocols to test the selected biohazard in raw milk show that official sampling schemes for some pathogens (i.e. L. monocytogenes, VTEC) are lacking. In some countries there is no obligation to test raw milk, and only data for microbiological risks are those provided by national laboratories or some small milk producers do test raw milk before further technological procedure. Qualitative and quantitative data collected in the EU are difficult to compare because sample size, sample numbers, sampling location, sampling frequency and analytical method used are different between countries.
The key elements coming out comparing the information collected on sampling protocols applied by official authorities and food producers in relation to meat products show that so far qualitative and quantitative data collected in the EU are difficult to compare because sample size, sample numbers, sampling location, sampling frequency and analytical method used are different between countries. Obtaining information regarding sampling schemes from official and food producers was difficult in many countries due to a

number of reasons: official testing can be carried out by a number of different official laboratories across the countries and most results are sent to the national databases but are incomplete and there is no access to results on the databases for the purpose of risk assessments or other research activities; food producers are reluctant to co-operate for fear of damaging consequences or fear that there will be a requirement to do more testing which will be more costly. In addition, results of the private testing by FBOs are confidential and there is only a requirement to show that such testing is being carried out when audits arise; there is also a lack of understanding as to why such testing is required in some FBOs.
All food producers test their meat products according to Reg 2073/2005 but there are many gaps that can result in inconsistencies between FBOs such as the following:
- Frequency: Frequency is usually determined through the FBO's HACCP plans and rely on risk analysis. As a result, some FBOs may test routinely while others do very little testing and may even rely on official testing by the authorities. The frequency of official testing can vary significantly as well
-Methods: Although all official testing is carried out with standardized ISO methods, some private laboratories that carry out testing for FBOs try to use rapid recognized tests to shorten testing times. Various other recognized methods particularly for L. monocytogenes can also be used across different private laboratories and there may be differences in results between official testing (uses ISO methods only) and the private laboratory testing. Sometimes there is also a lack of understanding from the FBO of the methods used and results obtained through private testing.
-Shelf life studies: In the case of L. monocytogenes it is difficult for FBOs to establish whether their product(s) can support the growth of L. monocytogenes. A product that is deemed to have no Listeria following dispatch and then found to contain L. monocytogenes during export to countries (eg. USA) with 'zero tolerance' is costly for the FBO. The US authorities are considering regulations similar to the EU This acceptance in the regulation of a low level of L. monocytogenes in certain circumstances supports the widespread occurrence of L. monocytogenes and that control strategies are essential.
FBOs which carry out substantial testing usually do so because of client requirements. Clients usually have stringent testing of products prior to purchase. Requirements for different countries, eg. zero tolerance for America and Swedish requirements for Salmonella also exist.

Salmonella sampling in meat products is performed applying the EU regulation 2073/2005 but food producers in many countries also do additional testing for export to countries such as Sweden. Official control sampling schemes still not include Campylobacter because of its high prevalence and concentration in meat products. The baseline survey performed at EU level to estimate prevalence and counts of Campylobacter in broiler carcasses established the step immediately after chilling as sampling step and the ISO 10272-1 and 10272-2 as cultural detection and enumeration methods. In Germany an ELFA system is also used as screening method and real time PCR as confirmatory or rapid screening method.
Listeria monocytogenes in meat products is tested applying the EU regulation 2073/2005. However, for products not included in the regulation national law are in place. The critical gaps in the sampling protocols are that it is not always easy to establish at which stage in the production chain samples are tested. Moreover, is not always so easy to classify the products between those supporting and not supporting the growth of L. monocytogenes even if SANCO guidelines on this task are quite detailed. The analytical methods mostly used in the EU countries are the ISO 11290-1 and 11290-2 along with other recognized methods that are varying between private laboratories. However, national regulations are based on alternative methods such as the Italian directive to test L. monocytogenes in non RTE food including MPN.

Official sampling schemes for VTEC in the food industry are required and it seems crucial to include in future sampling protocols. EFSA has recently produced a report outlining technical specifications for the monitoring and reporting of VTEC on animals and food for Member States, however, the development of standard methodology for the sensitive and accurate detection of the clinically significant VTEC serogroups is ongoing.

The real extent of the illegal use of growth promoters seems to be much less favorable than the one observed based on the positive findings on animals in the slaughterhouse and farm. The determination of residues of drugs in matrices of biological origin indeed is often very complex, while also precautions are taken by the manufacturers, distributors and users of these compounds. The difficulties observed in the analysis are that some beta-agonists are strongly metabolized and the metabolites might still be unknown or their standards not available; recovery or sensitivity may be low.

In relation to plant products, viruses, E. coli O157:H7 and acrylamide are not included in the surveillance programmes. Moreover, the sampling plans differ between countries, and even between regions in the same country (regarding frequency, number of samples and lots to be tested, location and sample size). In available sampling schemes (for pistachio) few responses from producers were achieved. In addition, in most cases there is not enough information (regarding location and frequency of sampling) to consider these data representative of the whole amount of producers/business operators in Europe.

The second main objective of the BASELINE project was to the assessment of relevance and suitable limit values of POs and FSOs for biological and chemical risks.
In seafood (WP1), Performance objective for L. monocytogenes to ensure the food safety (defined as “Listeria levels not above 100 cfu/g on the last day of shelf life”) of fresh salmon intended to be used raw as it is, as sushi or cold-smoked salmon, can be set up at the fresh salmon processing step using a combination of Listeria limit values in the range 2-10 cfu/g, intended use of the salmon, and expected time-temperature conditions during storage. Performance objectives and sampling plans to identify batches or production companies with the same or higher contamination levels than in the samples analysed in BASELINE have also been developed: for fresh salmon, a high reliability can be obtained by testing 7 samples, using 0.976 log cfu/g as performance objective and rejection criterion. For cold-smoked salmon, absence/25 g in 88.8% samples of lot is a suitable performance objective. The sampling plan at retail (microbiological criteria) to achieve this PO is n=6, absence/25 g. Differentiated sampling plans and rejection criteria can be set up for different analysis according to their detection levels. Abuse temperature conditions, both at retail level, transport of products from retail to the consumer and, interestingly, during transport of samples to the lab are found to be important parameters to consider in development of safety criteria. Conditions leading to significant growth of Listeria have been observed both in the north and south of Europe. The prevalence of Listeria in surimi was found to be extremely low, but the growth rate is very high. Therefore, ways to ensure that no recontamination occurs between heat treatment and packing of surimi seems more important than analyzing large quantities products.
For biotoxins in shellfish, performance objectives have been developed for raw shellfish that are going to be cooked before consumption. The levels of toxins changes during cooking, but the change is both toxin and shellfish species specific due to different liquid loss and lipophilicity of the toxins. Mercury compounds in tuna have also been studied. These compounds are inert, and limit values which can be considered as food safety objectives have already been set up. Performance objectives are in this case the same as the

food safety objectives
It has not been possible to set up such criteria for viruses and Vibrio, for two reasons: 1. there is a lack of quantitative data due to the lack of quantitative methods to obtain such data, and 2. Risk assessments which are the basis for development of Food safety objectives, which in turn are the basis for development of performance objectives, have not yet been performed. However, it was found that Vibrio sp are common in the areas sampled in Croatia and Italy, but only a fraction of Vibrio contain pathogenicity markers. Furthermore, it was found that pathogenicity markers in Vibrio sp, as analysed by hybridisation methodology, are well suited as test parameters in areas with a high prevalence of Vibrio. Criteria based simply on Vibrio species would lead to copious waste of safe shellfish. For virus in shellfish, the results are similar as for Vibrio.

In relation to eggs and Salmonella(WP2), in the EU, no Salmonella of five named serotypes should be present in breeding flocks for laying hens producing table eggs and laying flocks should be free from S. Enteritidis and S. Typhimurium. Consequently, the product itself should be expected to be free from these serotypes. No PO can be recommended that can control this at a point near the consumer. Control in the primary production is the only realistic choice, but this cannot specifically distinguish between the named serotypes and other serotypes. An alternative a FSO would be absence of all Salmonella serotypes, but the public health benefit of this needs to be balanced against the economical burden it will mean to the producers. Heat-treated products should not contain Salmonella. Since non-compliance is very rare, a suitable PO based on microbiological values is not possible. Critical control of temperature during heat treatment is therefore crucial. With liquid pasteurized products, results of the current study can be used to formulate a final product, where re-growth due to re-contamination is not likely to happen.

Regarding milk and dairy products (WP3), according to current regulations (EC No 2073/2005 on microbiological criteria for foodstuffs criteria) for L. monocytogenes in ready to eat food is defined at dispatch (M=100 cfu/g; n=5; c=0), before the food has left the immediate control of the food business operator, who has produced it. This criterion shall apply if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life. The operator may fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life. Or: M= 0/25g; (n=5; c=0) - before the food has left the immediate control of the food business operator. In both cases PO = < 100 CFU/g. In case of unpasteurized cheeses (boiled cheese) stored at different temperatures (4 °C during 15 d + 12 °C during 13 d) as 95th percentile exceeds the established limit of 100 cfu/g, our recommendation is that PO should be fixed at the maximum allowable concentration not exceeding the FSO = 1 cfu/10g. For cheeses stored at higher temperatures (8 °C during 15 d + 12 °C during 13 d) as 95th percentile exceeds the established limit of 100 cfu/g, PO should be fixed at the maximum allowable concentration not exceeding the FSO = 5 cfu/200g. For VTEC in unpasteurized cheese no microbiological criteria are established, but our recommendation is zero tolerance because of the high risk and estimated low infective dose (PO = < 100 CFU/g). It should be taken into consideration that only with proper heat treatment of milk (at least 3 min at 75 °C) the production process will be under control. Cheeses made from raw milk or milk that has undergone a lower heat treatment than pasteurization have limits of absence of Salmonella spp in 25 g n=5; c=0 (EU regulation), and criterion applies for products placed on the market during their shelf-life (excluding products when the manufacturer can demonstrate to the satisfaction of the competent authorities that, due to the ripening time and aw of the product where appropriate, there is no Salmonella

risk). For raw milk there is a criterion of absence of Salmonella spp in 25 ml (n=5, c=0) if it is attended for consummation with no thermal treatment.

Regarding meat products (WP4), for Campylobacter in broiler chickens, a deterministic approach was selected to derive a potential FSO to be used as benchmark to elucidate POs for Campylobacter in broiler carcasses after chilling. POs were determined according to the maximum allowable prevalence and/or concentration not to be exceeded to be applied after the specific food chain step where samples were taken (i.e. after chilling).
Different factors were evaluated on the potential risk they pose on Campylobacter prevalence and concentration in broiler carcasses, based on the EFSA survey performed in 2008. The theoretical concentration under the detection limit (Cdet, log10 cfu/g) decreased when sample size was higher, thus increasing the probability of detecting positive samples. For the capacity of the slaughterhouse 74.9% of data were positive for those slaughterhouses processing more than 107 broilers/year. Incidence of positive carcasses for Campylobacter was slightly lower for slaughterhouses processing less than 107 broilers/year (72.0%). The percentage of censored data i.e. positive carcasses with a concentration less than 10 cfu/g, was significantly higher for those slaughterhouses processing more than 107 broilers/year (17.7% against 5.7% for slaughterhouses processing less than 107 broilers/year). No significant differences were denoted in the estimated parameters for the distribution of Campylobacter concentration ranging the mean values from 1.32 to 1.57 log10 cfu/g. The effect of thinning was more remarkable since the estimated mean concentration of Campylobacter for thinned carcasses was higher than those non-thinned (2.38 and 1.04 log10 cfu/g respectively). The effect of the factor time between sampling and analysis was not considered significant for the levels selected since N+ values were similar (75.0% and 65.5% for samples analyzed in more and less than 36h respectively) Mean concentration levels estimated by the MLE method ranged between 0.87 and 1.10 log10 cfu/g. Contamination level of carcasses were estimated between 2.48 (N+ = 88.7%) and -0.99 (N+ = 25.9%) log10 cfu/g for those countries with high and low contamination levels of Campylobacter, respectively. The estimated distribution parameters (mean (μ), standard deviation (σ)) were considered as the initial contamination level of Campylobacter after chilling of carcasses.
Basing on the knowledge of log-normal distributions estimated derivation of POs and microbiological criteria were performed. According to the risk factors selected PO values were derived in such a way those more than 99% units of the lot would comply with a specific concentration level. For low concentration data, variables sampling plans are not recommended since the probability of detection would require an unrealistic number of samples. Therefore, prevalence distribution was simulated and different percentiles were chosen to derive the minimum number of samples required to accept the lot at 5% CL. FSO was subsequently set as the proportion of contaminated units in the lot that would be accepted with a 95% probability.

To obtain accurate estimations of Salmonella and L. monocytogenes growth potential in naturally contaminated meat products the variability of the pathogens, food characteristics and storage conditions must be taken into account. This is important because there is considerable variability in naturally contaminated lots, so that single-point estimations do not reflect the potential growth which may occur during or at the end of the shelf life of a given food product. Instead of using single-point estimates of these variables, distributions characterizing the full range of potential values and their likelihood of occurrence are used as model inputs. Data of presence of Salmonella and L. monocytogenes under the scenarios

[(after packaging (S 1); after transport and storage at retail (S 2); after final storage at 6°C (S 3); after final storage at 14°C (S 4)] after were processed using Microsoft Excel v2010. The number of samples to test in order to verify the compliance to the POs was calculated using the binomial distribution at 95% confidence level.
The Salmonella prevalence found in the scenarios evaluated was 0.40 [0.31-0.49] for S1; 0.47 [0.38-0.56] for S2; 0.33 [0.25-0.42] for S3 and 0.26 [0.18-0.34] for S4. The theoretical concentration under the detection limit of the technique was calculated basing on the expected prevalence, yielding values between -1.59 and -1.92 log10 cfu/g.
POs for L. monocytogenes were considered as different target values from the pathogen prevalence. The median of the distribution (50th percentile) was chosen to subsequently derive two-class attributes sampling plans (c=0). FSOs allowed the estimation of the maximum percentage of lot units accepted with a 95% probability.
A quantitative risk assessment model for clinically significant VTEC in beef was updated and adapt in Baseline project. Based on the simulated model output, performance objective was set at various stages in beef chain. The model highlights the importance of setting PO at various stages of beef chain to increase the hygienic practice and ensure safety criteria for humans. The application of PO for carcass or beef trimming or freshly ground beef stages significantly lowered the probability of illness for raw, medium and well-done beef.
The tetracycline /beef matrix was used as an example to demonstrate how probability distributions of tetracycline contamination in beef based on surveillance data can be used in a spread sheet Monte Carlo simulation to estimate the risk that the population or an individual will consume tetracycline exceeding ADI. The model is dynamic and can easily be adapted to different countries' consumption patterns. In terms of human safety, using ADI for quantitative risk assessment is more useful than prevalent more simplistic approach of calculating the fraction of samples that exceeded MRLs.

Regarding plant products (WP5), results obtained for the different combinations of biological hazards and foods suggest the great influence of the specific conditions at factory on determination of potential PO and PC. Specially, the contamination levels at factory enabling to meet a specific FSO strongly depended on the contamination dispersion level in food and the efficacy of applied reduction steps (washing or disinfection). From that, the approach followed to derive POs and PCs was based on those values obtained at worst conditions of contamination (i.e. high dispersion or prevalence), so providing higher PO and PC values with higher safety margin. In the case of initial contamination in raw material entering factory (i.e. unprocessed product), POs for E. coli O157:H7, Salmonella and L. monocytogenes were quite similar, with values of -5.70 -5.25 and -2.80 log cfu/g, respectively. The POs established at a subsequent step, after washing and packaging, in finished product, showed higher values, with -6.80 -6.20 -5.50 log cfu/g for E. coli O157:H7, Salmonella and L. monocytogenes, respectively. Owing to those levels seem difficult to test by sampling plans or tailor-made microbiological criteria, as it would require a large number of samples, the establishment of PCs for the washing step was considered as suitable measures to reach the FSO in fresh-cut leafy green products. The reductions leading to the proposed FSOs corresponded to 2.7 1.7 and 0.5 log cfu/g for E. coli O157:H7, Salmonella and L. monocytogenes, respectively. These reduction levels could be verified by using indicator microorganisms such as coliforms and mesophylic bacteria or process parameters. Nonetheless, it is worthy to mention that results here presented could vary depending on the used models, for example, tiny variations in the model parameters for reduction by washing or growth during shipping and distribution could result in different predictions. Therefore, the

derived POs and PCs should be exclusively linked to the mathematical model conditions applied in our study.
Concerning fresh apples, POs were defined on prevalence values and not on concentration values as performed for previous risk combinations. This was due to the lack of information about concentration, since not data are reported about this issue. The simulations demonstrated that prevalence levels in unprocessed apples equal or below 0.1% would lead to meet the proposed FSO for Salmonella(-3.21 log cfu/g) proposing this value as a potential PO. On the contrary, for processed apples, the derived PO corresponded to 0.08 % provided that contamination levels in contaminated apples are equal to or below 4 log cfu/apple. These values were greatly dependent on the specific conditions at factory (e.g. initial concentration and efficacy washing steps) and also on the model approach used for deriving the above mentioned values. However, it seems that those prevalence values would be difficult to be tested by practical samplings schemes. A first approach indicated that the probability of detecting a contaminated sample taking n=10 when prevalence level is fixed to 0.1% was lower than 0.01 which means that a high number of samples (>2000) would be needed to reach a more acceptable confidence level. Besides that, according to the results obtained in the experiment performed with apples (prevalence = 3%), sponges and water showed a higher contamination percentages when contaminated apples were processed irrespective to whether or not cross contamination took place. These levels would enable to carry out a more effective sampling plan on environment requiring lower number of samples to detect the potential presence of contaminated apples. Nonetheless, this hypothesis should be proved or validated at lower prevalence levels as those used in the simulated model (<<3 %).
For studying the distribution of residues, the coverage of 97.5th percentile of contamination is recommended at 95% probability level. (These criteria are suggested as they are used by FAO/WHO and EFSA for estimation of short term intake to be compared with the acute reference dose). This will require the analysis of ≥119 primary samples For planning official control programme, the targeted performance criterion is the ≥98% compliance with MRLs. The probability level of testing compliance will depend on the estimated level of risk.
The present study demonstrates that the emerging risk management metrics, FSO, PO and PC, might be also applied to the mycotoxin hazard. The example here presented underlined the need for better and more structured information on the impact of the storage and processing steps on mycotoxins accumulation. Moreover, the problem of the impact of uncertainty in PO and FSO compliance was brought up. FSO was considered equivalent to the maximum EC limits (Commission Regulation (EU) No 165/2010) and to the PO after processing. However, the determination and compliance with such levels is totally dependent on the validity of sampling and analytical procedures.
Acrylamide in foods is largely derived from heat-induced reactions between the Ramino group of the free amino acid asparagine and carbonyl group(s) of reducing sugars such as glucose. Secondary sources include analogous reactions in which several other free amino acids and carbonyl compounds participate. Reduction of asparagine and/or glucose content in unheated foods is expected to result in low-acrylamide foods. The available information on adverse manifestations of acrylamide and its major metabolite glycidamide indicates that neurotoxicity is a documented effect in human epidemiological studies; reproductive toxicity, genotoxicity, and carcinogenicity are potential human health risks only on the basis of animal studies. The cited conclusions and interpretations will undoubtedly be modified in the future as more information becomes available about what nature intends for acrylamide. Advice on consumption can be a complementary way of reducing the intake. This indicates the need to consider a management strategy combining regulatory means with consumption advice and risk communication activities.

Industrial processing and catering are the places in the food chain where mitigation efforts would be most efficient. At the home cooking level, avoiding overcooking is probably by far the most important action to recommend. Authorities must also consider national differences both regarding diet, cooking practices and risk perception. Home cooking advice is probably most efficient when combined with general consumption advice.

In relation to the objective regarding the evaluation of the need for new or adapted methods for sampling and testing of the risk factors identified in order to produce data to risk analysis, in seafood, an improved methodology for L. monocytogenes detection in smoked salmon by wet pooling test has been developed and tested. Two protocols for low detection levels have been successfully developed and tested. Such methods are needed before performance objectives at early process steps can be implemented. Two approaches have been developed in BASELINE and tested. One is an adaptation of the ISO method, giving detection level 2 cfu/g. It has been tested with good results in collaboration with the European reference laboratory for Listeria. The second is a MPN assay for rapid detection and enumeration of extremely low Listeria levels and uneven distribution of Listeria. The assay has been developed to prototype level and tested in industry. Even though some testing of the assay remains, we consider the potential for commercialisation of the assay as large. For new analysis methods for surveillance of toxins in shellfish, chemical analysis of the algal toxins from the passive sampling devices is less time-consuming than for shellfish. Sample preparation is rapid and simple, and few interfering components are present in the sample extracts. This technique could substantially reduce the need for a large proportion of routine shellfish monitoring tests by reducing the number of expensive chemical analysis or reducing the number of mouse bio assays by ethical reasons.

A qPCR assay for the quantification of Salmonella in egg and egg products was set up. Standard curves specific for each egg product were built. A competing flora in the range of 103 to 108 CFU as well as a burden of dead bacteria up to 106 Salmonella was shown not to interfere with quantification. This assay was demonstrated as a robust, rapid and sensitive method for the detection of Salmonella not only in egg and egg products but also in egg containing dishes (tiramisu and long eggs).

Real-Time PCR assays for detection and quantification of L. monocytogenes, Salmonella, VTEC in milk were developed, optimized and compared to reference culture methods. An analytical strategy was developed based in enrichment in half Fraser and a subsequent DNA extraction and L. monocytogenes-specific real-time PCR for a sensitive detection of this pathogen, using higher amounts. It was demonstrated that following this approach the limit of detection was 1-10 cfu per sample, but using samples of 50 g instead of 25g. This increases the analytical sensitivity and therefore could also increase the chance of detecting the pathogen. An optimized qPCR protocol for Salmonella Dublin detection in raw milk was developed and demonstrated a good reproducibility for artificially contaminated raw milk samples and a detection limit from 1 to 1x104 cfu/ml. The comparison of VTEC quantification by MPN-PCR method (five 1 to 10th dilution series of three tubes with enrichment in BPW for 24 hours) showed a correlation coefficient R2 in a range between 0.947 and 0.993. The two methods were therefore equivalent, but the qPCR method was faster and less time consuming.
Two Real Time PCR protocols for the rapid detection of Salmonella and Listeria monocytogenes in pork meat were optimised by testing different extraction protocols as well as by using minor amounts of pre-enrichment broth and shorter times incubation, in order to reduce costs and times. The new methods

showed a good performance when compared to standard ISO reference methods. In particular for L. monocytogenes PCR method, we have demonstrated that all the samples incubated for 24 h produced positive results also in reduced amount of Half Fraser Broth (HFB), while the sample incubated for both 5 h and 8 h produced negative results for each of three volume of HFB. The statistical analysis of the data does not show a statistical significance between the two volumes of pre-enrichment 1/5 w/v and 1/10 w/v at both levels of inoculum (8.4 and 84 CFU/mL), while showed a statistically significant difference for 1/3 w/v of HFB dilution at both levels of contamination. In this latter combination, in fact, the very small quantity of HFB creates a gelatinous consistency matrix, that it is very difficult to withdraw forming a very inhomogeneous extraction volume. The same experiments performed by ISO methods showed that all the samples tested at two different levels of contaminants, three volumes pre-enrichment broth and three incubation times produced positive results. This data demonstrated that the detection of L. monocytogenes by ISO method could be performed using shorter time of incubation.

Quantitative detection methods for Listeria monocytogenes and Salmonella enterica in plant products using EMA-qPCR were designed and optimised. The methodology described has shown an exceptional performance with excellent analytical sensitivity (down to 10 cfu/g of RTE lettuce) and with quantification capacities (linearity and PCR efficiency close to the ideal ($R2 > 0.998$) and PCR efficiency close to 100%).

The results regarding the calculation of POs and FSOs as well as the new or adapted methods for sampling concurred to the definition of new or improved sampling plans for selected food/risk combinations.
For the studied seafood (WP1), sampling programs often are carried out with fewer samples per batch than the official sampling plans indicate. This is probably due little awareness of the in-homogenous distribution of risk agents in a batch, and that analysis of many samples is expensive. Two ways to obtain improved and more cost effective sampling may be to 1) make sure that representative samples are selected and 2) to use pooled samples. The prevalence and levels of L. monocytogenes in fish sent from the producer vary with the time of the day it has been processed. The levels are very low immediately after processing, but increase during storage. Most of the bacteria seem to remain attached to the positive samples, but a fraction is transferred to the samples in direct contact with the positive samples and the samples below. Water released from the muscle and melting ice seem to be the main contamination route from positive to originally negative samples in a box. A sampling protocol for harvesting Listeria from large samples or pooled subsamples has been developed. Fish samples are washed with a growth medium for Listeria and the liquid fraction is collected and analysed. The protocol gives reliable and rapid detection of extremely low levels of Listeria in batches even if only 1 of 10 pieces is contaminated as low as 2 cfu/g. The protocol can be adapted to various detection levels and POs suitable for fresh fish, double-frozen fish, other fish species, raw material control for fish intended is used for production of sushi, carpaccio and cold smoking. Two protocols for detection of low levels of L. monocytogenes have been successfully developed. One is an adaptation of the ISO method, giving detection level 2 cfu/g. This method is currently being validated by the European reference laboratory for Listeria. The other is a MPN method derived from the ISO method and has detection level 1 cfu/5 g of fish.
Okadeic acid has been used as model toxin in studies related to biotoxins in shellfish. Knowledge about the distribution of this toxin has been obtained from reviews of literature and earlier monitoring programs. The impact of sample size and number of samples has been assessed using the Whitaker method. It is found that the best fit sampling plan is taking two samples of 30-40 samples each.

As for toxins, a lot of data about levels and distributions of mercury compounds are available in the literature. These have been collected and assessed using models. In conclusion, pooled samples from 10 tunas seems to be the best trade-off between reliability and costs for all fresh species except albacore tuna which one pooled sample from 5 tunas is sufficient and skipjack tuna which one pooled sample from 2 tunas is the best sampling plan. For canned tuna, pooled samples with 5 cans seem to be the best trade-off between reliability and costs.

Regarding egg and egg products (WP2), improved sampling plans for table eggs and powdered eggs have been evaluated. In particular studies were performed on: 1) the effect of pooling on the detection of Salmonella by comparing both ISO and real-Time PCR methods and 2) the best sampling strategy for powdered eggs starting from the assumption of an heterogeneous distribution of Salmonella cells within the egg product. According to a Monte Carlo simulation on the number of eggs to be tested in order to detect Salmonella in positive pooled table eggs with 95% certainty and assuming a Salmonella contamination ranging from 10 to 1000 CFU/pool of table eggs, Real-Time PCR was more sensitive and requires a lower number of eggs to be tested in comparison with the cultural method (160 vs 72 eggs tested in pools of 10 or 9 eggs each) However, with both methods, sampling needs to be very intensive to be sure not to falsely accept a positive lot, even when pooling is used. According to the Habraken approach for the evaluation of an effective fit-for-purpose sampling plan for powdered eggs, taking more and smaller sample units while keeping the total sample weight constant, improves the performance of sampling plans. Fit-for-purpose sampling plans for Salmonella detection in table eggs and powdered eggs act as alternative tools to obtain information about the expected prevalence of Salmonella in a food lot and how contamination can be distributed within this lot. Nevertheless, it must be pointed out that the intensity required for these egg-by products is not practical so they do not fully serve to discriminate between contaminated and non contaminated lots.

Regarding dairy products (WP3), the establishment of food safety criteria and sampling plans for L. monocytogenes in hard cheese is not practical. Considering hard cheese as a ready-to-eat food, if the established FSO =2 log cfu/g, a very high contamination of samples after production would be needed to cause listeriosis due to cheese consumption. Therefore, sampling plans may be useful as alternative tools to obtain information about the expected prevalence of L. monocytogenes in a food lot and how contamination can be distributed within this lot. Nevertheless, it must be pointed out that the intensity required is not practical so they do not fully serve to discriminate between contaminated and non-contaminated lots. Sampling strategies might be alternatively addressed to determine microbial prevalence and concentration on food-contact surfaces or aerosols in order to localize the main source of pathogen contamination. The level of listeria contamination in hard cheeses is very low. In that case the use of higher amount of sample is advisable. Within the WP3, partners have developed an analytical strategy based in enrichment in half Fraser and a subsequent DNA extraction and Listeria monocytogenes-specific real-time PCR for a sensitive detection of this pathogen, using higher amounts: We have demonstrated that following this approach the limit of detection was 1-10 cfu per sample, but using samples of 50 g instead of 25g. This increases the analytical sensitivity and therefore could also increase the chance of detecting the pathogen.
For boiled cheese sampling plan is based on the different storage scenarios. For the first scenario (15d stored at 4°C + 13d stored at 12°C) growth of L. monocytogenes exceeds the FSO at 25th storage day. This implies that microbial contamination should be lower in the final product and/or a lower storage

temperature may be required. The PO could be established just after elaboration considering the maximum concentration/frequency allowable to fulfill the FSO (2 log cfu/g) at the end of the shelf-life. In this case, it can be fixed to-1 log cfu/g (0.1 cfu/g = 1 cfu/10g). For the second scenario (15d stored at 8°C + 13d stored at 12°C) this value should be low to -1.6 lo g cfu/g (5 cfu/200g). Therefore, higher storage temperatures would need a lesser contamination level to achieve the same FSO. To estimate the number of samples needed to accept/reject a lot, presence/absence tests must be done in order to detect positive samples. With such a low mean concentration (1 cfu/10g and 5 cfu/200g), an enrichment protocol should be applied. Assuming the PO values established for the scenarios above mentioned 4 samples are needed assuming a storage of 15d at 8°C + 13d at 12 °C if a 25g sample is taken. For the scenario in which storage temperature is 4°C, 5 samples would be required to have the same discriminatory power. Nevertheless, it should be pointed out that the probability of detecting positives slightly increases from 10g to 25g sample. For the scenario of storage at 4°C, taking 5 samples of 8g each would produce the same efficiency as 5 samples of 25g. Likewise, for the scenario of storage at 8°C, 4 samples of 11g each would produce the same efficiency as 4 samples of 25g. To optimize sampling plans, sampling size can be reduced in both cases, since the number of samples to obtain a 5% probability of acceptance is the same.
Appropriate sampling schemes for VTEC in raw milk intended to be sold raw to final consumers need to consider that distribution in milk is not uniform and concentration is very low in positive samples. The number of sample units to be taken should ensure that a value below the infective dose could be reached with a ‘negligible’ probability. An estimate can be produced by modelling the data relative to the prevalence and concentration of microorganisms like non pathogen and enteropathogens strains E. coli O157 and O26. Mixing can only have the effect of reducing the chance of detecting the pathogens by lowering their concentration below the analytical detection level with currently used analytical protocol that require confirmation after isolation of target strains (O157, O26, O111, O145, O103,…) and characterization of their virulence traits. Alternative real-time and RT-PCR method, which was introduced for the detection in experimental studies, were evaluated in the framework of Task 3.4. As it was already mentioned in D.3.2 heat inactivation experiment was done by UNIBO, and repeated by Teagasc. Partners have concluded that there is no correlation between heat tolerance and serogroup but only six strains of VTEC were tested in the study.
Collected data of aflatoxin content in milk in Italy showed that approximately 2% of milk consignments have concentration above the maximum limit (EU Regulation 1881/2006). The data show that currently the controls at farm level are inadequate in controlling the aflatoxin contamination of feeds, therefore screening of raw milk prior to processing is needed. It is very important to monitor periodically all the farms supplying the milk and in order to protect the consumers the frequency of sampling should be adequate to ensure that the risk that contaminated milk batches are not inspected and accepted is low. A tolerance has to be defined (e.g. less than 5% of probability that the number of not compliant milk batches that are processed is equal or more than 2%. The not-compliant milk batches can be defined as the commingled milk consignments (represented by samples taken at the processing plants from the transport tankers) that have an AFM1 concentration higher than the Action Level (e.g. 0.04 µg Kg-1).
When samples indicate that samples are not-compliant, surveillance sampling plans has to be actuated in the production district that has supplied the milk and inspection by sampling and analyses of each consignment from that district (group of farms) must allow the detection of the source of aflatoxin contamination (e.g. feedstuffs). The frequency of sampling plans actuated for monitoring purpose should be higher during the seasons that historically have shown irregular consignments. The minimum frequency can be calculated on the basis of the prevalence of samples that historically (e.g. in the last five years)

were positive for AFM1 at a level higher than the Action Level.

Regarding meat products (WP4), practical examples on how to derive risk based metrics, like POs and FSO, from distribution values were shown as well as the statistical elaboration of sampling plans to verify the compliance of food lots to these safety criteria. The results showed the influence of processing variables and storage practices on the definition of sampling schemes. For Campylobacter, the existence of thinning method and/or high concentration on poultry carcasses would imply a less stringent sampling plan to detect positives and consequently, reject the food lot. For Listeria and Salmonella in pork cuts, abuse temperature (14 °C) or long storage periods (14d) could lead to higher prevalence and concentration values in the final product. Finally, for VTEC in ground beef variability in the serogroups studied was observed although most of the simulated values fell within the FSO established. With this information, food operators can define sampling schemes based on the maximum allowable number of units not exceeding the food safety criteria reported in this document. Different management strategies can be adopted to verify the compliance of food lots to these safety criteria according to the expected processing and storage conditions.
The risked based approach to select herds in a drug residue monitoring has a higher payoff and correspondingly a higher detection rate compared to the conventional proportional sampling approach as demonstrated by the tetracycline beef decision tree model. The magnitude of the difference depends on drug and meat matrix. As all European countries already have their own national residue programs, there is no added cost if they wish to move to the more cost efficient risked based probability sampling scheme. The model is dynamic as the prevalence and herd data will change from year to year. By using data obtained from a cost effective method, food safety authorities can better set FSOs and POs based on the different risk strata (husbandry type, disease profile, demographics, geographical) of the cattle industry.

Regarding plant products (WP5), in general, the required number of samples were higher than 10, and given the low prevalence, indicators or environmental sampling (sponges, water, centrifuge) could be important tools to detect the presence of enteric pathogens in processing facilities. Concerning Listeria monocytogenes, Performance Objectives could be tested or monitored by using sampling schemes even though, again certain assumptions were needed to derive attendant sampling plans.
The results of analyses of residue in representative crops together with the data published in scientific literature, provided experimental data for characterisation of the distribution of residues, estimation of sampling uncertainty and validation of the results obtained with model experiments. The absence of AFs in raw pistachio cannot be guaranteed, thus relying on industrial processes for a certain reduction is required. The food industry is responsible for setting up food safety management systems that deliver foodstuffs in compliance to the FSO.
According to the initial and final values proposed by European legal limits, processing (either selection or selection plus roasting or just roasting) is expected to decrease in 33 % the initial aflatoxin concentration in the raw pistachio. Our results suggest that about a 75 % of reduction may be achieved by the single roasting process, thus under the hypothesis of raw pistachio compliance with maximum level, the roasted pistachio must be safe. The underlying problem is the uncertainty associated to the aflatoxin levels reported in the present work and any other existing works; the high uncertainties due to sampling and sample preparation procedures may lead to unrealistic results, and this is an issue that needs to be solved. In the present work the major variability was associated with the subfraction selection and therefore increasing the number of the analysed subfractions could be an alternative for reducing uncertainty.

In relation to the objective to develop predictive mathematical models for biological risks and investigate and model sources and pathways of chemical contaminants to improve sampling schemes, during the project a systematic review of available mathematical models to predict microbial behaviour in culture media or food matrices comprising of logistic type models, mechanistic models, self-limiting models as well as a review on the predictive models included in ComBase, USDA Pathogen Modelling Program 7.0 and Seafood Spoilage and Safety Predictor (SSSP) have been performed. An excel template for the reporting of microbiological as well as chemical data have been edited for data collection from Partners along with guidelines for the sampling of microbiological and chemical contaminants. Finally, a BASELINE Sampling Terminolgy Codex has been agreed.
The results analysed allowed to identify the most relevant conditions influencing microbiological behaviour in a food matrix, such as the intrinsic factors, the microbiological and food matrix characteristics, and the extrinsic factors, which are temperature, pH, water activity (aw), processing time etc. This result has been reached for each specific food within the Hazard and Analysis of Critical Control Points (HACCP) system. The statistical distributions of the microorganisms at critical control points such as the slaughterhouse, at retail, and at consumption has been calculated as well as the association between food risk factors (temperature, storage time, time between sampling and analysing, season of sampling) and contamination level.
For chemical contaminants, pesticide and veterinary residues the critical control point (CCP) is the time when the food is marketed or offered for sale at the first time, hence, the corresponding data provided by WP3 and 5 to WP6 were generated at this point. The WP6 analysis of the data found two distinctly different situations for chemical contaminants and pesticide residues.

Based on data provided by WP1-5, predictive models for microbial growth, survival, growth/no growth and cross-contamination for the food/risk combinations selected in BASELINE project were developed. These models, together with models found by an extensive literature research, were integrated in the database of an internet based software* application developed by WP6 in collaboration with a subcontracting company, Optimum Quality, Cordoba. The results are reported in D6.5.The software application www.baselineapp.com comprises of a modelling module that integrates predictive models developed and found by research within the BASELINE project. It allows the user to input raw data, the modelling of data, the comparison and validation of models, and the export of data into different formats. The software offers a graphical user interface (GUI) where predictions from modelling can be used for the development of sampling plans and microbiological criteria for specific food/risk combinations under specific environmental conditions. Since then, the software tool has been continually subject to further development, the sampling plans module was continually developed by integrating a generic module, practical examples, specific sampling plans, a decision support system for the inexperienced user Furthermore, performance objectives and sampling plans were exemplified from data for several food and (microbiological) risk combinations. Finally, the software allows predicting microbial behaviour of different pathogens and food matrices with validated models, adding new models and data, providing an easy derivation of Microbiological Criteria and Sampling plans from previously established risk-based metrics, offering a tools menu to perform scenario analyses, and including decision-support system to help non experienced users to choose the suitable option.
Different statistical methods were applied to accurately estimate the limit of detection (LOD) or limit of quantification (LOQ) of the analytical techniques. Once distribution parameters were estimated, PO values

and sampling plans were according to WP1-WP5 activities.
Regarding pesticide residues in primary crop units, single sample increments, and composite samples, experimental data available from the scientific literature were collected and complemented with specifically designed field studies carried out on carrot, parsley leaves, apples, lettuce and gooseberry. Based on the data, recommendations for performance objective concerning the compliance of chemical contaminants with legal limits were given. It was recommended that a 98% compliance level should be introduced in the EU legislations unless the food contaminant concerned could cause direct health risk. Under these conditions, food producers and traders could safely verify the compliance of their products at 95% probability level economically applying the concept of action limits (the method of calculation and practical application of the Action limits is demonstrated utilising the elaborated typical sampling uncertainty). Furthermore, on the basis of this data, the principle of planning risk based monitoring programmes was demonstrated. It involves a 2-level tiered approach procedure taking into account the health risk caused by the potential exposure of consumers over the acceptable short and long-term intakes, percentage violation rate of legal limits, number of supervised and monitoring samples analysed.

Finally an extensive database consisting of 26113 aflatoxin M1 measurement results in milk carried out by the Italian milk producers association during 2003-2010 was used for the elaboration of risk based monitoring and early warning programmes. The programme is based on the moving window concept taking 5 random samples each day from randomly selected collection points weighted according to the number of farms providing milk. Based on the results obtained within the 5-day window and applying an action limit of 40 ng/kg, the compliance of 98% of the produced milk with the 50 ng/kg legal limit can be verified with about 90 % probability. The applicability of the model was tested with computer simulation of likely AFM1 contamination applying the actually measured values. Since the compliance of agricultural commodities with legal limits can only be guaranteed if all stakeholders of the production chain act responsibly, the programe aims of verifying the effectiveness of a production control system if the production is getting out of control.
Regarding the validation and harmonization of the sampling schemes developed in the project and alternative detection methods, it was estimated the sampling uncertainty for chemical contaminants. The uncertainty of sampling was estimated by repeatedly drawing random samples of various sizes with replacement from the unit crop residue pesticides data, or based on the results of replicate samples taken from the supervised trial plots sites. The results obtained were compared with those obtained from the limited number of samples (332) taken from field trials carried out. Altogether the sampling uncertainty was estimated for 146 individual food and feed commodities. Recommended typical sampling uncertainties with upper confidence limits were defined for 22 commodity groups. The upper confidence limits of typical sampling uncertainties are recommended for use in calculating action limits because of the potential serious consequences of declaring a sampled lot as meeting the specification when it is actually not compliant. The results provide the basic data for the calculation of action limits in regular process control and in early warning sampling plans.
The number of random samples (n) to be tested for verifying specified level of compliance (βp) with selected probability (βt) was calculated based on the binomial distribution. The advantage of using the binomial distribution to decide on the required number of sample is that the sample size is independent from the number of lots (N) to be sampled if N >> n.
n=(lg(1-β_t))/(lgβ_p)
The applicability of the binomial distribution theory and the above equation for calculation of the required

number of samples to be taken from a commodity containing the contaminant over a wide range (> 2000 fold) and scattered at the upper end was tested utilising the experimental data on aflatoxin M1 contamination in milk. The computer modelling performed with sample sizes between 29 and 299 with 1000 and 10000 repeated random sampling with replacement confirmed that the applicability of the method, even for the critical 40-50 ng/kg AFM1 concentrations range.
During the project possible microbiological criteria (MC) for Campylobacter in broiler carcasses were provided and a sampling plan to verify compliance with such criteria were suggested. Data were gathered on the presence and concentration of Campylobacter in broiler carcasses collected in three different Italian slaughterhouses, labeled as A, B and C. The sampling plan to be validated in each slaughterhouse included the analysis of three different carcasses collected immediately after chilling from 30 different lots, for a total of 90 samples per slaughterhouse. The number of positive samples containing above 100 CFU/g and above 1,000 CFU/g throughout the 30 tested lots was determined to estimate between-lot variability. Based on this information, the performance of four MC was evaluated for lot compliance: i) n=3; c=0; m=100 CFU/g; ii) n=3; c=0; m=1,000 CFU/g; iii) n=3; c=1; m=1,000 CFU/g and iv) n=3; c=2; m=1,000 CFU/g. Positive Campylobacter samples were found in 60% of the lots tested in slaughterhouses A and C and in 73.3% of lots from slaughterhouse B. The differences among the three slaughterhouses in the mean Campylobacter levels found in positive samples were not significant and were used to evaluate the performance of the MC. The level of lot compliance to different MC was calculated and for the most stringent one (n=3; c=0; m=100 CFU/g) was 40% at slaughterhouses A and C but only 26.7% at slaughterhouse B. The results of this study show a possible approach to establish MC for Campylobacter in broilers. According to (1) Campylobacter prevalence and concentration in Italy, (2) applied experimental plan and (3) selected slaughterhouses, the number of compliant lots to the suggested MC ranged between 26.7 and 100%.
Many alternative methods for different combination pathogen/food matrix, included and not included in Regulation EC 2023/2005, have been defined during the project and in house validated. Among foodborne pathogens and food matrices listed in Regulation EC 2073/2005 (Anonymous, 2005), we chose to perform a European validation of a molecular method (Real-Time PCR) for the determination of Listeria monocytogenes in soft cheese and Salmonella spp in pork meat. The validation approach is based on a collaborative and inter-laboratory study in accordance with the ISO 16140:2003, using a simple detection strategy based on ISO compatible enrichment coupled to a DNA extraction and a consolidated real-time PCR methods for the rapid and specific detection of pathogens in foods. In addition, differently to other validation study that was focused on one step only (e.g. PCR conditions) and thus gave no information on how the validated PCR protocol would work in routine analysis, we have performed our validation process evaluating the whole process from the enrichment step of the microorganism in an appropriate medium to the evaluation of the results obtained by cultural method and by alternative methods. The approach of all of the Real Time PCR methods have to be: to use the same enrichment broths (Half-Fraser for Listeria monocytogenes, Buffered Peptone Water for Salmonella spp.) of ISO methods, having a rapid protocol (next-day results), as easy and simple as possible (easy to be implemented in a routine lab), use an easy and simple bacterial DNA extraction protocols, compatible with ISO standard (i.e. the ISO standard must be continued once the results are obtained by the alternative methods), not too expensive (similar price as standard).Thirteen laboratories from seven different European Countries participated in the trials. The results obtained in these ring trial studies that the performance of the Real-Time PCR method is equal or higher to that using the reference ISO methods. As the two steps for fully validation indicated in that ISO standard (in house validations using different matrices and interlaboratory study) has been successfully

conducted, we can conclude that the Real-Time PCR method meets the requirements of a diagnostic PCR and has the potential to become a standardized method for the rapid detection of Listeria monocytogenes and Salmonella in diagnostic laboratories. The Real-time PCR method to detect Listeria monocytogenes in soft cheese and the Real-time PCR method to detect Salmonella spp in pork meat, based on the European validation, have been harmonized in order to be adopted as international standards and in alternative improved sampling scheme developed in the project.
A sampling plan for Salmonella spp in table eggs using Real-Time PCR has been evaluated. The proposed sampling plan in order to achieve a 95% certainty of not falsely accepting a contaminated lot, needs at least 16 pools of 10 eggs each to be tested by ISO 6579, while the minimum number of pools to be tested was reduced to 8 pools of 9 eggs when Real-Time PCR was applied, as the analytical method, because Real-Time PCR showed to be more sensitive than ISO 6579 in detecting low level of Salmonella spp contamination in pools of eggshells.
Detection of Listeria monocytogenes in smoked salmon was improved by using the wet pooling test. Controlling Listeria monocytogenes in smoked salmon is still a challenge for food business operators. The reported low levels and uneven distribution of the pathogen make it necessary to apply good sampling plans and analytical procedures in order to detect contaminated batches. However, analyses are expensive and due to current economic situations, usually only one sample per lot is checked instead of the n = 5 samples established in Regulation (EC) No 2073/2005. This work proposes the investigation of Listeria monocytogenes by pooling pre-enriched Half Fraser from several units from a batch (n = 6). The results obtained from a preliminary in-house validation (n = 51 pools) showed that the performance characteristics (sensitivity and specificity) are very high (N95%). This high reliability, together with reduced costs (nearly half), suggests that the wet pooling test could be a good cost-effective approach for investigating Listeria monocytogenes in smoked salmon.

To share and disseminate the scientific knowledge deriving from the project to stakeholders the following dissemination activities were carried out:
1. Set up of collaboration and exchange of knowledge/ experiences between SMEs and scientists inside the project
2. Dissemination of research findings to food Authorities through the organisation of two targeted workshops (October 2012, September 2013)
3. Dissemination of research findings to policymakers through the realisation of a White Paper
4. Dissemination to stakeholders (scientific community), through: web-site with freely downloadable deliverables, 31 scientific publications on 9 relevant journals, organisation of 2 workshops, 1 International Conference (12/11/2013), 1 webinar (two separated sessions) and 5 training courses and the participation with visibility to 27 International scientific events. (Conferences/Symposia/Workshops/Forums/Scientific Committees meetings/Research Days)
5. Reinforcement of the basis for a wider industrial involvement through new recruitments and contacts with EU Food Federations/Organisations/Associations,
6. Reality checks carried out contacting more than 220 between industries and labs
7. Training of 22 people (11 inside and 11 outside the Consortium) on the Baseline software tool through the two sessions of webinar organised in April 2013 by Optimum Quality
8. Training of 187 external technicians/researchers during the five practical courses organised respectively in Norway, Italy, Spain (2), Croatia.

Potential Impact:
POTENTIAL IMPACT
At European level, in 2002, the Regulation (EC) 178 of the European Parliament and of the Council states that, in order to achieve the general objective of a high level of protection of human health and life, food law shall be based on risk analysis, except where this is not appropriate to the circumstances or the nature of the measure. However, the Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs requires that food business operators ensure that foodstuffs comply with the relevant microbiological criteria which are not based on risk analysis.
Since there are differences between the microbiological criteria reported in the European Regulation No 2073/2005 and risk based metrics formulated by the CAC and, at European level, there is the need to base the food laws on risk analysis, the results collected during the BASELINE project should contribute to fill this gap presenting a possible approach to calculate POs for specific food/hazard combinations. To this aim a dedicated software tool was also provided at the end of the project.
The impact of the project the project results is clear at different levels:

HEALTH IMPACT
The definition of risk based metrics such as the performance objectives support the regulatory authorities in the implementation of new food EU Regulations based on those metrics. The implementation of a risk-based approach to food safety requires specific knowledge, competencies and tools, but the expected benefits justify past and future efforts. Implementing a risk-based approach to food safety, instead of a pure control system for verifying the compliance to an imposed specific limit value, often defined at the end of the shelf-life when it is no longer useful to prevent foodborne illnesses, is fundamental to reduce the probability and/or impact of foodborne illness. The BASELINE results allow to support this approach providing new knowledge of risks along the food chain to ensure that food safety criteria, at time of production, guarantees the appropriate level of protection at retail and finally, at the time of consumption. The improved knowledge of food-chain steps and potential hazards can help to identify the critical control points and to establish monitoring “upstream” to guarantee food safety.
Within the Baseline project for each food/risk combination specific POs were defined based on FSOs theoretically calculated. In particular specific Performance Objectives were determined for the following chemical and biological risks: L. monocytogenes in fresh salmon intended used raw as it is or as sushi or cold-smoked salmon; biotoxins in raw shellfish to be cooked before consumption; aflatoxin M1 and VTEC in raw milk; Campylobacter in poultry carcasses; Salmonella and Campylobacter in pork cuts, VTEC and tetracycline in beef. Moreover theoretically POs for VTEC, Salmonella spp. and Listeria monocytogenes in ready-to-eat leafy green vegetables were identified.
The BASELINE Suggestions for an implementation of current European Regulations on Food Safety concern the improvement of sampling plans. The review on existing sampling scheme for biological and chemical risks in different food categories, outlined that for most of the food categories investigated, no European Regulations are available (i.e. Salmonella in table eggs, L. monocytogenes and VTEC in raw milk, Campylobacter in poultry carcasses, viruses and E. coli O157:H7 in plant products, etc). Moreover, when available, the sampling plans have been set up without a mathematical approach taking into account the distribution of the hazard along the food chain. BASELINE used available published data and collected new data all along the food chain for modelling the distribution of survival and growth of most important biological hazards. The modelling results were used to define the best sampling plans able to verify the compliance to specific POs related to selected hazards. The achievement of the POs will allow to reduce

the number of potentially contaminated food lots on the market reducing as a consequence the number of foodborne diseases.
The current EU Regulation on Food Safety states that Food Business Operators are in charge for safety of their products. In this context, the application of microbiological criteria, when available, or the application of the risk based metrics developed in the BASELINE project, such as the POs represent a crucial instrument for FBOs to verify the compliance of their lots to place on the market. Effective sampling plans for safety criteria are a crucial tools for the food business operators because they can be applied to check the hygienic status of processing plants and technological procedures. In fact, changes in the percent of lots not compliant with the defined safety criteria (i.e. MC or POs) represent the signal that something in the HACCP or GMP is not working properly. This possibility support the food companies to introduce rapid changes to improve biological and chemical safety of their products before placing unsafe products on the market reducing the large costs related to food recalls.

ECONOMIC IMPACT
The presence of dangerous biological and chemical risk agents in food products commercialised within the European Union has a negative economic impact on both food producers and general society. The large application of the sampling schemes developed during the BASELINE will increase the probability to detect chemical and biological risks in foods before their distribution to consumers decreasing the negative economic impact due to food recalls and foodborne diseases.
The quantitative impact of the BASELINE project outputs on those costs is very difficult to estimate at this time. However, it will be easy to measure when the EU regulations will be implemented with the risk based metrics suggested in the project as well as the corresponding sampling plans. To guarantee the correct formulation of the POs a dedicated software tool is available Moreover, training sessions on the application of the project results have been organised and followed by technicians working within the food companies.
Regarding the new analytical methods suggested in the project they concern quantification of low levels of L. monocytogenes in seafood; quantification of Salmonellain table eggs, pasteurized egg products and commercially available egg containing dishes using qPCR after a short non-selective 8 h pre-enrichment; detection of up to 1 cfu/g of Salmonellaand L. monocytogenes in meat products by RT-PCR in 27h; RT-PCR methods for detection of HAV and noroviruses GI and GII in vegetables; multi-residue methods to detect pesticide in plant products; method for determination and quantification of acrylamide in nuts; EMA-qPCR methods for detection and quantification of Listeria monocytogenes and Salmonellaenterica in plant products. The new methods improved in the BASELINE project are mainly based on molecular techniques which are generally more fast of the cultural methods. Therefore, their application should represent a huge economical advantage for the food companies to detect a food hazard.

SCIENTIFIC IMPACT
The BASELINE results were presented in public Deliverable available in the web site, in scientific publications in International journals with impact factor as well as during national and international conferences. Moreover, dedicated training sessions on safety criteria defined for seafood, eggs and egg products, milk and dairy products, meat products, plant products were performed live and are available in the web for general public. The results collected in the project improved knowledge on weakness and strength of current sampling schemes applied in the EU for seafood, eggs and egg products, milk and dairy products, meat products, plant products; on biological and chemical hazards in selected food chains,

taking into account transport and storage at retail but also transport and storage by consumers; on distributions of biological and chemical hazards in the different steps along the food chain up to consumption; on mathematical and statistical approach to define risk based metrics such as PO and FSO; on management of variability and uncertainty during data modelling; on new analytical methods and sampling procedures.

EUROPEAN ADDED VALUE

Contribution to standards

The revised Directive 2003/99/EC requires EU member states to collect relevant and comparable data to identify and characterise hazards, assess exposures and characterise risks related to zoonoses and zoonotic agents. Monitoring must take place at the stages of the food chain most appropriate to the zoonosis or zoonotic agent concerned. The hygiene package of five laws adopted by the EU in 2004 aims to merge, harmonise and simplify complex hygiene requirements previously scattered over seventeen EU directives. These laws will apply at every point in the food chain, in line with the EU's "farm to fork" approach. Key to the new legislation is that all food and feed operators have primary responsibility for ensuring that food put on the EU market meets required safety standards. Those responsible for manufacture of a product will also be required to conduct studies to investigate compliance with microbiological and chemical criteria as described in many European directives (2073/2005; Commission Regulation (EC) No 1881/2006). With the establishment of a common health certificate for food and feed products entering the EU, imports will also face less red tape Importers bringing supplies into the EU would find it easier to do so but it could also result in increasing competition within Europe from non-EU producers and manufacturers. This emphasises the need for rapid, sensitive methods of pathogen- and toxin detection for food imported from the non-EU countries. In this context BASELINE developed and disseminated standardized and validated analytical methods and tools relevant to food risks, available for food authorities and food producers. The application of these methods and tolls will support the collection of comparable data among EU countries to be included in risk assessment models.

Contribution to policy developments

The BASELINE consortium, including Partners having responsibilities for provision of advice and consultancy to their respective national governments on aspects of human health risks and food safety, focused on the definition of quantitative POs to be applied along with microbiological criteria to check the lot compliance with reference to selected biological and chemical hazards. Developing meaningful microbiological criteria for a food or ingredient is a complex process that requires considerable efforts and resources. Therefore, a microbiological criterion should be provided only when there is a need and when it can be shown to be effective and practical. In fact, in the Commission Regulation 2073/2005, microbiological criteria have been defined for specific biological risks in selected food products. Thus, food control authorities serve as risk managers and, through the risk analysis process, may ultimately decide that a microbiological criterion is necessary for a food at one or more points in the food chain. Ideally, the standards will be based upon a tolerable level of risk for the biological hazard of concern, but this correlation is out of scope for Regulation 2073/2005. In fact, the current health status of a population is evaluated conducting a Quantitative Microbiological Risk Assessment (QMRA) for a product or product group to which a pathogen is associated. A QMRA can give an absolute or a relative indication of the health status, i.e. provide an absolute numerical expression of the risk at population level or a relative or benchmarked expression (e.g. a ranking), respectively. Importantly, QMRA studies can be developed on

many levels of detail, depending amongst many others factors on the complexity of the issue, the urgency for obtaining the risk estimate and the data available. A novel area where quantitative microbiological risk assessment is increasingly used, is the setting of risk based food safety standards, such as FSOs and POs. In this context, the contribution of the BASELINE project was to show the scientific and statistically based approach to derive POs for different food/biological hazard combinations. Such approach was also implemented in a software tool available in the project web site (www.baselineeurope.eu). The software shows also how to establish microbiological criteria basing on risk-based metrics and predictive models. The Baseline “software” is a practical tool which, together with specific guidelines to be developed by food authorities (e.g. DG SANCO, EFSA, etc.), can represent a solution for accelerating the adoption of risk-based metrics for hazards management.

Contribution to Industry perspective
The project developed a mathematical approach to calculate, case by case, the proper level of Performance Objectives along the farm-to-fork chain. As a result, modelling “software”, able to establish food safety criteria and related sampling plan for each of the five food chain case studies, was developed. This tool has open access and is useful to Food Business Operators to model and evaluate their specific food/risk combinations. Link: http://www.baselineapp.com Sampling plans tailored to food safety criteria are practicable for most of the food chains.
Quantitative data to improve risk assessment models for the main microbial and chemical hazards related to the different foods can be provided through the sampling strategies proposed by the project. In particular for those less well-known chemical and biological hazards, it is fundamental to collect as much data as possible. It should be understood that the collection of risk data is the most important phase in a risk-based approach and will represent most of the effort in developing a common and shared European food safety strategy. Food Business Operators should fully understand that contributing to the risk analysis by providing unbiased data is part of their duties, in a mechanism which wants to improve their future performance and not to point out their weaknesses.
To control hazards, where sampling is not always financially practical, a risk-based system allows for alternative solutions to sampling plans while assuring the same level of safety. In some cases, approaches other than sampling can be used to assess the prevalence and levels of food risk agents. Sampling of products can be simplified by analysing pooled samples, production and water environments can be sampled instead of the products, and parameters correlated with 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 high risk foods to certain market segments can be used to ensure food safety. Furthermore, use of more specific and sensitive analytical methods provide lower detection and quantification limits which may reduce the number of samples in the sampling plans and make them more efficient. 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 under normal or abuse conditions, or the risk can be easy eliminated by the final consumer during preparation of the food.
Risk assessment in the food industry can be improved by a deeper knowledge of different processing steps as well as transport and storage. It requires assessment and monitoring of the risks to consumer health along the entire food chain, from farming practices, through the raw materials, the food processing activities and the transportation and storage up to consumption. Transportation and home storage cannot be excluded in a risk assessment because changes in temperature during some seasons and/or countries

may allow significant growth of some human pathogens in some cases.

DISSEMINATION ACTIVITIES

Dissemination to food authorities

UNIBO organised two workshops for food authorities. The 1st BASELINE workshop for Authorities was held in Brussels, which was carried out on 18 October 2012 at Regione Emilia Romagna – Ufficio di Bruxelles. In this period a web-page was drafted and managed in order to disseminate the workshop and register the attendees: the main information were published at: http://www.unibo.it/Portale/Ricerca/Baseline.htm. The news was also uploaded on the BASELINE project web-site, for a wider visibility. The page described the event, including the agenda, the registration form and further information about the venue and Brussels.

The workshop was successfully carried out. 50 people attended the event, 10 representatives of authorities. The discussion was profitably moderated by the Coordinator, who invited the attendees to express their opinions and experiences in the field in more occasions. A round table closed the event. The minutes of the workshop was circulated to all the pre-registered people, to inform a larger audience on the treated issues and the project results.

The 2nd BASELINE workshop for Authorities was held in Parma the 26th of September 2013. UNIBO designed a web page in its website in order to disseminate the workshop http://www.unibo.it/Portale/Ricerca/Baseline_second_workshop_2013.htm.

As shown in the Figure below, the page described the event, included the draft WS agenda, the registration form and further information about the venue and Brussels. The news was also uploaded on the BASELINE project web-site, for a wider visibility. UNIBO realized also a dedicated flyer (first announcement) and printed 200 copies which were distributed before and during the event.

All the speakers contributed in setting up the Agenda and were called to contribute to the dissemination of the event. University of Bologna, Itacyl and TCA also selected the potential interested audience and drawn out a list of 405 total contacts to invite at the event.

27 people attended the event, 10 external to the Consortium. The discussion was profitably moderated by the Coordinator, who invited the attendees to express their opinions and experiences in the field in more occasions. The minutes of the workshop was circulated to all the pre-registered people, to inform a larger audience on the treated issues and the project results.

These two events gave the possibility to the project to collect some feedbacks about authorities perception of project results. Authorities showed interest in the technical project results and asked for more details during the presentations. In particular, it emerged a big interest in the web-based software tool realized into the project. Baseline proposes a software tool based on predictive models, which can help end-users to derive and evaluate sampling plans for microbiological hazards in foods. In the questionnaire filled-in by Authorities at the end of the 2nd workshop it emerged that this is considered the most interesting and promising project resultS.

Moreover a White Paper for policymakers was realised and 150 copies distributed during the 2nd workshop and the Baseline Final Conference.

Dissemination to scientific community

A total of 60 publications have been published by the consortium :

31 peer reviewed publications on 9 relevant Journals (Food Control, Italian Journal of Food Safety, Food Analytical Methods, Food Microbiology, Journal of Environmental Science and Health, International

Journal of Food Microbiology, Journal of Dairy Science, Food and Public Health, The Croatian meat journal);
24 papers in proceedings; 4 articles/sections in books; 1 University publication
Besides, participation with posters/oral presentations to 27 International events on the topic (Conferences, Symposia, Workshops, Forums, Scientific Committees meetings/Research Days);
15 poster presentations at EU and International level;
80 speeches in 18 public scientific event
1 press-release to inform media about the Final Conference event;
7 videos on project results recorded and uploaded on the project website and on Youtube;
(http://www.baselineeurope.eu/video.asp) and on Youtube at the links: http://www.youtube.com/watch?v=8wiHlwpCtac
http://www.youtube.com/watch?v=pIYxF-WYB3I
http://www.youtube.com/watch?v=w-_Fc3PU1Qc
http://www.youtube.com/watch?v=PLspzH0v-fo
http://www.youtube.com/watch?v=lbWbxZe2LTM#t=18
http://www.youtube.com/watch?v=Pu0j4bsJ-f0 http://www.youtube.com/watch?v=mya1NzGmpbM
100 copies of Baseline flyer distributed
5 advertising initiatives through own Newsletters, Social media.
1 Final Conference: it was held on November the 12th, at the Regency Hotel in Bologna. Sixteen international speakers followed one another in the day, in four sessions moderated by four different representatives of the project, in order to present to the audience the project findings. Great success of the initiative that has seen more than 80 experts from 16 different countries of Europe, but also from all over the world (Nigeria, South Africa, America, Argentina, South-Western Asia), cluster around the interest on this topic. Industrial representatives, as well as the Italian Ministry of Health, attended the event.

Dissemination to other stakeholders
Webinars: two Webinars for training the partners and the interested stakeholders in using the software tool were organised to improve the participation:
Session 1: April 19th, 2013
Session 2: April 26th, 2013
Duration: 2 hours in length, followed by 30 minutes OF question/answer time.
22 attendees participated in the Webinar (10 during the first session, 12 in the second one), 50% of which were external to the Consortium.

Training courses: Five training courses on BASELINE research findings have been successfully carried out were attended by 187 technicians/researchers from Brazil, Bulgaria, Croatia, Egypt, Italy, Lithuania, Norway, Poland, Russia, Spain, Thailand, USA:
9. Seafood, Oslo: June, the 6th 2013
10. Plant products, San Adrián: June, the 25th 2013
11. Local Croatian training course: July, the 19th 2013
12. Meat and eggs, Bergamo: September, the 15th 2013
13. Dairy Products, Valladolid: September, the 23rd 2013
14. Statistic training, Budapest: within MoniqA meeting 2012

Applying the rules established into the “evaluation plan and Indicators”, the courses were evaluated (as an average) with a score of 4.5 on 5 (considering a scale from 1 “poor” to 5 “excellent”).

Reality checks with industries and labs
From June 2013 to November 2013, more than 220 between food industries and private analysis laboratories, plus public health laboratories, have been contacted in Europe. On more than 220 contacted units, about 12% returned back the filled-in questionnaires or answered by direct contact to the requests. 88% of them have a laboratory for analysis (even if not always used for analysis of foodborne pathogens, which can be outsourced). 75% of interviewed Companies have an internal QA laboratory for analysis. Most of them (50%) are large enterprises.
It emerged that it took some time before the concept of “performance objective” is understood and accepted by European food industry. A preventive / risk-assessment based approach is difficult to understand, since it requires time, investments and it doesn’t give immediate benefits and Food Companies are principally oriented to solve other and contingent problems. People working on food production don’t know how to implement these new concepts in practice, consequently training and gain of knowledge to Food Business Operators and other interested parties (e.g. labs) are necessary actions to foresee in the forthcoming future. Also the approach to new/alternative analytical methods is initially not considered useful, especially from companies that have not had previous experience with such methods. Differently, some large companies have already invested in PCR machine for in-house routine monitoring activities, with satisfaction and advantages. Furthermore, the progress suggested by Baseline project is not without risks: more sensitivity and more efficiency assured by new alternative methods may lead to finding more positives than now and Companies would be frightened by this. Next obliged step is then to provide reassurances and concrete guidelines for managing the eventual non-compliant samples. In other cases, excessive procedural weighting not tied to a real benefit for the company, will never be accepted by industry.

Baseline web-site
The website address is www.baselineeurope.eu/. It will continue to be online for five years after the end of the project.

Public area: calendar of events and several templates and documents related to the project have been regularly updated by UNIBO; moreover after approval by EC, all Deliverables have been uploaded in the public area of BASELINE website for their free consultation and are reachable at the link:
http://www.baselineeurope.eu/documents.asp?t=2
At the following link you can check all details related to this issue:http://stats9.nowhere.it/9/
Baseline web-site was always used as a tool for giving visibility to each of these initiatives.
Mail was used to inform and to invite people such as Food Authorities (up to 405 invitations), Baseline Stakeholder Platform (48 members), industry and the scientific community (up to 2.000 contacts for the Final Conference advertisement).

“STAKEHOLDERS PLATFORM”
Here below are summarised the food industries involved in the Stakeholder platform
INDUSTRY TYPE NUMBER
Food SMEs, included laboratories 12

Services SMEs 1
Large Enterprises 15
Agriculture Cooperatives 1
Consultant (freelancers) 4
Research Organizations 7
Government Institute 5
Association 3
SECTOR
Meat 5
Fruits and vegetables* 8
Dairy 4
Eggs 1
Fish 1
RTE/canned meals/other sectors 6
Laboratories 3
COUNTRY
Italy 20
Spain 10
Norway 6
Austria 3
Sweden 2
Finland 1
Germany 1
Turkey 1
Egypt 1
New Zealand 1
Croatia 2
*in addition, Coexphal Asociaciòn represents 100 industries of the sector

Moreover Federations for Food were involved
15. FoodDrinkEurope office in Brussels,
16. Norwegian Seafood Federation, which is organising most seafood producers in Norway, and recruited it also as BASP member.
17. Croatian Food Agency
18. COEXPHAL (Asociaciòn de Organizaciones de Productores de Frutas y Hortalizas de Almerìa), which represents 100 industries of the sector, and recruited it also as BASP member.

EXPLOITATION OF RESULTS
Improvement of specific sampling plan and derived performance objectives for specific food/risk combinations
POs values, identified within Baseline project, for selected food/risk combinations and related sampling plan able to verify the compliance of food lots represent a practical reference to be used by food industries as well as by food authorities in order to ensure safe food for consumers. Designed sampling plan are

ready for risk managers and may be further implemented and updated when new data will be collected in the future. The risk based approach used to identify the POs values can be applyed to the identification of FSOs by regulatory authorities when the legislation will move to this new approach

New/revised analytical methods
The following analytical methods have been set up within Baseline project:
L. monocytogenes detection in smoked salmon by wet pooling test
MPN assay for rapid detection and enumeration of extremely low Listeria levels and uneven distribution of Listeria in smoked salmon
A qPCR assay for the quantification of Salmonellain egg and egg products was set up.
Real-Time PCR assays for detection and quantification of L. monocytogenes, Salmonella, VTEC in milk and/or soft cheese.
Two Real Time PCR protocols for the rapid detection of Salmonellaand Listeria monocytogenes in pork meat
Quantitative detection methods for Listeria monocytogenes and Salmonellaenterica in plant products using EMA-qPCR
Real-Time PCR method for detection HAV and noroviruses GI and GII to vegetables
Multi-residue method to detect pesticide in cabbage, lettuce and RTE-lettuce
Protocol for the determination and quantification of acrylamide in plant products with a high content of starch and aminoacids

These methods are developed and fully described in the corresponding deliverables so that they can be implemented in any laboratory. The laboratory that wants to implement the method should carry out an internal validation in order to assure they work correctly
Next steps after the end of the project, implementation of these methods by involvement of partners of Baseline project which can collaborate with different stakeholders references (laboratories, private laboratories, food industry)
In particular among these analytical methods two REalt-Time PCR assay for detection of Salmonellain pork meat and Listeria monocytogenes in soft cheese were validated by an interlaboratory study and their protocols were harmonized and structured as an ISO standard already available for scientific community.

Software tool
Software web-based tool was created within the project BASELINE. The objective was to develop of a software tool based on predictive models, which helps end-users to derive and evaluate sampling plans for microbiological hazards in foods. The Software has been generated with the results of the elaboration of the mathematic models as outcome of the research of partners involved in the development of predictive models (UNIBO, NVI, CNTA, DKFZ,UBO,ISS, ITACyL, VETFAC, UCO, UCPH, UN, ANSES, TEAGASC).
The entities involved in software development were:
The German Institute of Cancer Research (DKFZ, Germany) who has supported the technical and statistical parts regarding the sampling plans design
The University of Cordoba (UCO, Spain) who has helped in the software design, development of predictive models and elucidation of practical examples for the determination of Microbiological Criteria in the foods/risks matrices selected.
A subcontracted SME (Optimum Quality, Spain) who took charge of the software development basing on

intelligent systems to be better applied by end-users.

Software utilization and maintenance
After the end of the BASELINE project further dissemination and exploitation plans will be carried out. A joint agreement for the software exploitation will be signed among the implicated parties stating the parties' share of ownership (proportional to the intellectual contribution invested in generating the foreground) and further exploitation measures. It is envisaged that IPR will be registered in the next period in order to protect the software invention and generated knowledge.
Find below a proposal on exploitation of results. In a first stage, the main features of the software will be thoroughly revised to assure a good performance. It is expected to incorporate additional improvements such as generation of new predictive models, sampling plans and derivation of alternative Microbiological Criteria for validation processes in food industries.
Training courses might be carried out to food assessors, stakeholders, food authorities and those interested parties that intend to design and establish a more efficient decision-making process.
Additionally, webinars, workshops and communications in congresses and symposia related to food safety will complement this exploitation strategy.

General expected regulations for user's access: Two profiles for users are expected for the software tool:
Basic users: those that can access to the web platform and only visualize and perform scenario analyses, without edition capability.
Advanced users: those who can access to the advance menu thus making the possibility to edit predictive models and sampling schemes.
The software is expected to be a free web-based tool.

List of Websites:
http://www.baselineeurope.eu/
Project Coordinator: Prof. Gerardo Manfreda Email: gerardo.manfreda@unibo.it Ph. +39 051 20 9 7854
Project Team member: Prof. Marcello Trevisani Email: marcello.trevisani@unibo.it Ph. +39 051 20 9 7330
Project Team member: Alessandra De Cesare Email: alessandra.decesare@unibo.it Ph. +39 051 20 9 7853

Related documents

final1-annexes-description-of-the-main-scientific-and-technological-results-and-beneficiaries.pdf

Last update: 5 February 2015

Permalink: https://cordis.europa.eu/project/id/222738/reporting