BASELINE

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

PROJECT DELIVERABLE

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Executive Summary

Within "Baseline" project, workpackage 4 focuses on "Selection and optimisation of sampling plan for the different risk target in poultry, pork and beef raw meat. The main objectives of WP4 are (i) to review current sampling schemes for selected poultry, pork and beef raw meat products, (ii) to identify risk factors and their POs and FSOs in selected meat products and (iii) to assess the need of new or adapted analytical methods to ensure meat products safety. The outcome of the studies conducted is to provide safety criteria and sampling schemes for the different biological and chemical risk-product combinations selected for meat products: *Campylobacter* and poultry, *Salmonella* and pork cuts, *Listeria* and pork cuts, VTEC and beef, Tetracycline and beef (table 1).

Table 1. Risk product combinations and the investigations conducted for safety criteria definition

Biological/chemical Risk	Product	Investigations and data collection
Campylobacter spp.	Poultry products with skin	Prevalence studies from the EU MS's – EFSA data (Anses, Unibo, UCO) Risk fators during handling and transport (Anses, TiHo)
Salmonella spp.	Pork cuts	Unibo – Itacyl – ISS
Listeria monocytogenes	Pork cuts	Unibo – Itacyl – ISS
VTEC	Ground beef	Teagasc
Tetracycline	Beef	Vetinst

This deliverable focuses on possible sampling plans to provide a statistically-designed approach for determining safety criteria in relation to the product-risk combinations reported in table 1. Based on such criteria food industries would check the acceptability of their batches whenever limits for these combinations will be stated in the EU Regulations.

General on safety criteria

The Risk Analysis framework described by Codex Alimentarius provides a structured approach to the management of the safety of food. In the Codex document on Microbiological Risk Management and in ICMSF's ''Microorganisms in Foods 7: Microbiological Testing in Food Safety Management" (ICMSF, 2002), the establishment of a food safety objective (FSO) is described as a tool to meet a public health goal such as an appropriate level of protection (ALOP). More recently, an FAO/WHO expert consultation re-emphasised the original definition for ALOP that was part of the Sanitary and Phytosanitary (SPS) Measures Agreement, namely that it is the ''expression of the level of protection in relation to food safety that is currently

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achieved. An FSO specifies the maximum permissible level of a microbiological hazard in a food at the moment of consumption. Maximum hazard levels at other points along the food chain are called performance objectives (POs). The current definitions for FSO and PO are that an FSO is ‘‘the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of (health) protection (ALOP)” while a PO is ‘‘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 an FSO or ALOP, as applicable”. Although FSOs and POs are expressed in quantitative terms, they are not microbiological criteria which are defined as the acceptability of a product or a food lot, based on the absence/presence or number of microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot (ICMSF, 2002). Often, however, this is not known and an assumed distribution is used because it provides the basis for establishing a mathematical relationship between FSOs, POs and microbiological criteria as shown below.

Safety criteria for *Campylobacter* in poultry products with skin

The reduction of human campylobacteriosis has become a priority in the European Union since it represents the most reported zoonoses. It has followed a significant increasing five-year trend since 2006, and in 2010 a total of 212,064 confirmed cases have been reported. The proportions of *Campylobacter*-positive food and animal samples remained at similar levels as in previous years, with the occurrence of *Campylobacter* continuing to be high in broiler meat (EFSA, 2012).

The reduction of human infections might be achieved commercializing a lower proportion of positive carcasses and/or carcasses with a lower concentration of *Campylobacter* cells in terms of CFU/g or CFU/carcass. The acceptable prevalence and concentration values in terms of human risks can be expressed as Performance Objectives (POs). Such POs represent clear targets the poultry production industries could try to achieve.

In accordance with Commission Decision 2007/516/EC5, in 2008 all Member States carried out a harmonized baseline survey on the prevalence of *Campylobacter* spp. in broiler flocks and broiler carcasses. On carcasses, both qualitative and quantitative analyses were performed. The survey provides reference values, comparable between member states, in order to consider future performance objectives/targets along the broiler meat production chain.

The Commission has two legal bases to consider performance objectives/ targets:

In accordance with Regulation (EC) No 2160/2003 on the control of *Salmonella* and other specified food-borne zoonotic agents, targets for the reduction of the prevalence of *Campylobacter* can be adopted at the level of primary production and, where appropriate, at other stages of the food chain.

In accordance with Regulation (EC) No 852/2004 on the hygiene of foodstuffs, microbiological criteria can be adopted for broiler meat.

In this project the data collected during the baseline survey performed in 2008 were analyzed to suggest possible PO for *Campylobacter*.

At present, food safety standards in the EU include microbiological criteria (MC) and targets in primary production. Targets can be considered equivalent to Performance Objectives and are aimed at reducing the prevalence of *Campylobacter* in the broiler flocks, thus mitigating the risk for human campylobacteriosis at its main reservoir.

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Safety criteria for *Salmonella* in pork cuts

Salmonella is recognized as an important zoonotic pathogen of economic and public health significance. Although important efforts have been taking in place as *Salmonella* control programmes in animals and a significant decrease of human cases was registered in 2010 in comparison to previous years, salmonellosis is still the second most reported zoonosis with 99,020 confirmed cases in EU in 2010 (EFSA and ECDC, 2012). *S.* Enteritidis and *S.* Typhimurium are the serovars most frequently associated with human illness. Human *S.* Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat, while *S.* Typhimurium cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat. In 2010 in Europe pig meat was reported as the most relevant food vehicle of *S*. Typhimurium encountering for the 29,8% of strong evidence outbreaks (EFSA and ECDC, 2012), confirming pig meat as an important food vehicle of *Salmonella*.

Commission regulation No 2073/2005 prescribes rules for sampling and testing, and set limits for the presence of *Salmonella* in specific food categories and in samples from food processing, often corresponding to absence in 10 or 25 g. To obtain accurate estimations of *Salmonella* growth potential in naturally contaminating meat products during transport and storage at retail as well as at the consumer's house in WP4 prevalence of Salmonella in ten lots of naturally contaminated loin chops, produced from the same company over a one-year period, was evaluated. Pathogen monitoring was conducted under four different scenarios (S) over the product shelf life: immediately after packaging at the processing facility (S1); after transport and storage at retail up to day 4 or 5 of the product shelf life (S2), total of 7 days; after storage at retail, transport at car temperature and storage at 6 (S3) or 14 (S4) °C up to the end of the product shelf life, established as one week after packaging. Furthermore, the effect of different cooking procedures on pathogen inactivation was assessed. The results collected in the survival and inactivation trials were used to formulate a possible POs for *Salmonella* in fresh pork meat intended to be eaten cooked. Such PO should be verified after packaging at the production facility.

Safety criteria for *L. monocytogenes* in pork cuts

Pork is the most frequently consumed meat in the European Union (Devine, 2003). Management of hazards transmitted to humans by consumption of pork is therefore of major health and economic significance. *Listeria monocytogenes* is one of the hazards having the highest scores of risk for pork consumers (Fosse et al., 2008). In 2010, 1.601 confirmed human cases of listeriosis were reported in the European Union resulting in a case-fatality rate of 17 % for those confirmed cases where sufficient information is available (EFSA, 2012). The incidence of human cases of listeriosis attributed to the consumption of pork has been estimated at 0.042 cases per 100,000 habitants per year in Europe (Fosse et al., 2008).

L. monocytogenes is thought to be harbored in the intestinal tract of presumably healthy pigs. The prevalence of the pathogen in pig faecal samples ranges from 0 to 47%, with the highest prevalence reported in Eastern Europe (Fenlon et al., 1996). Carcasses can be contaminated when the large intestine is ruptured during evisceration (Skovgaard & Nørrung 1989), as well as through contact between infected tonsils and the tongue and the other viscera and carcass. Furthermore, chilling and cutting significantly increase the contamination of pork meat and pork based products such as fresh and fermented sausages. Cross contamination may occur at any stage between the meat processing plant to the final consumer at home (Reij & De Aantrekker, 2004). Contamination in the home often occurs when the same cooking utensils, such as cutting boards, are used on raw contaminated foods and cooked or ready to eat foods. The

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contamination levels in foods associated with *L. monocytogenes* human infections are between 10^2 and 10^6 cfu/g or ml in the majority of cases (Dawson et al., 2006).

The Regulation (EC) No 2073/2005, amended by No 1441/2007, does not include microbiological limits for *L. monocytogenes* in fresh pork meat intended to be eaten cooked. Therefore, few data are available on prevalence and concentrations of *L. monocytogenes* in fresh pork products leaving the processing facilities. In Europe, according to the literature review conducted by Mataragas et al. (2008) to define risk profiles of pork meat and risk ratings for various pathogens including *L. monocytogenes*, the pathogen prevalence in raw pork products at retail was calculated at 9.9% and in ready to eat pork products at 3.2%. Finally, in Italy, 10.3% of raw pork samples submitted for official analyses during 2001 to 2002 were *L. monocytogenes* positive (Busani et al., 2005).

To obtain accurate estimations of *L. monocytogenes* growth potential in naturally contaminated meat products the variability of the pathogen, 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 (Pouillot et al., 2010). The use of statistically-based techniques allows for estimate distribution parameters to be derived from microbial concentration data. In WP4 prevalence and concentrations of *L. monocytogenes* in ten lots of naturally contaminated loin chops, produced from the same company over a one-year period, were evaluated. Pathogen monitoring was conducted under four different scenarios (S) over the product shelf life: immediately after packaging at the processing facility (S1); after transport and storage at retail up to day 4 or 5 of the product shelf life (S2), total of 7 days; after storage at retail, transport at car temperature and storage at 6 (S3) or 14 (S4) °C up to the end of the product shelf life, established as one week after packaging. Furthermore, the effect of different cooking procedures on pathogen inactivation was assessed. The results collected in the survival and inactivation trials were used to formulate a possible POs for *L. monocytogenes* in fresh pork meat intended to be eaten cooked. Such PO should be verified after packaging at the production facility.

Safety criteria for VTEC in ground beef

VTEC (E. coli O157 and other selected serogroups (O26, O103, O111, O145) are important pathogens which can cause serious and potentially fatal human illness. Sporadic and outbreak cases cause a huge economic burden to people infected (lost work time, child care if excluded from nursery etc) and to the agri- food sector through loss of reputation and business opportunities. In 2010 in the EU, almost half of the cases reported were related to serogroup O157 (41.1 %). The most important human pathogenic VTEC serogroup O157 was isolated by the MS in various food categories including bovine meat, cow's milk, cheese, pig meat and sheep meat. As in previous years, most of the recoveries were from cattle and food of bovine origin. There is currently no regulation to sample for the presence of VTEC in specific food categories but in 2009 EFSA published a report describing a harmonised monitoring approach for VTEC in animals the food chain. This highlighted that ruminant animals and their foods, in particular beef are an important vehicle of VTEC transmission and outlined that this was an important area to focus sampling equally. It purported that O157 should be the main focus for monitoring with those MS with capability to include the other 4 serogroups. However, the O104 outbreak in 2012 has put new focus on need to monitor for non O157 serogroups.

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In Baseline, studies were conducted to investigate the survival of VTEC in the process and distribution part of the chain including the impact of thermal treatments (at 58 and 62◦C), pH (organic acids at PH 3) and retail and abuse storage temperatures (5 and 15 C) on a range of strains from 5 clinicially significant serogroups. This data together with a beef slaughter process model for clinically significant VTEC in beef (completed in another EU project *Prosafebeef* but available to Baseline) allows a PO to be set for VTEC in the beef chain. The most useful place for this PO is in ground beef which may then be consumed in a meal with ground beef, used in burger formation or in a fermented sausages.

Safety criteria for Tetracycline in beef

It is the population risk or proportion of population that would likely consume tetracycline in beef exceeding the acceptable daily intake (ADI). The risk is measured as a probability based on the national beef tetracycline surveillance program, and the eating habits of the population.

The food safety objective or **FSO** for chemical risks is proposed to be the acceptable probability or proportion in the population that the ADI will be exceeded for the chemical risk and meat matrix based on the quantitative risk assessment model**.**

Each country has to decide its own risk level for each chemical/food matrix or the acceptable probability that ADI for that chemical/meat matrix will be exceeded in its country's population.

Material and methods

Campylobacter and poultry

Description of the dataset

Campylobacter survey results from EU countries were received in a Microsoft Excel spreadsheet format. The dataset used in this study was a part of the anonymised raw dataset of the EU-wide baseline survey on the prevalence and counts of *Campylobacter* in broiler carcasses carried out in 2008 (EFSA, 2010). A total of 10,162 carcasses from 3,400 batches belonging to 25 EU countries were selected. The remaining two countries were excluded from the analysis due to the lack of data (<30). Although the EFSA survey considered prevalence of *Campylobacter* in caeca samples, in this study, data from broiler carcasses were selected since the establishment of an MC was assumed to be set immediately after chilling of carcasses.

Data pre-processing

As the description of the uncertainty of the number of positive batches within the same country, a Beta distribution was used for prevalence of *Campylobacter*-contaminated broiler carcasses (P) with two parameters ($\alpha = s+1$, $\beta = n-s+1$); being *n* the total number of samples in the batch and *s* the positive samples. This distribution is widely used as the description of the uncertainty or a random variation of prevalence (Vose, 2008).

Counts of *Campylobacter* corresponding to each country surveyed, contained data with numerical concentration (> 10cfu/g), left censored data (those positives with a concentration below the limit of quantification of the technique (LoQ), i.e. 10 cfu/g) and negative values. Before submitting the dataset of each country to the statistical analysis, left censored data were assumed to be defined by the Poisson distribution, with a parameter (λ) equal to LoQ/2. Negative values of *Campylobacter* counts in broiler

carcasses were estimated as a value representing the pathogen theoretical concentration of samples under detection limit (C_{det}, log cfu/g). The mean concentration of samples under detection limit was calculated by the equation (Jarvis, 2000):

$$C_{\det} = -\left(\frac{2.303}{w}\right) \cdot \log\left(\frac{S_{neg}}{S_{tot}}\right) \qquad \text{eq 1}$$

where w is the weight of the analytical unit (e.g. 10g), S_{neg} (number of samples tested as negative), and S_{tot} (total number of samples analyzed).

Analysis of qualitative factors associated to *Campylobacter* counts in broiler carcasses

The most representative qualitative factors were selected to evaluate the impact on *Campylobacter* counts in broiler carcasses, as described in Table 2.

Table 2. Description of qualitative factors and numerical codes used in the multivariate regression analysis

Factors considered	**Abbreviation**	**Codes**
Country	-	access to the original file
Capacity of the slaughterhouse (1-6) (broilers slaughtered/year)	Cap	1 = <100,000; 2 = 100,000-499,999; 3 = 500,000-999,999; 4 = 1,000,000-4,999,999; 5 = 5,000,000-9,999,999; 6 = >10,000,000
Thinning (yes, no unknown)	Thin	1 = yes, 0 = no, 3 = unknown
Production type (conventional, free range standard, free range organic)	Prod	1 = conventional, 2 = standard, 3 = organic
Type of chilling of carcasses (air, inmersion or spray)	Chil	1 = air, 2 = inmersion, 3 = spray
Time between sampling and analysis	Time-samp	access to the numerical codes of EFSA
Campylobacter enumeration in carcasses (log cfu/g)	log c	access to the original file

Factors selected were classified according to numerical codes for the elaboration of the multivariate regression analysis. Data were transferred to, and all statistical analysis carried out using the software Statistica for Windows v10 (Statsoft Iberica, Portugal). The explanatory variables included in the regression models were 'capacity of the slaughterhouse', 'thinning', 'production type', 'chilling', 'time between sampling and analysis'; while the dependent factor used corresponded to '*Campylobacter* counts in broiler carcasses (log cfu/g)'. A univariate regression analysis with main effects was built to study the influence of categorical predictors (use factorial ANCOVA to include continuous predictors) on a single continuous dependent variable (*Campylobacter* counts, log cfu/g).

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Variables with more than 20% of missing data and those for which there was no variability were removed time between sampling and laboratory testing was the same in all samples (e.g. same code for thinning, chilling or production type reported for one country) (Hue et al., 2010). Only factors significantly associated with *Campylobacter* contamination ($p < 0.05$) were considered for further analysis.

Estimation of the distribution for *Campylobacter* counts in broiler carcasses

For the enumeration results, data groups were built basing on the univariate regression analysis results. For some countries evaluated, a division into more than one group was done since the categorical factors considered in the univariate analysis were significant ($p<0.05$). Once groups were constituted, a parametric distribution (i.e. log normal) was fitted to the data, as it has been reported as a representative distribution in previous studies for representing microbial contamination data (Crépet et al., 2007). Parameters of the log normal distributions (mean, μ and standard deviation, σ) of *Campylobacter* counts over the 3,400 batches belonging to the 25 EU countries were calculated using maximum likelihood estimation (MLE) method (Busschaert et al., 2010; Commeau et al., 2012).

Goodness of fit indices used to evaluate the adjustment of the distributions to the observed data, were the Kolmogorov Smirnov (KS) and the Anderson Darling (AD) tests.

The AD test focuses more on the goodness of fit at the tail level and the KS test focuses more on the goodness of fit at the center of the distribution. The test value is expressed as a p-value, indicating the probability that a dataset randomly sampled from the hypothetical distribution would be equally or even more extreme than the observed dataset. As the p-value decreases to zero we are less confident that the fitted distribution could possibly have generated the original experimental data set. A P–P plot (probability–probability plot or percent–percent plot) was used to calculate how closely the two datasets of the observed counts and the estimated distribution agree, by plotting the two cumulative distribution functions against each other.

Estimation of food safety criteria

The results obtained from the distributions for *Campylobacter* counts could be directly 'translated' into Performance Objectives (POs). In the present study, a PO could be the prevalence and/or the concentration of the pathogen that should not be exceeded after chilling of samples (when samples were taken for the EFSA survey). As such broiler carcasses should be analyzed by the manufacturer in order to prove that they comply with the specified limit of prevalence and/or concentration. For this purpose, microbiological criteria (MC) can be applied to ensure that POs are not being exceeded. MC is one of the potential control measures to reduce risk (Reij and Zwietering, 2008). When the distribution of the pathogen of concern is known, industry specific MC, aimed to verify compliance with a PO, could be developed using statistical methods. As log normal distribution provides the basis for establishing a mathematical relationship between PO and MC (van Schothorst et al., 2009), POs were estimated as the percentage of units in the batch that should not exceed a specified limit.

Salmonella and pork cuts

Ten different samplings were conducted between December 2010 and November 2011 at the processing facility of Partner CST in Italy. During each sampling, 20 packs (samples) containing 300±20 g of loin chops each and a temperature-logger, were vacuum packaged at the processing facility. The loin chops collected during different samplings belonged to different lots. However, those collected during the same sampling

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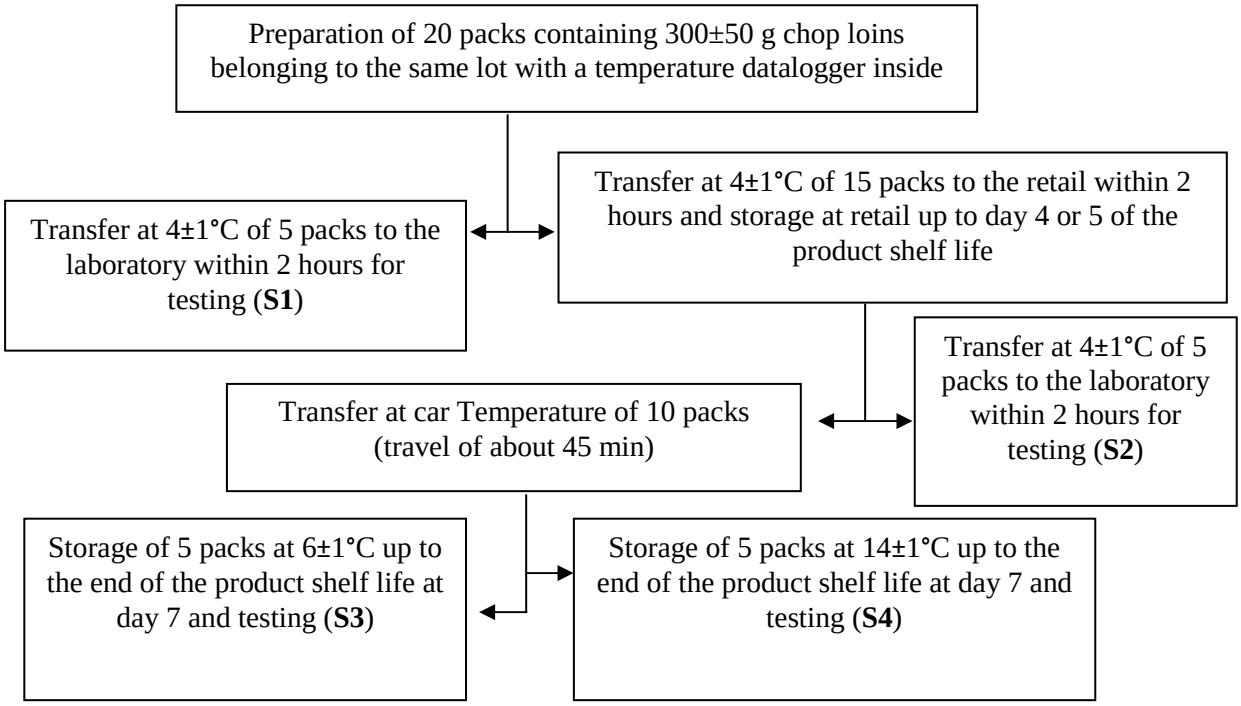
time were obtained from the same lot, meaning from animals reared in the same house and slaughtered at the same age.

For each lot, prevalence of *Salmonella* on chop loins was evaluated under four different scenarios, labelled as S1, S2, S3 and S4, throughout the product shelf life:

- within two hours from packaging (S1);
- after delivery at retail and storage at retail up to the fourth or fifth day of the product shelf life that was set at one week (S2);
- after delivery and storage at retail, transport at car temperature for 45±10 minutes and storage at 6±1°C, up to day seven of the product shelf life (S3);
- after delivery and storage at retail, transport at car temperature for 45±10 minutes and storage at 14±1°C, up to day seven of the product shelf life (S4).

A general scheme showing the experimental design followed and the number of sample units tested at each scenario is represented in Figure 1. The final storage temperatures (i.e., 6 and 14°C) were selected as a mean and consumer abuse refrigerator temperatures, according to the results of a non-representative survey to determine the temperatures of consumer refrigerators in the region where the study was conducted (data not shown). Moreover, other authors showed that the temperature in home refrigerators is often closer to 9°C than 4°C (Sergelidis et al., 1997).

Figure 1. Experimental design of loin chops analysis



During each sampling, the outer wrapper of each pack containing the loin chop portions was disinfected with 70% ethanol. After slitting the package with a flame-sterilized knife, each meat portion was aseptically

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cut into several pieces. Afterwards, a sample unit of 25 g was obtained from different parts of each sample. Finally, the temperature logger from each pack was collected and connected to a PC to upload the registered data.

Each 25 g sample was aseptically transferred to a BagFilter® (Interscience), diluted 1:10 in Buffer Peptone Water (BPW, Oxoid, Milan, Italy) and homogenised by stomaching for 1 min in a Stomacher 400 (Seward, Worthington, UK) at normal speed. Samples were incubated for 18±2 h at 37±1°C for a pre-enrichment. From the 18h pre-enriched BPW, 0.1 ml were transferred into 10 ml of Rappaport Vassilliadis broth (Oxoid) and incubated for 24 ± 3 h at 37 ± 1 °C. One ml of the BPW was transferred in 10 ml of Muller Kaufmann tetrathionate novobiocin Broth (Oxoid) and incubated for 24 ± 3 h at 41,5 ± 1 °C. After incubation, broths were streaked in duplicate onto Xylose Lysine Deoxycholate (XLD, Oxoid, Milan, Italy) and Brilliant Green Agar (BGA, Oxoid). Plates were incubated for 24 ± 3 h at 37 ± 1 °C. Five suspected *Salmonella spp*. colonies per sample were subcultured in Nutrient Agar (Oxoid) and their identity was confirmed by qualitative PCR as previously described (Rijpens et al., 2002). Temperature at dynamic storage conditions was measured using a Escort iMiniPlus PDF temperature logger (Escort, Buchanan, VA, United States; accuracy from -10 to +70 ± 0.3°C) placed inside each loin chop package. Temperature was monitored every 15 min from the time of dispatch to Salmonella analysis period. Overall, four t/T profiles per lot were obtained, one for each scenario. For pH measurements, 10 g of loin chop were transferred to a BagFilter® (Interscience) containing 90 ml of sterile distilled water (pH=7) and homogenized for 2 min at normal speed (Stomacher 400, Seward, Worthington, UK). Then, the pH was measured with a pH meter 507 (Crison, Instruments Spa, Carpi, Italy). Descriptive statistics (mean, standard deviation, 5^{th} and 95^{th} percentiles) were calculated for each lot in each storage scenario.

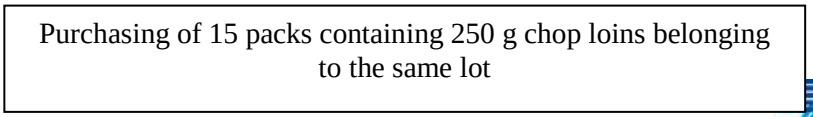
Twelve different samplings were conducted between May 2011 and April 2012 at a local retailer in Spain. During each sampling, 15 packs (samples) containing 250 g of loin chops each were purchased. The loin chops collected during different samplings belonged to different lots. However, those collected during the same sampling time were obtained from the same lot, meaning from animals reared in the same house and slaughtered at the same age.

For each lot, prevalence of *Salmonella* on chop loins was evaluated under four different scenarios, labelled as S1, S2, S3 and S4, throughout the product shelf life:

- after purchasing at retail (S1);
- after delivery and storage at retail, transport at car temperature for 15±5 minutes and storage at 6±1°C, up to day seven of the product shelf life (S2);
- after delivery and storage at retail, transport at car temperature for 15±5 minutes and storage at 12±1°C, up to day seven of the product shelf life (S3).

A general scheme showing the experimental design followed and the number of sample units tested at each scenario is represented in Figure 2. The final storage temperatures (i.e., 6 and 14°C) were selected as a mean and consumer abuse refrigerator temperatures, according to the results of a non-representative survey to determine the temperatures of consumer refrigerators in the region where the study was conducted (data not shown).

Figure 2. Experimental design of loin chops analysis



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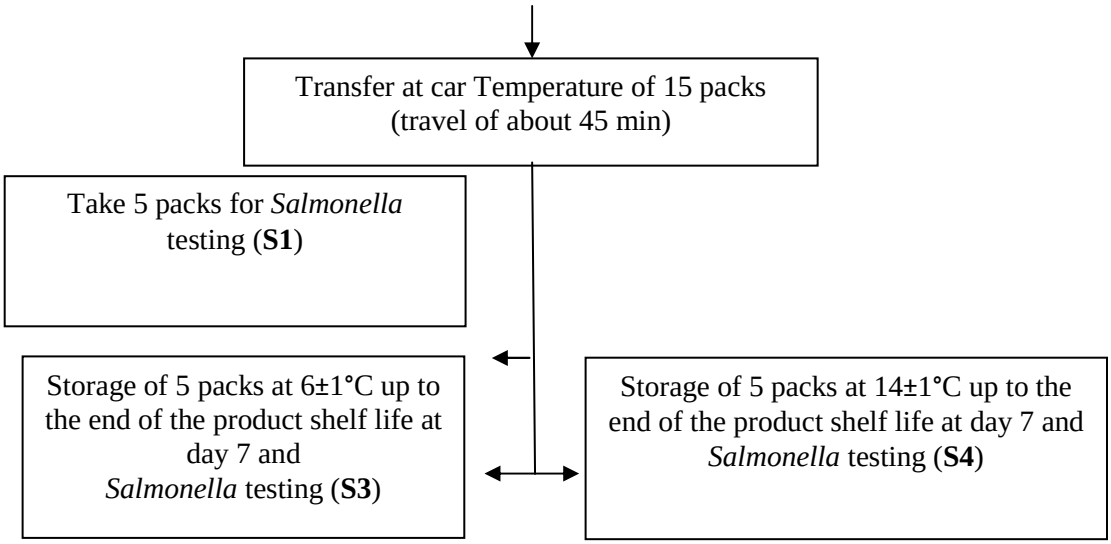

During each sampling, the outer wrapper of each pack containing the loin chop portions was disinfected with 70% ethanol. After slitting the package with a flame-sterilized knife, each meat portion was aseptically cut into several pieces. Afterwards, a sample unit of 25 g was obtained from different parts of each sample. The subsequent process was similar as described above. Descriptive statistics (mean, standard deviation,) were calculated for each lot in each storage scenario.

Listeria monocytogenes and pork cuts

The scheme presented for *Salmonella* (both for Italy and Spain) was also followed for *L. monoctogenes*, Each 25 g sample was aseptically transferred to a BagFilter® (Interscience), diluted 1:10 in Fraser broth (CM0895B, Oxoid) without supplements and homogenised by stomaching for 1 min in a Stomacher 400 (Seward, Worthington, UK) at normal speed. After storage at 20±1°C for one hour, 1 ml of the diluted sample was spread plated in duplicate onto three plates of Listeria Ottaviani Agosti agar (ALOA) (4016052, Biolife) and, incubated for 24 h at 30±1°C. The limit of quantification of the technique (LOQ) is 10 cfu/g. Subsequently, 5 ml of half-strength Fraser supplement (SR0166E, Oxoid) were added to the Fraser broth incubated for 24 h at 30±1°C for a first enrichment. At the end of the incubation period, the broth was streaked in duplicate onto ALOA agar plates and incubated as previously described. Furthermore, 0.1 ml of the half-Fraser broth was transferred into 10 ml of Fraser broth supplemented with 1% of Fraser supplement (SR156E, Oxoid) before incubation for 48 h at 37±1°C. At the end of the incubation period a second streaking was carried out in duplicate onto ALOA agar plates and incubated as previously described. Identity confirmation for at least three suspected *L. monocytogenes* colonies per sample was performed by PCR using the protocol published by Wesley et al. (2001) or Rodríguez-Lázaro et al. (2004). In each PCR run, *L. monocytogenes* ATCC 13932 and *Escherichia coli* ATCC 25922 were used as positive and negative controls, respectively.

Modeling approach to estimate the impact of cooking treatments on the establishment of Food Safety Objectives for *Salmonella* and *Listeria monocytogenes* in pork loin chops

The study aimed at designing suitable cooking procedures, simulating those practices made at home by consumers, in order to provide an estimation of the fulfillment of FSO for two foodborne pathogens, i.e. *Salmonella* and *Listeria monocytogenes*. Cooking treatments were performed with gas and oven and 40 independent replicates were done. Besides, three intensity levels were tested: raw (mild heating), medium heating and well-done.

Once trial results were obtained, data were submitted to be modeled. A probabilistic exposure assessment model was carried out in MS Excel, considering two different modules:

i) FSO module

ii) cooking module.

Positive samples after cooking (40 repetitions)				
Cooking levels	gas, non-stick pan		Oven	
	Salmonella spp	*Listeria monocytogenes*	*Salmonella spp*	*Listeria monocytogenes*
Raw	7	6	5	5
Medium	3	3	2	2
Well-done	1	2	0	0

i) FSO module

To provide an approximation of FSO, the approach studied by Perni et al. (2009) was followed. That study was focused on the establishment of risk-based metrics for various pathogens in steam meals (mixture of raw ingredients, packaged in MAP and cooked), thus, it was considered the most appropriate for this case.

FSO were related with the number of illnesses per 1,000,000 persons per year (ill) in the following Eq.

$$Ill = W * C * r * S * 1{,}000{,}000 \quad \text{Eq. 1}$$

where W: serving size (250g), S: No. servings consumed per person per year (365), r: dose-response parameter and C: microbial concentration (CFU/g). The log C was estimated as the FSO.

The parameter ill was dependent on the fatality rate (f) (i.e. the maximum number of cases/1,000,000 people) that can be assumed and the mortality rate (m). The parameter f was fixed to 1.

$$Ill = f / m \quad \text{Eq. 2}$$

With the information represented in the Excel spreadsheet, the estimated FSO for Lm = -0.33 log CFU/g while the estimated for Salmonella was -6.72 log CFU/g.

ii) Cooking module

The final number of positives (N +) was considered for modelling purposes. The inequation proposed by ICMSF was applied to calculate the number of reductions (log) achieved in each of the six treatments.

$$H_0 + sumI - sumR \leq FSO \quad \text{Eq. 3}$$

where H_0 is the initial contamination (log CFU/g), sumI is the sum of potential increments of the pathogen and sumR is the expected reductions.

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The parameter H_0 was estimated as a Pert distribution, with a minimum, mode and maximum value. Values obtained for H_0 ranged from 7 to 7.5 log CFU/g.

SumI was not considered as the pathogen is expected to be destroyed as consequence of the application of the thermal treatment. SumR was calculated as the subtraction of H_0 and the log transformed theoretical concentration below the detection limit (C_{det}), which was assumed to be the remaining concentration after the applied treatment.

Estimation of C_{det}:

Firstly, the expected number of positives after treatment (N + est) was calculated with a binomial distribution (n, c) with n = 40 (number of assays) and c = N +.

C_{det} (CFU/g) was estimated assuming a Poisson process to calculate the concentration under the detection limit of the technique. The analytical sample was assumed to be 25 g (w).

$$C_{\text{det}} = \frac{\ln[n/n - N + est)]}{w} \qquad \text{Eq. 4}$$

A MonteCarlo simulation (#10,000 iterations) was performed in ModelRisk v4.3 considering H_0 as input and C_{det} and R as output distributions. The results obtained are in the sheets "*Listeria* oven", "*Listeria* gas", "*Salmonella* oven" and "*Salmonella* gas".

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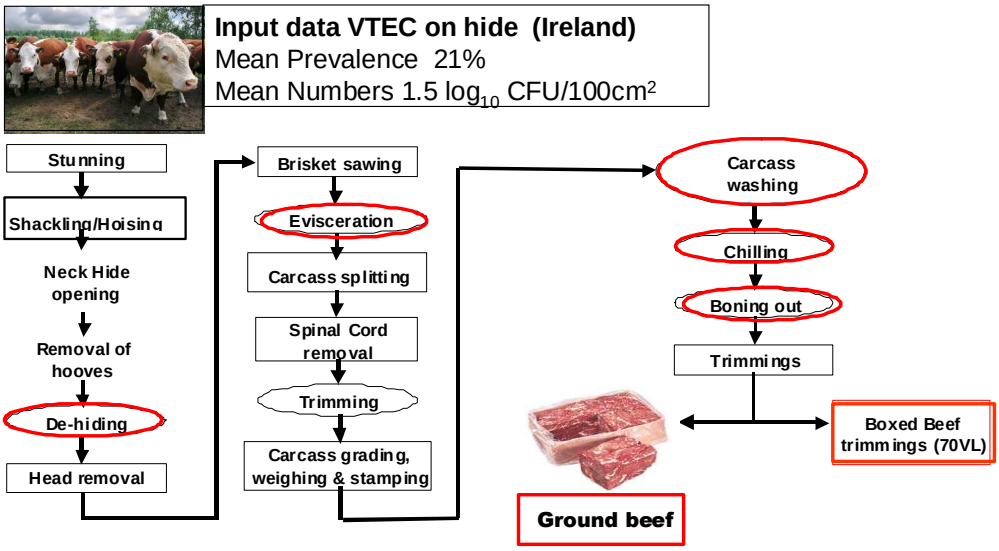
VTEC and ground beef

The figure below shows the stages in involved in beef slaughter to ground beef and high risk area for transmission are highlighted in red.

Slaughter chain : Input and stages impacting on VTEC contamination



It shows the input data to the model was the prevalence and concentration of clinical significant VTEC on bovine hide.

A beef process chain model using Monte Carlo simulation was developed for contamination of boxed beef trimmings in Irish abattoirs was used to predict the distribution and concentration of VTEC in ground beef and is shown in the Table and Figure below. The model was run for 1,000 iterations with a lot comprising of 350 animals. The model used Beta distributions for the hide, carcass pre-wash and carcass post wash prevalence, Uniform distributions were used for the concentration of *E. coli* O157 at these points. The predicted mean prevalence was 1.11 % and the predicted concentration of VTEC ranged from 0 to 4.82 log CFU/ g with a mean of 1.3 log CFU/g

Predicted prevalence of VTEC in ground beef by beef process model

Mean	**1.11%**
Mode	1.81%
Median	0.90%
5%	0.00%
95%	3.01%

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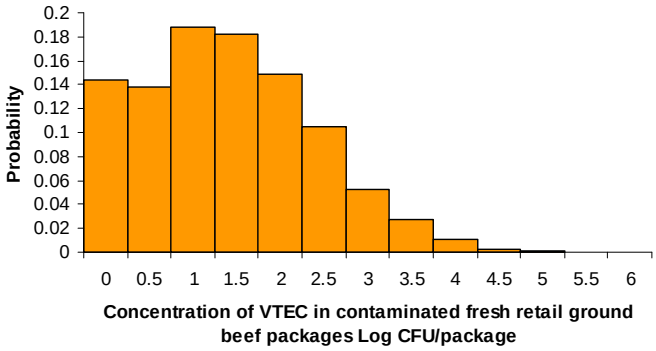

The results obtained from the distributions for VTEC counts can now be translated into a Performance Objective (POs) for ground beef. In the next months the PO target and the sample plan at this point will be completed.

Tetracycline and beef

Data:

1. Ten year (2001-2010) prevalence and quantitative data of chemical risk (tetracycline) for meat (beef, pork and poultry) from the Bundesamt für Verbraucherschutz (Federal Office of Consumer Protection and Food Safety).

2. Consumption data of population e.g. per capita consumption, frequency of consumption, age, weight, ethnicity and sex of consumers.

Model:

Stochastic model using *excel* spread sheet and Monte Carlo simulations using *@Risk* to generate probabilities that a consumer in the population would consume the hazard (tetracycline) exceeding the ADI (1 µg /kg bodyweight) for that hazard.

Table 3. Variables in the model and their input values in probability distributions.

s/n	Variables	Probability distributions	Parameters
1	Prevalence contaminated	Exponential or binomial	Mean or prevalence (probablilty of residue present)
2	Residue level (ug/kg fresh meat)	Lognormal	mean, SD
3	Residue level (LOD included) (µg /kg fresh meat)	Beta General	a1,a2, min max
4	Weight and age correction	GammaAlt	Quantiles, 5%, 50%,95%
5	Frequency of consumption	Triang	min, most likely, max

6	Consumption per capita (kg/person)		Country data

Quantification of risks can be stratum specific to age, sex, nationality and ethnicity.

The model uses the matrix of tetracycline and beef as example. This prototype quantitative risk assessment model has been created to calculate the population risk or likelihood that the chemical hazard consumed will exceed the acceptable daily intake or ADI on a diet of the specific meat type. Stratum specific risks can be calculated based on information about the consumer, e.g. age, sex, ethnicity, nationality and weight. Inputs from the data collected are entered as probability distributions to reflect the variability of each input (table 3).

Results

Campylobacter and poultry

The results obtained from the multivariate regression analysis showed that the variables 'capacity of the slaughterhouse', 'time between sampling and analysis' and 'thinning method' were significant ($p<0.05$) for some EU countries evaluated. Results are presented in Table 4. It should be noted that countries No. 10 and 11 were not included in the analysis since they contain less than 30 data belonging to one category for each individual factor, thus making the analysis not representative. For other countries, not significant differences were obtained.

Table 4. Significant variables ($p<0.05$) obtained in the multivariate regression analysis for the *Campylobacter* counts in broiler carcasses for each EU country evaluated.

Country No.	Variables	MSE[1]	F value	*p* value
2	Cap[2]	10.117	9.632	0.002
4	Cap	2.605	4.306	0.014
5	Cap	6.933	8.867	0.000
5	Time-samp[3]	3.266	4.177	0.007
7	Thin[4]	5.732	8.213	0.005
9	Cap	2.650	4.481	0.013
9	Thin	5.634	9.527	0.002
13	Thin	9.389	9.644	0.003
18	Thin	32.589	34.490	0.000
19	Cap	2.543	4.686	0.010
19	Time-samp	2.479	4.569	0.000
21	Cap	8.298	11.759	0.000
25	Time-samp	7.186	7.163	0.000

[1] Mean Square Error
[2] Capacity of the slaughterhouse
[3] Time between sampling and analysis
[4] Thinning method

According to this procedure, the whole dataset was split up into the corresponding categories, following the criteria of Table 2. For instance, if the variable 'thinning method' was found as significant, then data were divided into two sub-datasets: those catalogued as '0' (no thinning) and those named as '1' (thinning was followed), for each EU country where these differences were found, for further analysis and distribution fitting.

Once datasets were obtained, descriptive statistics were calculated, as shown in Table 5. In total 3,400 batches were evaluated in this study (10,162 broiler carcasses). The average number of batches per country evaluated ranged from 12 to 144. Prevalence of *Campylobacter*-contaminated broiler carcasses had a large variability between the evaluated countries, ranging between 4.9% (country No. 10) to 100% (country No. 18b-thinning method applied), and the highest number of censored data (concentration below the LoQ, 10 cfu/g) was 82 for country No. 16. The highest number of positives carcasses (obtained from detection and enumeration methods) corresponded to the country No. 6 (370 out of 422 carcasses were positive for *Campylobacter* spp), followed by countries 23 and 14 (360 and 348 positive carcasses, respectively). The country No. 11 obtained the highest number of non detects (N_{det}=376), followed by country No. 20 (N_{det}=355). The theoretical concentration under the detection limit (C_{det}, log cfu/g) was calculated according to eq 1. It can be seen that most of the values obtained were below -1 log cfu/g = 1 cfu/10g. These data were further used to fit log normal distributions by the MLE method.

Table 5. Descriptive statistics of *Campylobacter* counts in broiler carcasses: No. batches; No. carcasses, positive carcasses (N_+), non detects (N_{det}), censored data (N_{cens}), mean concentration under the limit of quantification (C_{det}, log cfu/g) and mean concentration of the analyzed samples (C_{mean}, log cfu/g) ± CI (95%) between brackets.

Country No.	N_batches	N_carcasses	N_+	N_{det}	N_{cens}	C_{det} (log cfu/g)	C_{mean} (log cfu/g)
1	132	396	123	273	29	-1.83	-0.57 [-0.79;-0.36]
2a	124	371	287	84	63	-1.23	1.42 [1.24; 1.60]
2b	13	37	33	4	4	-1.05	2.56 [2.00;3.12]
3	127	380	198	182	6	-1.53	0.69 [0.46;0.93]
4a	98	294	202	92	2	-1.33	1.85 [1.58;2.13]
4b	21	63	25	38	0	-1.69	0.49 [-0.21;1.19]
5a	59	177	115	62	12	-1.38	0.84 [0.55;1.12]
5b	82	244	197	47	14	-1.18	1.83 [1.60;2.06]
5c	84	252	168	84	22	-1.36	0.95 [0.71;1.18]
5d	57	169	144	25	4	-1.12	2.13 [1.88;2.39]
6	141	422	370	52	50	-1.08	1.87 [1.65;2.10]
7a	44	130	105	25	42	-1.18	1.23 [0.96;1.50]
7b	76	226	211	15	33	-0.96	2.36 [2.18;2.55]

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8	141	422	295	127	78	-1.32	1.34 [1.12;1.56]
9a	69	205	136	69	44	-1.36	0.86 [0.61;1.10]
9b	46	136	108	28	20	-1.20	1.86 [1.55;2.18]
9c	31	93	66	27	10	-1.31	1.60 [1.15;2.05]
9d	105	315	222	93	66	-1.31	1.20 [0.83;1.57]
10	34	102	5	97	3	-2.70	-2.49 [-2.84;-2.13]
11	132	396	20	376	0	-2.68	-2.47 [-2.64;-2.31]
12	94	280	126	154	9	-1.62	0.35 [0.06;0.64]
13a	54	162	82	80	5	-1.55	0.35 [-0.01;0.71]
13b	12	34	24	10	0	-1.31	1.61 [0.75;2.47]
14	123	367	348	19	1	-0.93	3.37 [3.17;3.57]
15	41	122	41	81	0	-1.79	-0.41 [-0.76;-0.06]
16	144	432	267	165	82	-1.41	0.79 [0.60;0.98]
17	140	419	339	80	17	-1.18	2.08 [1.90;2.26]
18a	39	117	113	4	0	-0.87	2.16 [1.94;2.38]
18b	52	156	156	0	0	-	3.04 [2.88;3.21]
19a	103	307	256	51	123	-1.14	1.02 [0.88;1.16]
19b	36	106	77	29	22	-1.29	1.11 [0.79;1.43]
19c	50	150	118	32	72	-1.21	0.69 [0.42;0.96]
19d	88	263	215	48	73	-1.17	1.21 [0.93;1.49]
20	137	410	55	355	18	-2.24	-1.73 [-1.90;-1.56]
21a	84	252	176	76	9	-1.32	1.36 [1.12;1.61]
21b	57	170	139	31	0	-1.17	1.94 [1.62;2.27]
22	125	374	172	202	60	-1.61	-0.08 [-0.28;0.13]
23	130	389	360	29	0	-0.98	2.71 [2.56;2.86]
24	143	429	162	267	15	-1.72	-0.15 [-0.36;0.06]
25a	75	224	101	123	16	-1.62	0.39 [0.08;0.70]
25b	57	169	104	65	42	-1.42	0.32 [0.05;0.58]

Figure legend:
2a- Cap=6; 2b-Cap=2-5; 4a- Cap=6; 4b-Cap=2-5; 5a- Cap=6; 5b-Cap=2-5; 5c- Time-samp=24-36 h and 60-72 h; 5d- Time-samp=36-60 h; 7a- Thin=0; 7b-Thin=1; 9a- Cap=6; 9b-Cap=2-5; 9c- Thin=0; 9d-Thin=1; 13a-Thin=0; 13b-Thin=1; 18a- Thin=0; 18b-Thin=1; 19a- Cap=6; 19b-Cap=2-5; 19c- Time-samp=36-72 h; 19d-Time-samp <36 h and 72-80 h; 21a- Cap=6; 21b-Cap=2-5; 25a- Time-samp < 36 h and 60-72 h; 25b- Time-samp= 36-60 h and 72-80 h.

Overall, mean concentrations (C_{mean}, log cfu/g) estimated by the MLE method ranged between 3.37 log cfu/g (country No. 14) and -2.49 log cfu/g (country No. 10) (Table 3). The adjustment of the fitted distributions to data observed can be visualized in figure 3 through the probability-probability (P-P) plots and the goodness-of-fit statistics calculated (Kolmogorov-Smirnov and Anderson-Darling tests) represented

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in Table 6. As can be seen, p-values were higher than 0.05 for all fitted distributions, so normality was assumed for *Campylobacter* counts.

Figure 3. Probability–probability plot (P-P plot) representing the theoretical cumulative distributions (red line) against the observed counts of *Campylobacter* in broiler carcasses

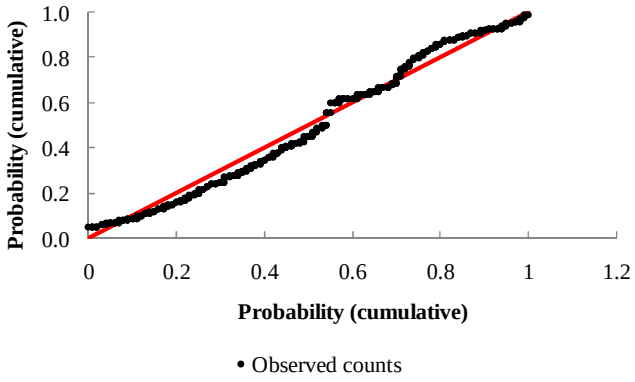

Figure 4 represents the relationship between the 50th percentile of the Beta distributions for *Campylobacter* prevalence (number of positive carcasses / total number of carcasses) and the mean concentration (C_{mean}, log cfu/g) estimated for the log normal distributions. A strong linear relationship was observed (R^2=0.86) showing that a high prevalence of *Campylobacter* in a batch does imply the presence of heavily contaminated carcasses.

Figure 4. Relationship between mean concentration of *Campylobacter* (C_{mean}, log cfu/g) in broiler carcasses estimated by the MLE method, and 50th percentile of the Beta distributions for prevalence in the EU countries evaluated.

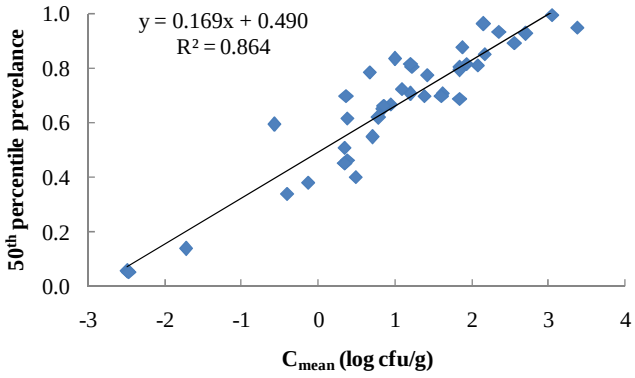

Table 6. Goodness-of-fit indices obtained for the fitted log normal distributions of *Campylobacter* counts in broiler carcasses: Kolmogorov-Smirnov test (K-S) and Anderson-Darling test (A-D) together with mean concentration, μ (log cfu/g) and standard deviation, σ (log cfu/g).

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Country No.	K-S	K-S *p* value	A-D	A-D *p* value	μ (log cfu/g)	σ (log cfu/g)
1	0.07	0.06	1.86	0.11	-0.57	0.95
2a	0.06	0.11	1.82	0.12	1.42	1.79
2b	0.14	0.41	0.69	0.56	2.56	1.69
3	0.03	0.80	0.70	0.56	0.70	0.65
4a	0.08	0.03	2.30	0.06	1.85	0.62
4b	0.08	0.74	0.47	0.78	0.48	0.57
5a	0.08	0.17	1.77	0.12	0.84	1.94
5b	0.04	0.76	0.41	0.84	1.84	0.68
5c	0.07	0.14	2.41	0.06	0.95	1.92
5d	0.06	0.47	0.43	0.82	2.17	0.89
6	0.10	0.33	0.92	0.40	1.87	1.11
7a	0.11	0.10	1.80	0.12	1.23	1.56
7b	0.06	0.41	0.73	0.53	2.36	0.64
8	0.07	0.08	1.69	0.14	0.36	0.72
9a	0.08	0.15	1.59	0.16	0.86	1.81
9b	0.05	0.79	0.48	0.77	1.84	0.59
9c	0.05	0.94	0.31	0.93	1.62	0.73
9d	0.10	0.34	1.02	0.35	1.20	1.79
10	0.12	0.09	1.76	0.12	-2.49	1.83
11	0.06	0.14	2.23	0.07	-2.47	1.67
12	0.04	0.74	0.90	0.42	0.34	0.72
13a	0.06	0.78	0.37	0.87	0.33	0.61
13b	0.14	0.52	0.95	0.38	1.61	2.46
14	0.07	0.62	0.58	0.67	3.37	0.72
15	0.11	0.08	2.30	0.06	-0.41	1.97
16	0.06	0.05	3.08	0.02	0.79	2.03
17	0.04	0.65	0.49	0.75	2.07	0.71
18a	0.09	0.33	0.94	0.39	2.16	1.18
18b	0.09	0.30	1.08	0.32	3.04	0.90
19a	0.12	0.00	2.84	0.03	0.99	0.77
19b	0.08	0.49	0.44	0.81	1.09	0.68
19c	0.12	0.07	1.15	0.29	0.66	1.15
19d	0.12	0.08	1.66	0.14	1.21	1.46
20	0.06	0.08	3.65	0.01	-1.73	1.78
21a	0.04	0.88	0.34	0.91	1.38	0.71
21b	0.04	0.96	0.40	0.85	1.93	0.63
22	0.04	0.44	1.33	0.22	0.38	0.72
23	0.05	0.22	1.02	0.34	2.70	0.91
24	0.05	0.23	1.10	0.31	-0.14	0.81

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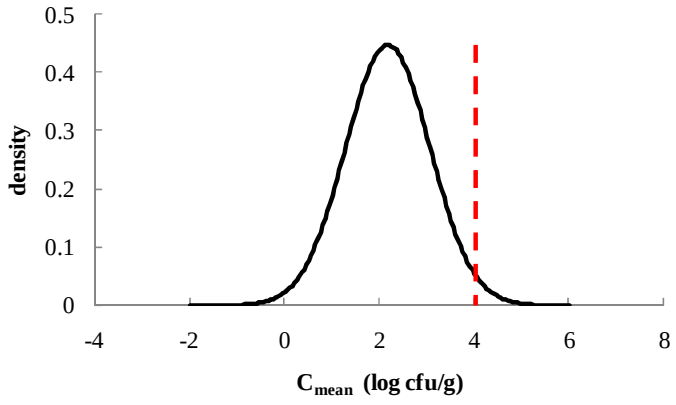
25a	0.05	0.69	0.53	0.72	0.34	0.81
25b	0.09	0.09	1.01	0.35	0.38	1.06

2a- Cap=6; 2b-Cap=2-5; 4a- Cap=6; 4b-Cap=2-5; 5a- Cap=6; 5b-Cap=2-5; 5c- Time-samp=24-36 h and 60-72 h; 5d- Time-samp=36-60 h; 7a- Thin=0; 7b-Thin=1; 9a- Cap=6; 9b-Cap=2-5; 9c- Thin=0; 9d-Thin=1; 13a-Thin=0; 13b-Thin=1; 18a- Thin=0; 18b-Thin=1; 19a- Cap=6; 19b-Cap=2-5; 19c- Time-samp=36-72 h; 19d-Time-samp <36 h and 72-80 h; 21a- Cap=6; 21b-Cap=2-5; 25a- Time-samp < 36 h and 60-72 h; 25b- Time-samp= 36-60 h and 72-80 h.

The *Campylobacter* performance target (CPT) was introduced as a regulatory tool to verify the effectiveness of interventions to reduce levels of *Campylobacter* contamination during the processing (slaughter and dressing) of broiler chickens. To estimate potential Food Safety Criteria to be applied after the specific food chain step where samples were taken (i.e. after chilling) POs were determined according to the maximum allowable prevalence and / or concentration not to be exceeded. For this specific case, in those countries where mean concentration was established above the LoQ (10 cfu/g), POs were set as target concentration levels so that a certain percentage of contaminated units is allowed. This is illustrated in figure 5. For the country No. 5d (Time-samp=36-60 h). In this case, parameters of the log normal distribution are μ=2.17; σ=0.89 log cfu/g. If a target concentration of 4 log cfu/g is chosen, then the maximum percentage of units above this value is 1.99%. Therefore, the PO can be formulated as P(log cfu/g>4; 1.99%), in other words no more than 1.99% of units will be contaminated with a concentration higher than 4 log cfu/g. The next step would be to implement a MC so that the samples taken comply with this criteria.

Figure 5. Log normal distribution (μ=2.17; σ=0.89) estimated for the country No. 5d (Time-samp=36-60 h) of *Campylobacter* concentration in broiler carcasses. The dashed line represents the target concentration (4 log cfu/g) above which a certain percentage of contaminated units in the lot (1.99%), is allowed. This corresponds to the area at the right side of the dashed line.

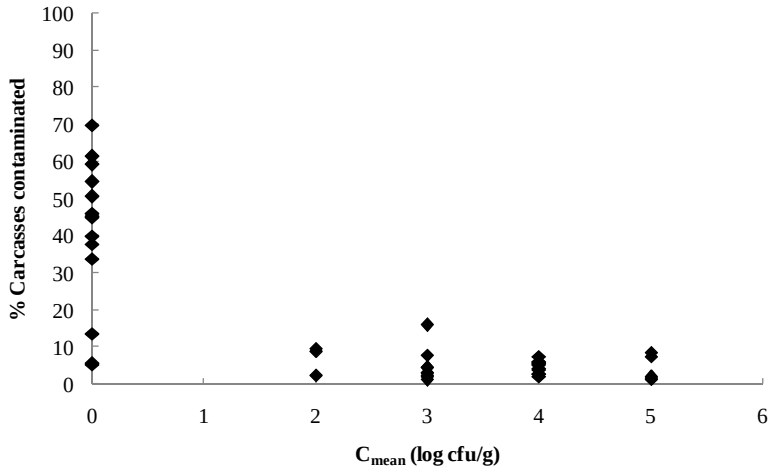


On the other hand, when estimated mean concentration of *Campylobacter* was below the LoQ, PO were based on prevalence values. These data were obtained by considering the 50th percentile of the Beta distributions for prevalence, even though other target limits could be hyphotetically set by a risk manager. A graphic representation of estimated POs for *Campylobacter* in broiler carcasses for the EU countries

evaluated is shoen in figure. 6. It should be noted that points at the y-axis when log cfu/g = 0 are referred to 'presence' in a certain percentage of contaminated carcasses (0 log cfu/g = 1 cfu/g).

Figure 6. Graphic representation of estimated Performance Objectives (PO defined as maximum allowable percentage of contaminated units below a certain limit of concentration in log cfu/g) for *Campylobacter* in broiler carcasses for the EU countries evaluated.

Points at the y-axis when log cfu/g = 0 are referred to 'presence' in a certain percentage of contaminated carcasses (0 log cfu/g = 1 cfu/g).



Salmonella and pork cuts

In Italy, *Salmonella* was detected in 6 of the ten lots tested and whenever present the pathogen did not disappear along the product shelf life. The prevalence of the pathogen in the scenarios under study ranged between 52% prevalence detected in samples tested after transport and storage at retail to 34% prevalence detected at the end of the product shelf life after storage at abuse temperature (Figure 7). In Spain, *Salmonella* was detected in 4 of the 12 lots tested (33.33%). In total 180 samples were tested and 15 were positive (8.33%). The prevalence of the pathogen in the scenarios under study ranged between 25% prevalence detected in samples tested immediately after arriving to the lab to 33.33% prevalence detected at the end of the product shelf life after storage at normal refrigeration temperature (Figure 8).

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Figure 7. Prevalence of *Salmonella* in the samples tested under the different scenarios examined (Italy)

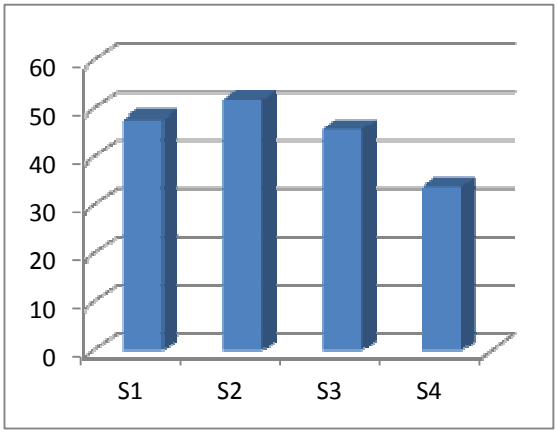
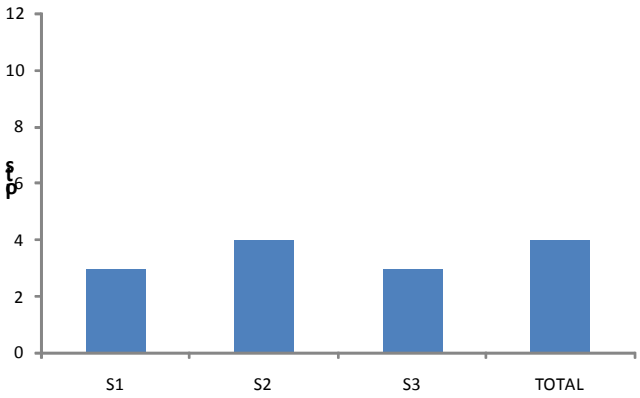


Figure 8. Prevalence of *Salmonella* in the lots tested under the different scenarios examined (Spain)



Even if the variability between numbers of positive samples at each scenario was high the positive lots after packaging remained positive whereas the negative lots after packaging were negative up to the end of the shelf life in Italy (Figure 9).

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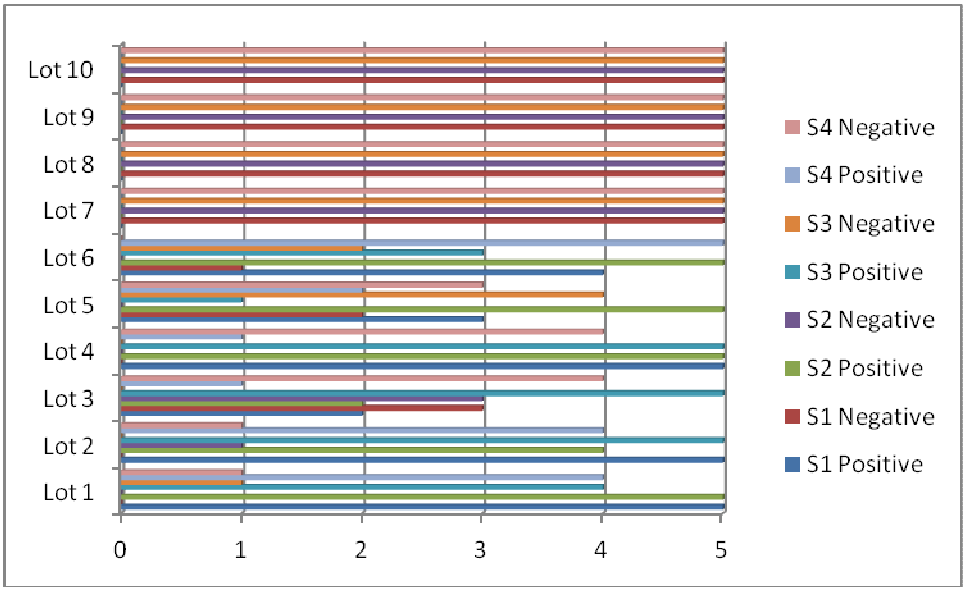
Figure 9. Positive and negative samples in each lot for each scenario



Listeria monocytogenes and pork cuts

At each sampling point, dynamic t/T profiles, product pH, prevalence and populations of *L. monocytogenes* were determined. Mean values together with standard deviations are summarized in table 7a for Italy and table 7b for Spain. Overall in Italy, prevalence of *L. monocytogenes* immediately after packaging (i.e., S1) was 49% and increased to more than 70% during storage at retail (i.e., S2) and to 72% during storage at temperatures mimicking mean and abuse temperature conditions in the consumer's refrigerators (Table 7A). High variability in *L. monocytogenes* prevalence between lots was found. In fact, storage scenario S3, lots 1, 8, 9 and 10, had less than 4 samples positive, while for lots 3, 4, and 10 all samples were positive for *L. monocytogenes*. After a final storage at temperature abuse conditions (S4), lot prevalence of *L. monocytogenes* increased and in lots 5 and 6 all tested samples were positive. In comparison to the prevalence levels reported by the authors cited in the introduction, those estimated in this study are much higher. However, the papers described in the literature refer to a range of pork products collected from different processing facilities or retail shops often located in different localities. In contrast, the *L. monocytogenes* prevalence levels found in this study refer only to loin chops processed in the same facility through a one year period.

Overall in Spain, prevalence of *L. monocytogenes* was 41.67% (5 lots positive out of 12). The prevalence after arriving to the lab (i.e., S1) was 16.67% and increased to more than 33.33% during storage at abuse temperature conditions in the consumer's refrigerators (Table 7B). Although the samples were positive, they were below the limit of quantification.

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Table 7.

A - Mean values ± standard deviation of storage temperature (T_{mean}, ºC), pH (pH_{mean}) and concentration of *L. monocytogenes* (Conc, log cfu/g), together with percentage of samples showing a positive result in the enumeration method, i.e. > 10 cfu/g (P_{en}, %)

Scenario[1]	T_{mean} ºC	pH_{mean}	Conc (log cfu/g)	P_{en} (%)
S1	3.31±2.89	6.22±0.37	2.02±2.15	5
S2	5.50±1.72	6.21±0.24	2.46±2.55	15
S3	6.14±0.66	6.04±0.21	2.38±2.35	20
S4	11.18±2.58	6.05±0.31	3.14±3.35	39

B - Mean values ± standard deviation of storage temperature (T_{mean}, ºC), and lots positives for *L. monocytogenes*, together with percentage of samples showing a positive result in the enumeration method, i.e. > 10 cfu/g (P_{en}, %)

Scenario[1]	T_{mean} ºC	pH_{mean}	Positive
S1	4.00±0.49	5.95±0.17	2/12
S2	5.20±0.52	5.85±0.11	2/12
S3	12.06±1.08	5.97±0.14	4/12
TOTAL	- - -	5.92±0.14	5/12

Modeling approach to estimate the impact of cooking treatments on the establishment of Food Safety Objectives for *Salmonella* and *Listeria monocytogenes* in pork loin chops

For Lm it was concluded that all samples were below the FSO (-0.33 log CFU/g) so cooking treatment was sufficient to fulfill the FSO, even though unrealistic contamination levels were used (7 log CFU/g). The highest concentration reached was -1.7 log CFU/g when a raw treatment was applied.

However, for *Salmonella*, all samples were above the FSO (-6.72 log CFU/g) regardless the treatment applied. A reduction of 14D would be necessary to achieve the FSO. This measure is not practical, since maximum reduction was assumed as 12D (R_{max}). Therefore, the maximum allowable contamination for the meat before cooking was estimated as PO.

H_0 – sumR ≤ FSO

H_0 – 12 ≤ –6.72

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Assuming a 100% effective treatment, H_0 = 5.27 log CFU/g.

However, it must be considered the very high contamination levels for the raw meat and the presence of positive samples of Salmonella after cooking, which was probably caused by cross-contamination procedures with the cooking utensils.

Assumptions in this approach:

- Independence of replicates

- Random contamination of samples

- Final number of microorganisms Poisson distributed

- FSO estimated following a deterministic approach. No variability and uncertainty in r, m and f was considered

- Public heath objectives taken from literature

VTEC and ground beef

A scenario analysis was conducted to see the impact of including a carcass decontamination (Acid wash) on predicted distribution and concentration of VTEC on ground beef (Table 8 and Figure 10). A mean count of 0.66 log cfu/g was predicted on treated carcass and 1.2 log cfu/g on non-treated carcasses. In the next phase a PO and sample plan will be calculated for VTEC on ground beef.

Table 8. Use of Process model to see effect a carcass decontamination step (acid wash) on prevalence of VTEC on fresh retail ground beef

	No carcass Decontamination	**Carcass Decontamination**
Mean	**1.11%**	**0.56%**
Median	0.90%	0.05%
Percentile 5%	0.00%	0.00%
Percentile 95%	3.01%	2.41%

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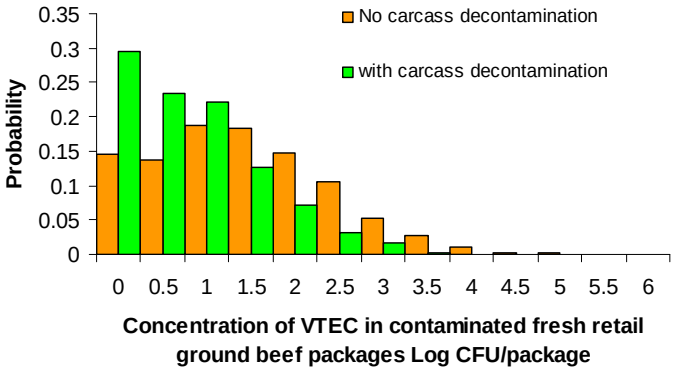
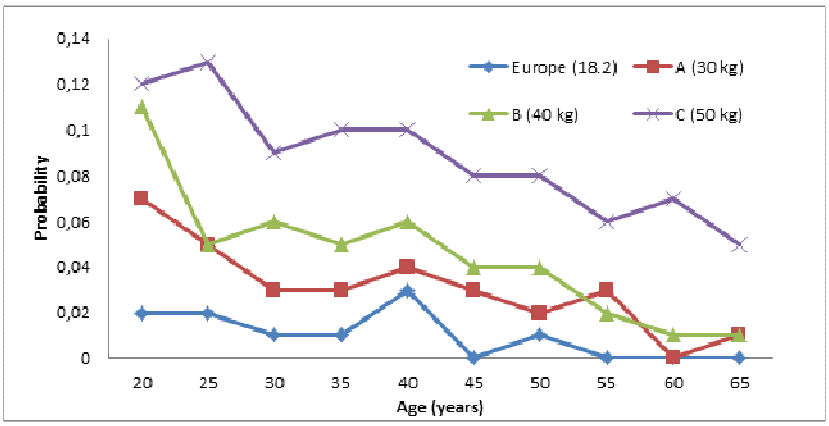
Figure 10. Concentration of VTEC in contaminated fresh retail ground beef packages LOG CFU/package



Tetracycline and beef

A simulated output based on the model is seen in the graph below in 4 regions with the 4 hypothetical annual per capita beef consumption patterns (Europe-18.2kg, 30kg, 40kg, 50kg) (figure 11).

Figure 11. Risk of exceeding ADI for tetracycline in 4 regions with different per capita consumption of beef (18.2, 30, 40, 50) (kg) patterned by age



Risk managers or food safety authorities can use this quantitative method to assess whether the FSO has been reached.

Conclusions

This deliverable describes the safety criteria for the different biological and chemical risk-product combinations selected for meat products: *Campylobacter* and poultry, *Salmonella* and pork cuts, *Listeria* and pork cuts, VTEC and beef, Tetracycline and beef.

For *Campylobacter* in broiler chickens, 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). For this specific case, in those countries where mean concentration was established above the LoQ (10 cfu/g), POs were set as target concentration levels so that a certain percentage of contaminated units is allowed.

For *Salmonella*, all samples were above the FSO (-6.72 log CFU/g) regardless the treatment applied. A reduction of 14D would be necessary to achieve the FSO. This measure is not practical, since maximum reduction was assumed as 12D (R_{max}). Therefore, the maximum allowable contamination for the meat before cooking was estimated as PO.

For *L. monocytogenes* it was concluded that all samples were below the FSO (-0.33 log CFU/g) so cooking treatment was sufficient to fulfill the FSO, even though unrealistic contamination levels were used (7 log CFU/g). The highest concentration reached was -1.7 log CFU/g when a raw treatment was applied treatment was applied. This approach could provide more accurate estimations by considering other inputs such as variability and uncertainty in the parameters used. However, the main purpose was to evaluate the impact of potential cooking treatments in raw meat before consumption and how food safety criteria can be applied into a risk analysis framework.

For VTEC a PO and sample plan are now being calculated for monitoring of VTEC on ground beef. A risk model and scenario analysis has shown that a carcass intervention (acid wash) can lower the VTEC contamination on beef product at the time of consumption reducing the level of risk for the consumer.

The tetracycline /beef matrix is 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.

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