Development of a model for *Listeria monocytogenes* in RTE foods

Prof. Dr Fernando Pérez-Rodríguez
University of Córdoba (Spain)
E-mai: fernando.perez@uco.es

Outline

- Introduction and model scope
- Selection of D-R models
- Exposure assessment
- Simulation and output
- An easy-to-use framework: Excel Add-in "Lis-RA"
- Conclusions

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- ❑ **Introduction and model scope**
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Introduction

Probabilistic risk assessment of *Listeria monocytogenes* for in RTE foods developed by EFSA in collaboration with the University of Córdoba (Spain) and IRTA (Spain)

Closing gaps for performing a risk assessment on *Listeria monocytogenes* in RTE foods: Activity 2, a quantitative risk characterization on *L. monocytogenes* in RTE foods; starting from the retail stage[1]

- packaged (hot, cold) smoked or gravad fish (not frozen),
- packaged heat-treated meat products (cooked meat, sausages, pâté)
- soft or semi-soft cheeses (excluding fresh cheeses)

The risk assessment scope covers from retail to home, considering Listeria growth up to consumption

1Contract number: OC/EFSA/BIOCONTAM/2014/02CT1

Listeriosis

L. monocytogenes is a psychrotrophic microorganism able to produce a foodborne diseases

Listeriosis is mostly related to relatively high doses and

Elderly population (>64) is most affected group, particularly >84 year (ECDC/EFSA, 2016)

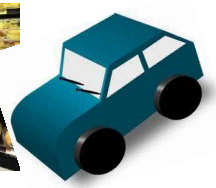
In 2014: “EU case fatality was 17.7% among the 1,524 confirmed cases with known outcome”

Quantitative Microbial risk assessment

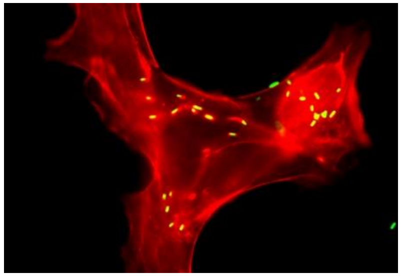
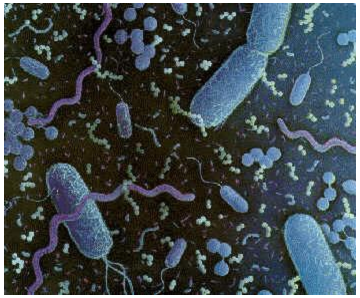
EXPOSURE ASSESSMENT



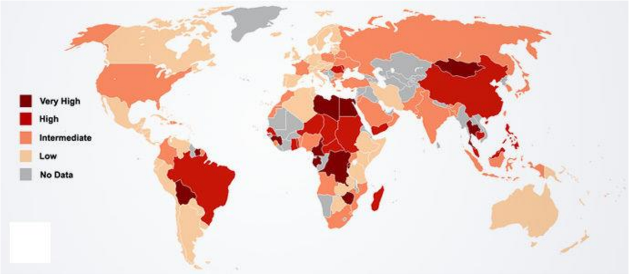


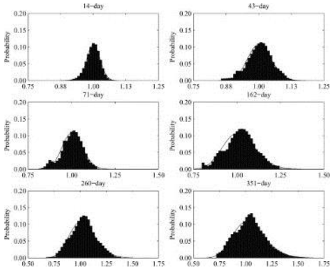




HAZARD CHARACTERIZATION


RISK CHARACTERIZATION





Model scope

Food chain step/s: **from retail to consumption**

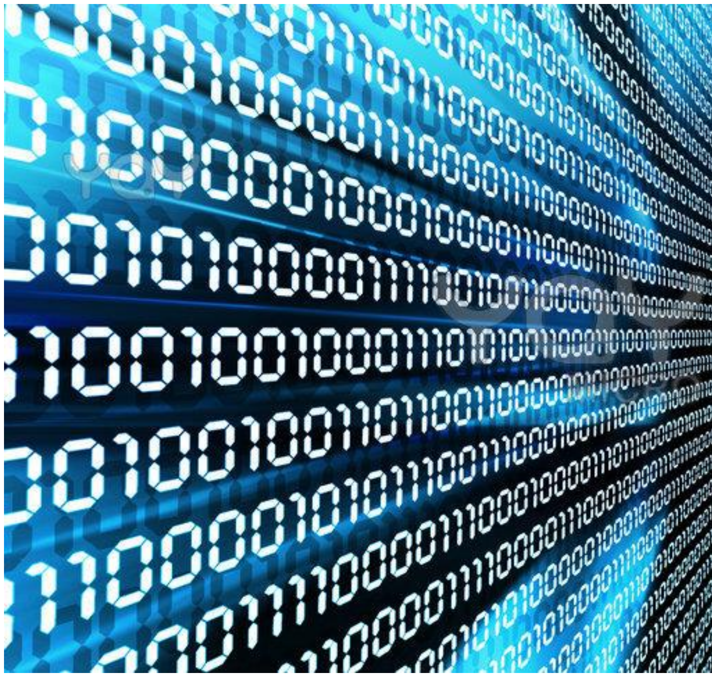
- Selected RTE **food categories**:
  - packaged (hot, cold) smoked or gravad fish,
  - packaged heat-treated meat products (cooked meat, sausages, pâté)
  - soft or semi-soft cheeses (excluding fresh cheeses)

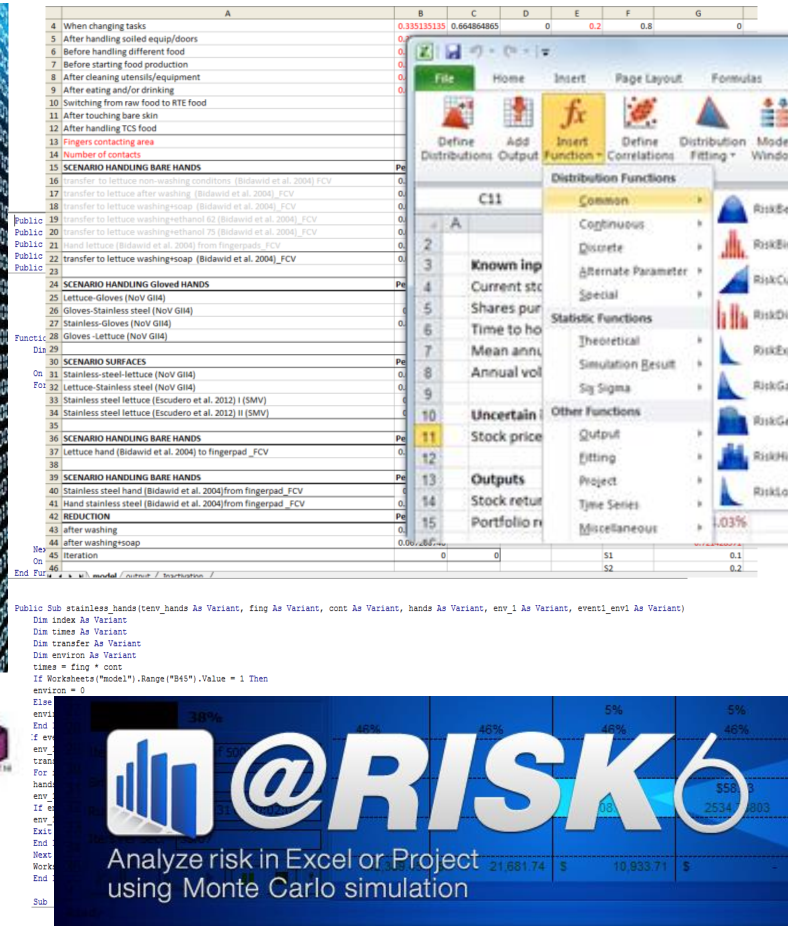
- **Post retail factors**: time and temperature

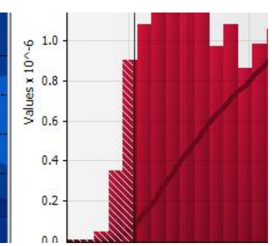
- Handling **scenarios**: slicing, packaging type, formulation with growth inhibitors,...

- **Population**: normal and susceptible (elderly and pregnant) population.

In-silico risk assessment model for listeriosis and RTE products







Raw data framework

European Food Safety Authority

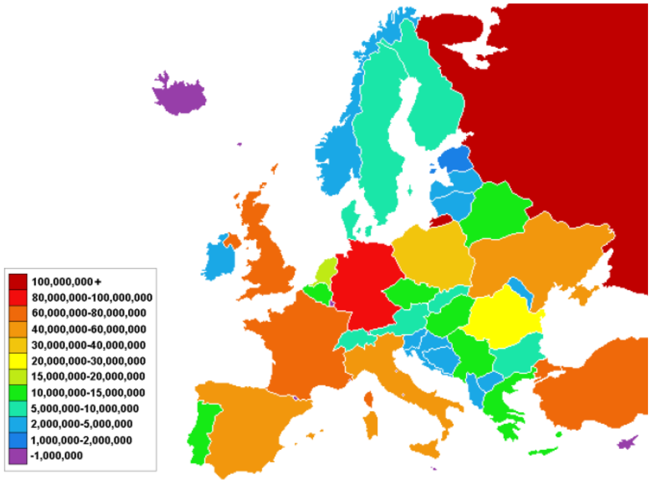
DATA COLLECTION BY EFSA

- Pesticide residues occurrence
- Contaminant occurrence
- Food Consumption
- Food additive usage and occurrence
- Veterinary medicinal product residues
- Zoonoses, antimicrobial resistance & foodborne outbreaks
- Molecular Typing (from 2014)

EU-wide Baseline studies

eurostat

Your key to European statistics



Food and Agriculture Organization of the United Nations

FAOSTAT

Data analysis/processsing tools

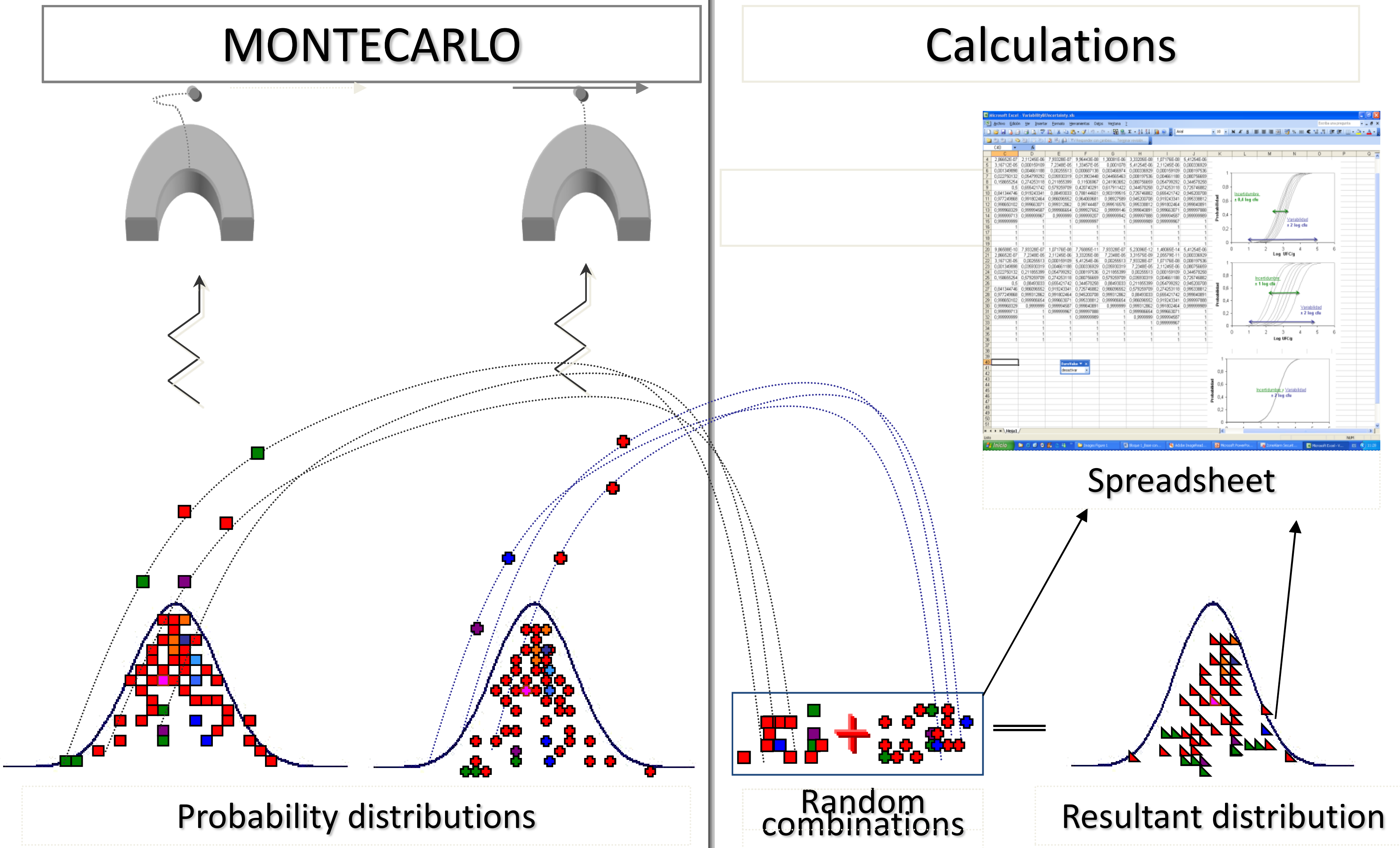
Systematic review

Data quality assessment

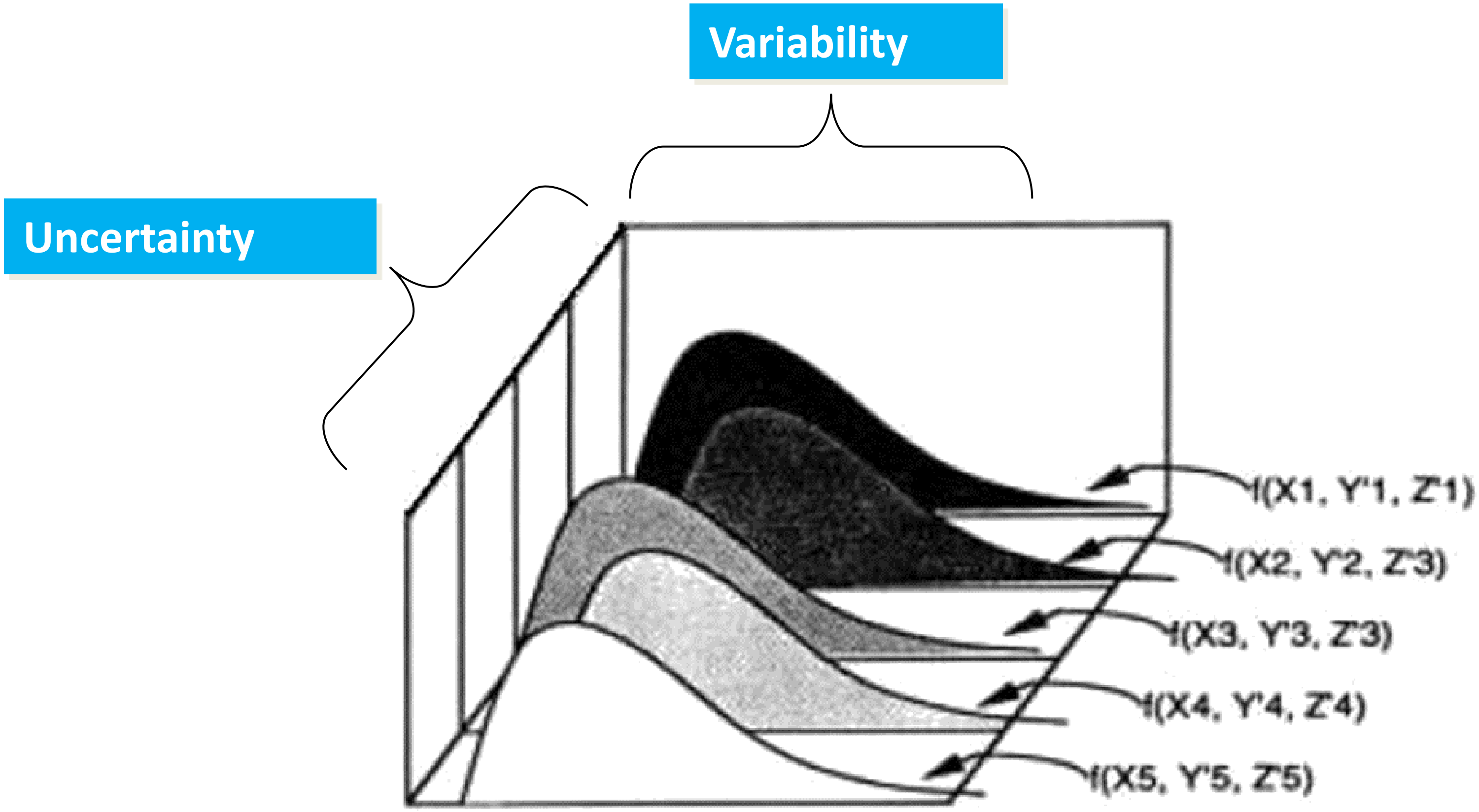
Expert knowledge elicitation

Statistical analysis
Numerical methods

Numerical techniques



Second order model



(adapted from Hoffman and Hammonds, 1994)

How to complete the risk picture?

DATA

PROCESSING DATA TOOLS

INPUT → **MODEL** → **OUTPUT**

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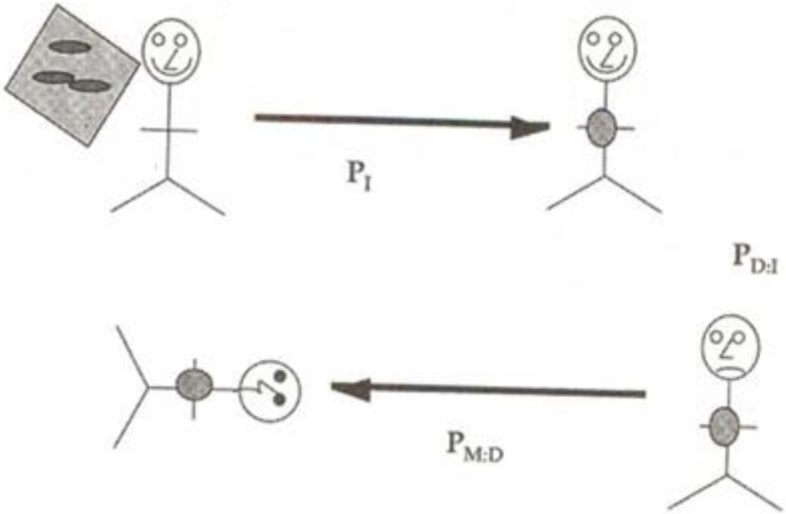
Dose-response (D-R) model

- Mathematical function that may be used to describe the relationship between dose and the magnitude of a response on a continuous scale in an individual.



Adapted from Haas et al. 1999

Selection of D-R models for risk assessment

Tool to evaluate the quality of the Exponential dose-response models currently available:

Application of Numeral Unit Spread Assessment Pedigree (NUSAP) system

The NUSAP system (Boone et al., 2009) is intended to assess data quality resulting from uncertainties that are hard to quantify such as methodological and epistemological uncertainties, and that are not systematically taken into account in scientific studies.

NUSAP scoring system

Objective scores
Assessors

Weights to Pedigree Criteria
Experts

Selection of D-R models for risk assessment

Pedigree criteria

Proxy:

- Year of publication of the dose-response model.
- Geographical origin of primary data. Not applicable for animal models.

Empirical basis:

- Primary source of data.
- Number of independent sources for the primary source of data.
- Number of subpopulation groups from which data were analysed.

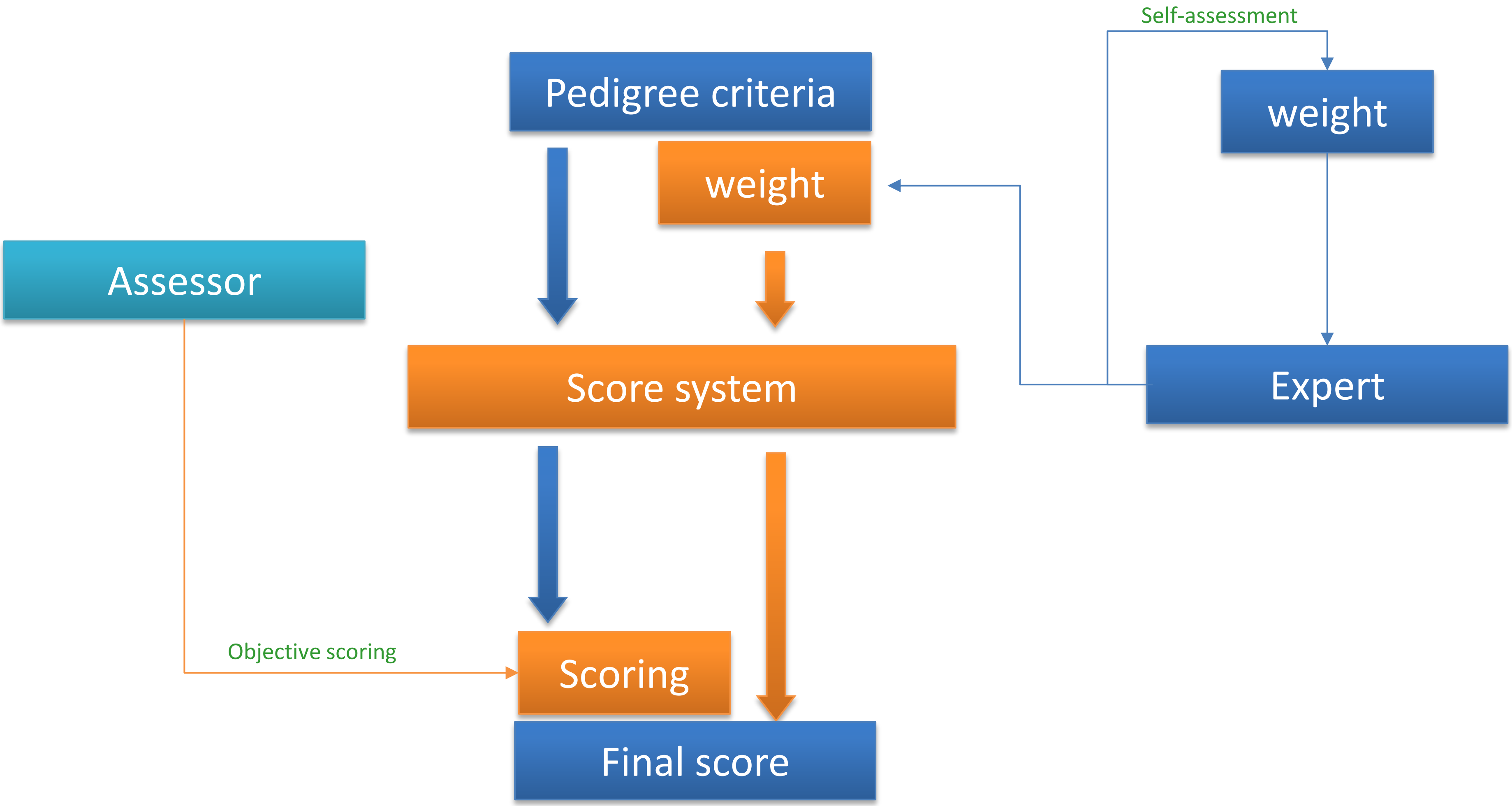
Methodological rigor

- Inclusion of variability and uncertainty.
- Statistical analysis. Not applicable for Buchanan et al. (1997) approach.
- Number and descriptions of endpoints.
- Publication source.

Validation:

- Validation of the dose-response model with other datasets.

Selection of D-R models for risk assessment



Selection of D-R models for risk assessment

Outcome:

- Arithmetic *versus* geometric sequence (arithmetic sequence, i.e. 1, 2, 3 and 4).
- General agreement in the difficulty of FDA/FSIS model to be implemented; the model is neither readily reproduced nor readily defined
- the use of two dose-response models: Pouillot et al. (2015), representing a novel approach to describe *L. monocytogenes* dose-response relationship; and FAO/WHO (2004), an institutional approach internationally recognized and easy to reproduce.

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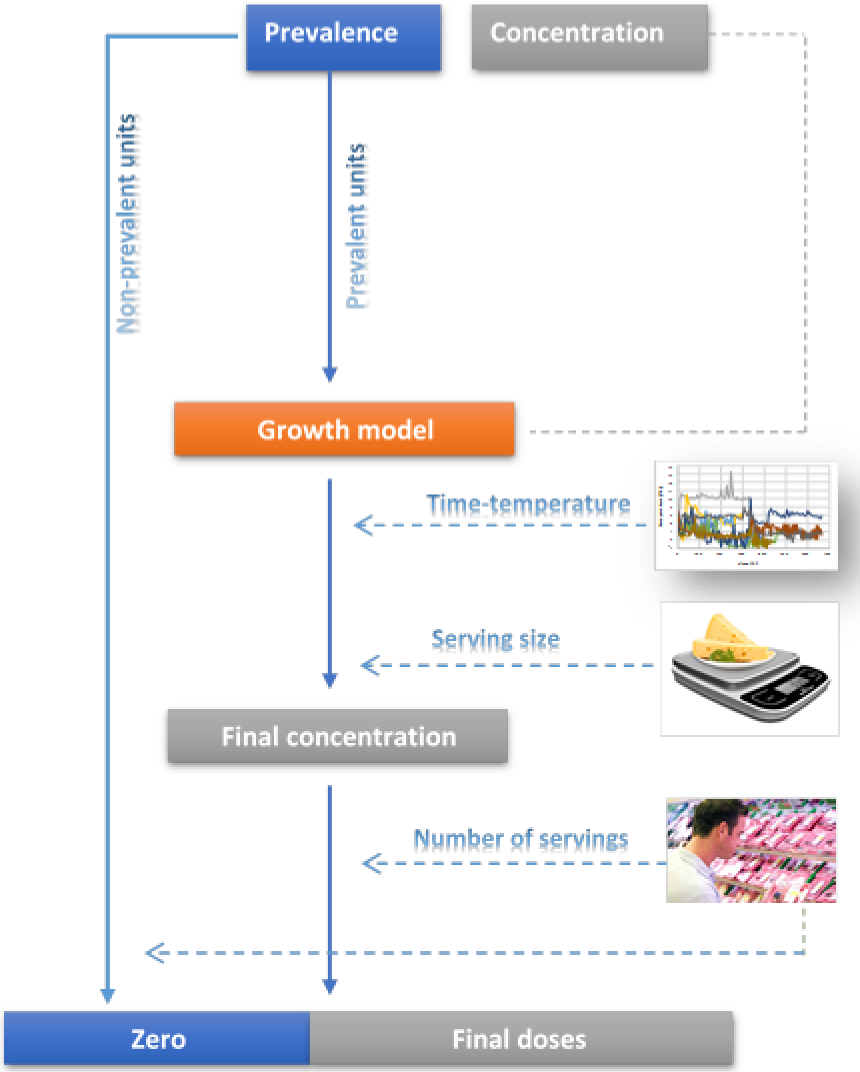
Exposure Assessment

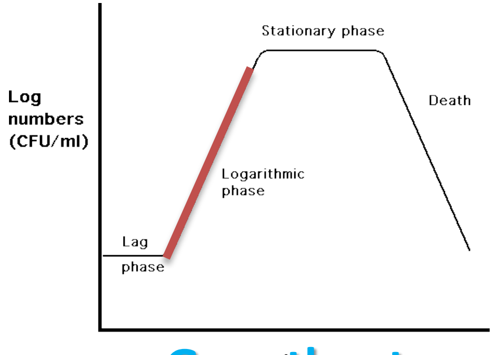


MAIN VARIABLES:

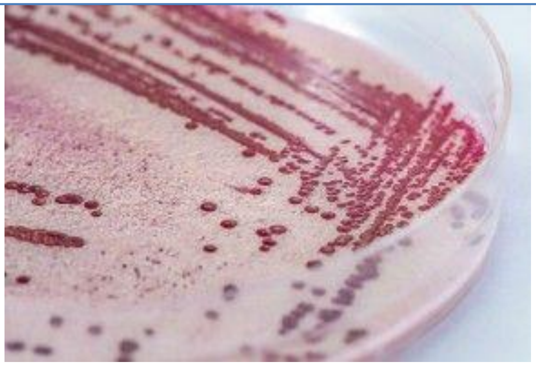
- Prevalence/concentration distributions of *L. monocytogenes*
- Stochastic model for the growth of *L. monocytogenes*
- Temperature-time profiles from retail to home
- Time to consumption
- Food serving size and number of serving per year

Structure of exposure assessment

Categories	Sub-Categories	Scenarios
Packaged heat-treated meat products	Cooked meat, sausage, Pâté	*ROP/normal; Sliced/non-sliced
Soft or semi-soft cheese	-	Sliced/non-sliced
Packaged (not Frozen) smoked and gravad fishs	Cold and hot smoked fish and gravad fish	ROP/normal; Sliced/non-sliced



Growth rate

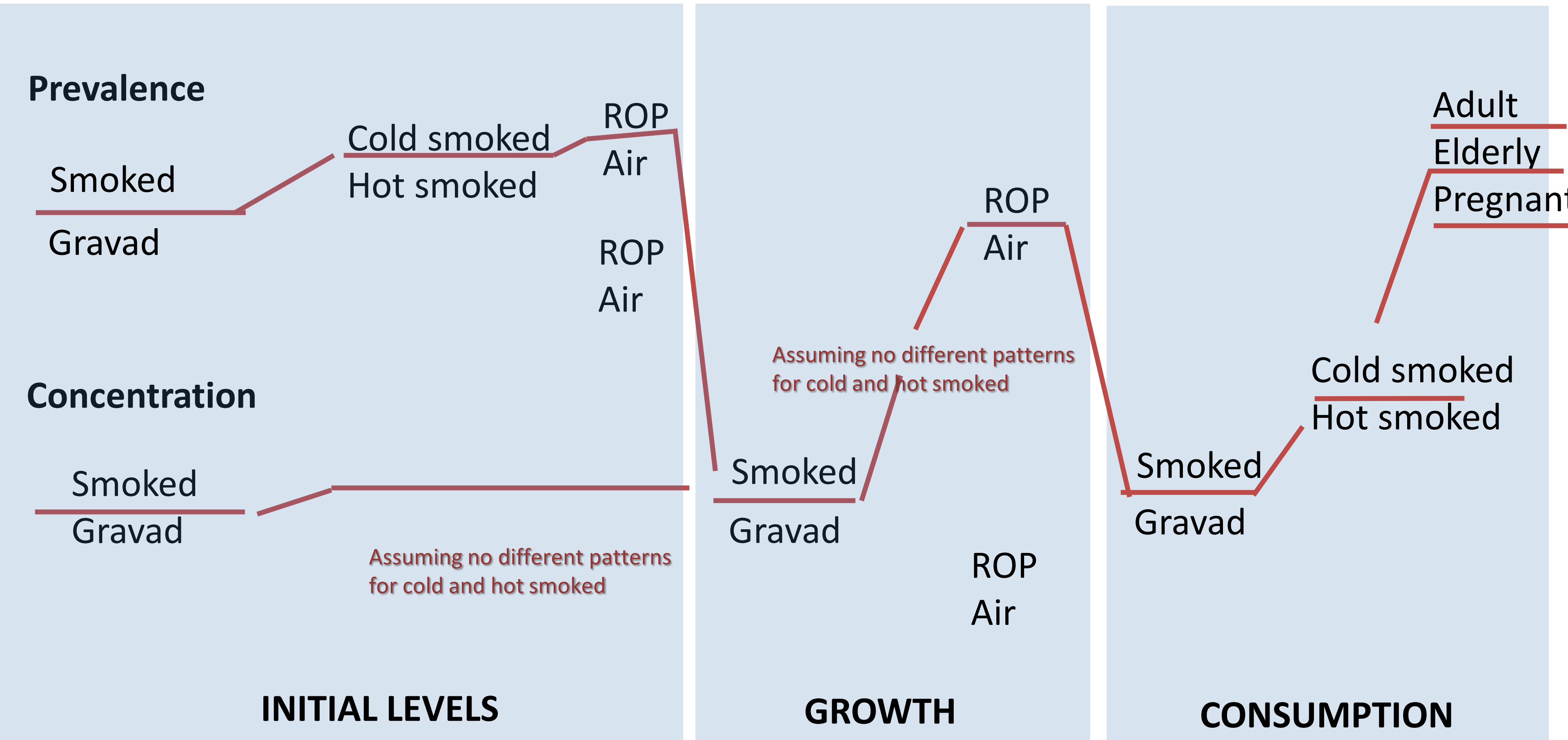


Prevalence & Concentration

*ROP: REDUCED OXYGEN PACKAGING

Structure of exposure assessment

Iteration *i*: COLD SMOKED FISH UNDER REDUCED OXYGEN PACKAGING



Prevalence categorization models

Food-based sub-categories for initial prevalence

Cooked meat*
Sausage*
Paté

Smoked fish (cold)
Smoked fish (hot)
Gravad fish

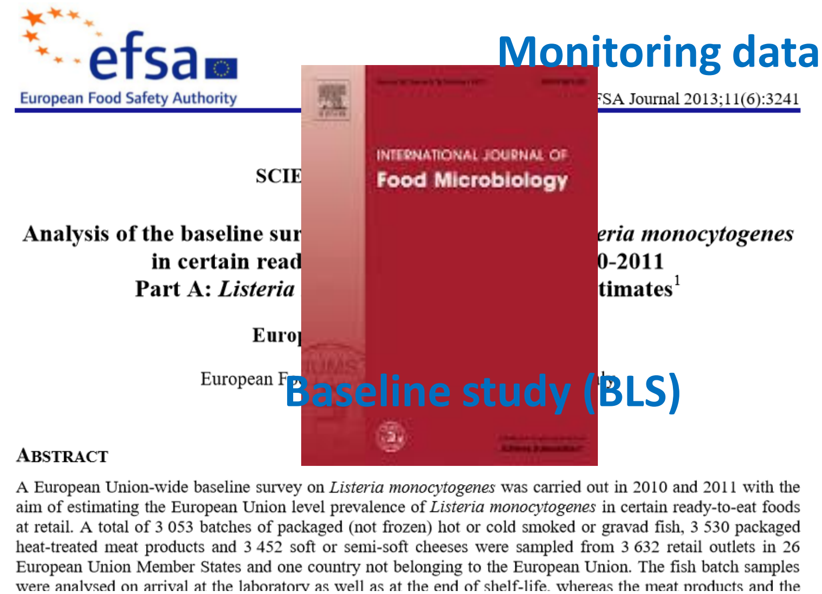
Soft/semi-soft cheeses

Meat produ

Fish produc

Cheese pro

Monitoring data



Baseline study (BLS)

Scenarios for initial prevalence:

- Packaging type for heat treated meat and smoked and gravad fish: Air (normal) and ROP (Reduced Oxygen Packaging)
- Slicing and non-slicing for heat-treated meat
- Hot and cold smoked fish

*Cooked meat and sausage were treated as individual subcategories for the BLS data. Monitoring data and scientific studies could not distinguish between both subcategories

Prevalence categorization models

Food category	Subcategory	Scenario	Fitted Beta distributions[(a)]	Mean [C.I. 95%]
RTE fish products	Cold-smoked fish	Sliced	Beta(76+1;511-76+1)	**0.151** [0.116-0.186]
		Non sliced	Beta(18+1;102-18+1)	**0.183** [0.103-0.270]
	Hot-smoked fish	Sliced	Beta(20+1;239-20+1)	0.087 [0.049-0.130]
		Non sliced	Beta(12+1;273-12+1)	0.047 [0.021-0.078]
	Gravad fish	Sliced	Beta(30+1;219-30+1)	**0.140** [0.091-0.194]
		Non sliced[(b)]	Beta(0+1;33-0+1)	0.029 [0.005-0.103]
RTE meat products	Cooked meat	Sliced	Beta(43+1;2297-43+1)	0.019 [0.013-0.026]
		Non sliced	Beta(3+1;193-3+1)	0.021 [0.003-0.045]
	Sausage	Sliced	RiskBeta(11+1;548-11+1)	0.022 [0.009-0.037]
		Non sliced	RiskBeta(2+1;214-2+1)	0.014 [0.001-0.034]
	Paté	Sliced	RiskBeta(7+1;114-7+1)	0.069 [0.023-0.125]
		Non sliced	RiskBeta(2+1;70-2+1)	0.042 [0.003-0.010]
RTE cheese products	Soft and semi-soft cheese	Sliced	RiskBeta(5+1;816-5+1)	0.007 [0.002-0.015]
		Non sliced	RiskBeta(8+1;2298-8+1)	0.004 [0.001-0.007]

(a) Beta distributions were defined as (σ =s+ 1; β=N-s+1), being s +1; N-s+1) being s the number of positives and N the total number of samples per RTE food subcategory.

(b) No positive samples were reported. A prior Beta (1,1) was considered for describing uncertainty in prevalence estimates.

Defining concentration at retail

Food category	Sub-category	Data source
RTE fish products	Cold-smoked fish	BLS/monitoring 11-14/Activity 1
	Hot-smoked fish	BLS/monitoring 11-14/Activity 1
	Gravad fish	BLS/monitoring 11-14/Activity 1
RTE meat products	Cooked meat	Monitoring 11-14/Activity 1
	Sausage	Activity 1
	Pâté	Monitoring 11-14/Activity 1
RTE cheese products	Soft and semi-soft cheese	Monitoring 11-14/ Activity 1

Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods:
Activity 1, an extensive literature search and study selection with data extraction on *L. monocytogenes* in a wide range of RTE food.

Contract number: NP/EFSA/BIOCONTAM/2015/04-CT1

Defining concentration at retail

Initial concentration simulated in the model represents for mean concentration variation between lots.

Collected data are assumed to come from different batches

Data from positive and negative samples (censored data) were considered for building probability distributions: e.g. 0 cfu/25g → < 0.04 cfu/g

Log normal was used to describe variability of lot mean concentration: distribution was fitted to collected data

Poisson distribution was used to simulate partitioning (sampling) in the model. In doing so, doses per serving size can be estimated.

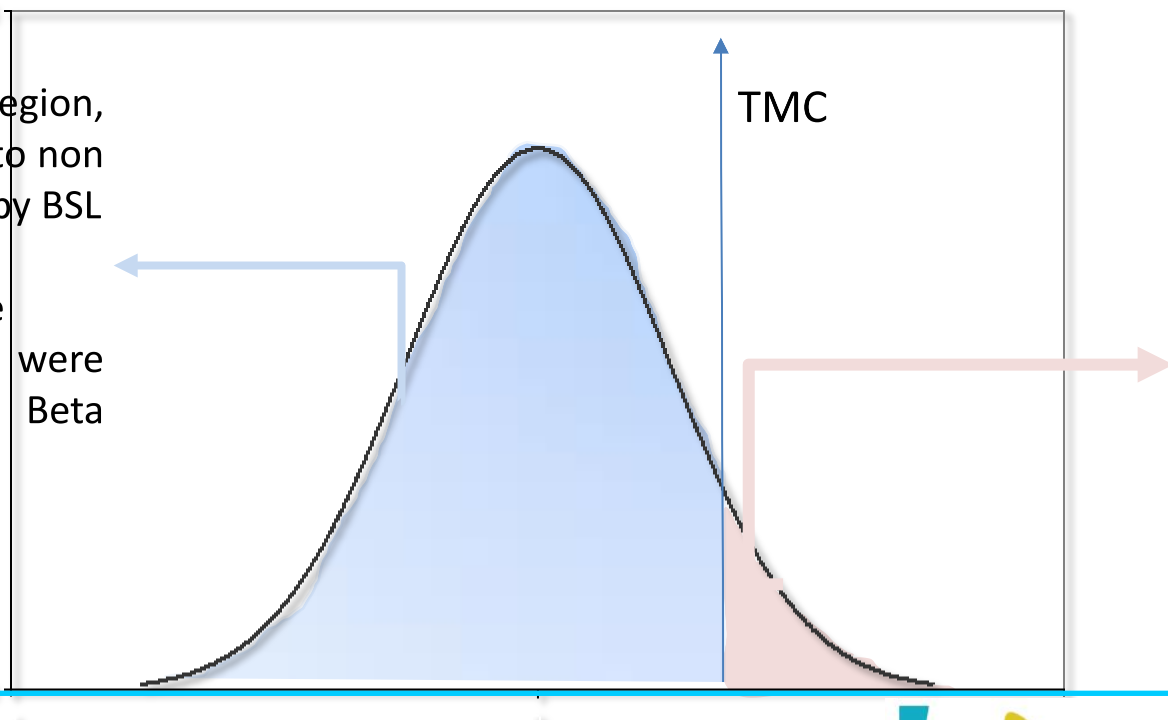
This distribution assumes an random contamination pattern.

Defining concentration at retail

As prevalence was modelled separately, lognormal distribution was truncated to the minimum concentration values resulting into positive servings. <u>This corresponds to the theoretical minimum concentration (TMC) for a positive sample (1 cell in 25g).</u>

This was applied to each food subcategory.

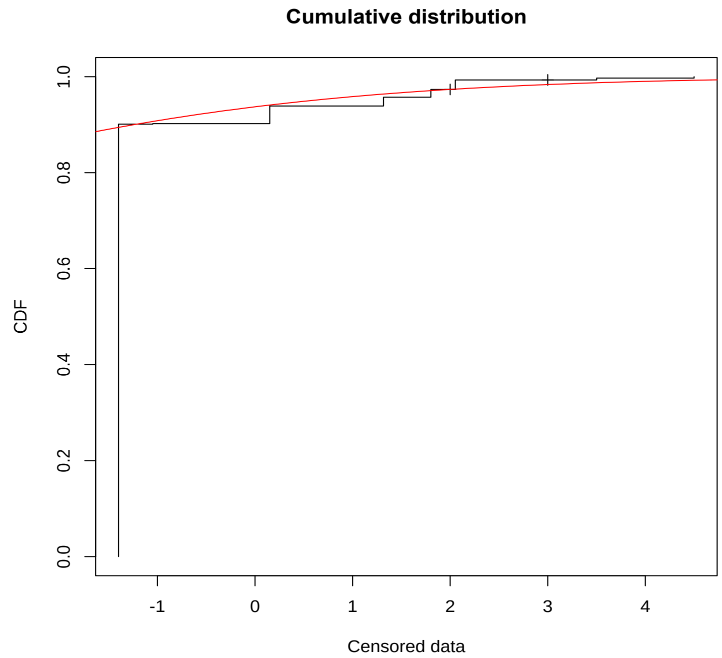
- Excluded region, corresponding to non prevalent data by BSL
- Non-prevalence samples were modelled by Beta distribution



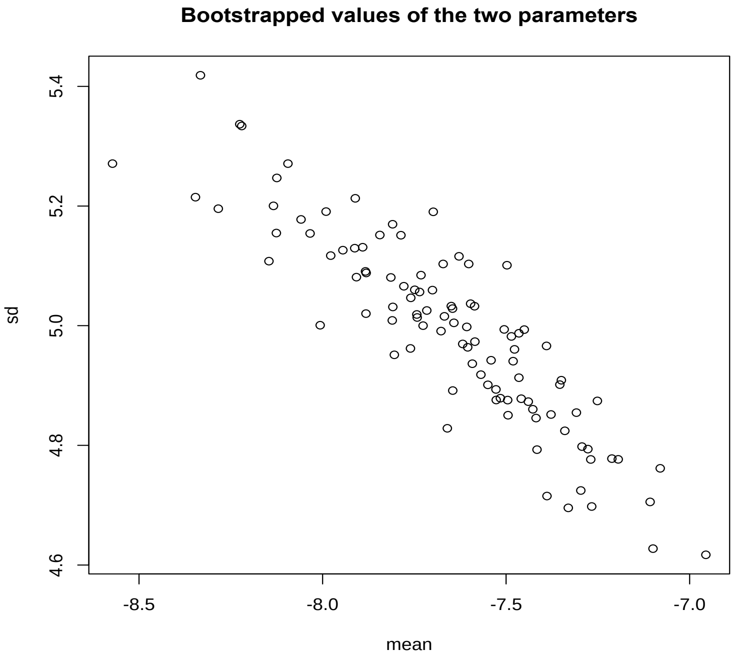
Simulated prevalent samples

Defining concentration at retail

A)



B)



Fitting of the log normal distributions to describe the initial concentration of *L. monocytogenes* of cold smoked fish at retail (A) and correlation between mean (μ) and standard deviation (SD) values resulting from the bootstrap of simulated data (B)

Defining concentration at retail

Food subcategory	Mean	SD	50th Perc.	5th Perc.	95th Perc.	LogL	AIC	BIC
Cold smoked fish	0.867	1.842	1.248	0.394	4.620	-1.50×10^{3}	3.12×10^{3}	3.14×10^{3}
Hot smoked fish	-0.271	0.943	1.318	-0.511	1.593	-1.79×10^{3}	3.59×10^{3}	3.60×10^{3}
Gravad fish	1.011	1.931	1.236	0.524	4.950	-2.39×10^{2}	4.83×10^{2}	4.92×10^{2}
Cooked meat	1.100	2.119	1.241	0.523	5.453	-7.10×10^{2}	1.42×10^{3}	1.44×10^{3}
Sausage	2.194	2.704	1.151	1.598	7.482	-3.22×10^{1}	6.84×10^{1}	7.53×10^{1}
Pâté	1.461	2.334	1.213	0.852	6.240	-1.86×10^{3}	3.73×10^{3}	3.74×10^{3}
Soft and semi-soft cheese	0.909	1.917	1.252	0.389	4.886	-3.14×10^{2}	6.32×10^{2}	6.46×10^{2}

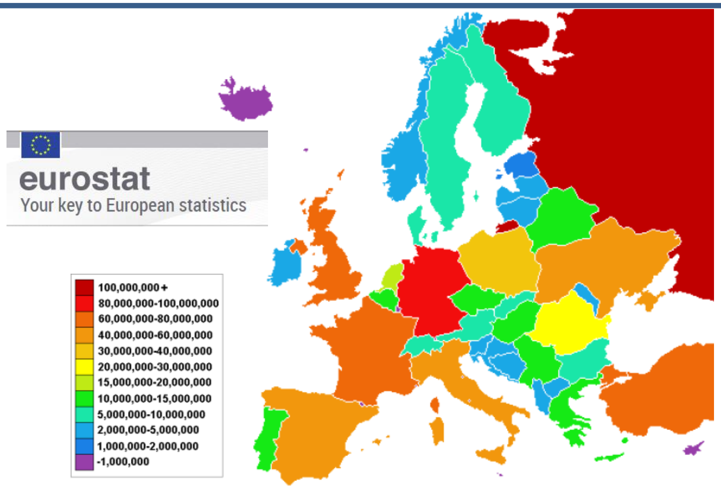
Serving size and number of servings

- Linear extrapolation:

[the value of numbers of serving /person] **X** the total subpopulation in the survey country.

	Austria	Denmark	Finland	Germany	Greece	Ireland	Latvia	Netherland:	Romania
TOTAL consumption	1.73E+10	9.48E+09	2.64E+10	3.61E+10	0.00E+00	7.87E+09	0.00E+00	5.35E+10	1.38E
Susceptible populatioi	NA	1.22E+09	NA	NA	NA	NA	NA	NA	NA
Healthy population	NA	7.48E+09	2.44E+10	NA	NA	NA	NA	NA	NA
Elderly population	3.78E+09	1.96E+09	2.00E+09	0.00E+00	0.00E+00	6.15E+08	0.00E+00	1.42E+10	3.14E
Healthy population	1.35E+10	7.48E+09	2.44E+10	3.59E+10	0.00E+00	7.25E+09	0.00E+00	3.93E+10	1.07E
Pregnant women	1.08E+08	1.51E+08	5.39E+08	#¡DIV/0!	#¡DIV/0!	2.00E+08	#¡DIV/0!	2.53E+08	5.30E
Total/person	2034.098026	1683.9433	4.84E+03	4.47E+02	0.00E+00	1.71E+03	0.00E+00	3.18E+03	6.92E
#Servings (50g)	3.46E+08	1.90E+08	5.28E+08	7.23E+08	0.00E+00	1.57E+08	0.00E+00	1.07E+09	2.76E
APROXIMATION									
Total consumption	1.76E+10	1.13E+10	2.19E+10	2.02E+10	6.52E+10	1.98E+10	1.14E+11	2.66E+10	5.74E
Total population	8506889	11203992	10512419	5627235	1315819	5.45E+06	8.08E+07	2.00E+06	1.68E
Elderly population	1.56E+06	1.03E+06	1.06E+06	1.68E+07	2.24E+06	5.81E+05	3.82E+05	2.92E+06	3.30E
Healthy population	6.87E+06	6.87E+06	4.34E+06	6.33E+07	8.59E+06	3.96E+06	1.60E+06	1.37E+07	1.65E
Pregnant population	7.83E+04	5.62E+04	5.84E+04	6.83E+05	9.41E+04	6.92E+04	2.04E+04	1.71E+05	1.81E

EFSA food consumption database per surveyed country and subpopulation

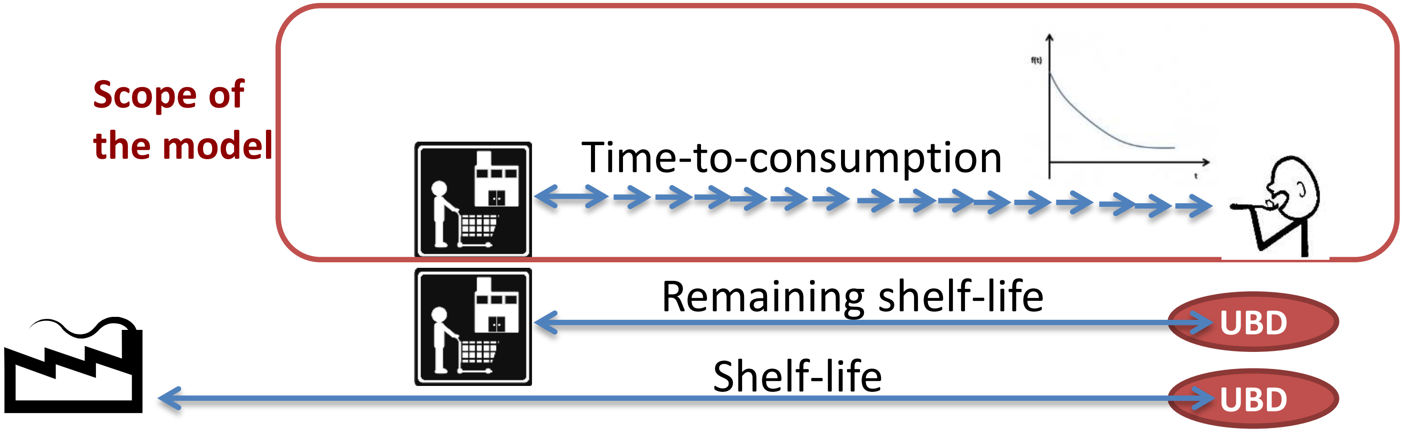


Demographic data per country and subpopulation

- *When there are missing population groups, the available groups are used for extrapolation to the rest*
- *When there are missing countries, the available countries are used for extrapolation to the rest: no pattern*

Time to consumption

- Some concepts need to be clarified:
  - ✓ Shelf-life: time elapsed from production date to use-by-date.
  - ✓ Remaining shelf-life: time elapsed from purchase date (PD) to use-by-date (UBD). It is calculated as UBD-PD.
  - ✓ Time-to-consumption (TTC): time elapsed from purchase date (PD) to consumption.
- No available data covering all food categories and subcategories
- Use of BASELINE data, in which use-by-date and purchase date are reported. Remaining shelf-lives were calculated as indicated above.
- **Exponential distribution to describe TTC by means of the 99% percentile** (a statistic from the remaining shelf-lives calculated) and a minimum value (uniform (0.01; 0.04) months as initial guess).



Time-temperature profiles

- Temperature-time profiles obtained from the FRISBEE project (http://frisbeetool.eu/FrisbeeTool/about.html) encompassing different RTE products such as deli-meat, cheese and vegetables were used as the basis to estimate *L. monocytogenes* growth from retail to consumption. Datasets were rearranged include temperature records every 5 hours and only from retail to consumption.
- **No data were available for fish, so those profiles for meats are assumed to be the same for fish.**
- Time in time-temperature profiles is truncated based on time-to-consumption output. Thus, a profile can never exceed the simulated time-to-consumption.

A	B	C	D	E	F	G	H	I	J	
	Time-temperature profiles FRISBEE database									
Record	Food product	Stage/step cold chain	Country	Packaging	Data collecting equipment	Accuracy	Position	Tmin	Tmax	Tmea
t/T data 11501	cooked ham slices	complete	Hungary	vacuum	miniature datalogger, miniNOMAD, OM-84-TMP, Omega Engineering Inc., Stamford, USA	± 0,1 °C	Inside the food	0.7	12.5	
t/T data 11502	cooked ham slices	complete	Hungary	vacuum	miniature datalogger, miniNOMAD, OM-84-TMP, Omega Engineering Inc., Stamford, USA	± 0,1 °C	Inside the food	1.4	19.1	
t/T data 11510	cooked ham slices	complete	Hungary	vacuum	miniature datalogger, miniNOMAD, OM-84-TMP, Omega Engineering Inc., Stamford, USA	± 0,1 °C	Inside the food	0.1	9.5	
t/T data 11536	cooked ham slices	complete	Hungary	vacuum	miniature datalogger, miniNOMAD, OM-84-TMP, Omega Engineering Inc., Stamford, USA	± 0,1 °C	Inside the food	1	10.2	
					miniature datalogger, miniNOMAD, OM-84-TMP,					

Cheese | Meat | RTE meat

Growth model

Cooked meat & sausage/Pate/ smoked and gravad fish/ soft and semisoft cheese

EGRs are assumed to include the effect of microbial microbiota in food: data are taken from products naturally contaminated

The effect of LAB on Maximum Population Density (MPD) of *L. monocytogenes can be simulated i) interaction term and ii) using a probability distribution for MPD obtained from experiments in naturally contaminated foods*.

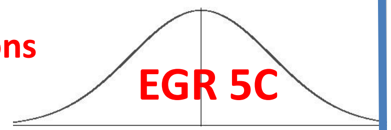
The deterministic secondary models used for LAB was deemed to be suitable for estimating the effect on MPD of Listeria

STOCHASTIC SECONDARY MODELS

DETERMINISTIC SECONDARY MODEL

LISTERIA MONOCYTOGENES

Refined **EGR 5C distributions**



$$EGR_{Y^\circ C} = EGR_{5^\circ C}\left(\frac{Y^\circ C - T_{\min}}{T_{5^\circ C} - T_{\min}}\right)^2$$

$$EGR_{Lact} = a\,(Lact_{max} - Lact)$$

(Devlieghere et al., 2000; Mejlholm et al., 2010; Mejlholm and Dalgaard, 2013; Mejlholm and Dalgaard, 2007; Østergaard et al., 2014).

INPUT variables (DIST)

EXPERT OPINION; LITERATURE AND MINTEL

OUTPUT

LACTIC ACIC BACTERIA

PRIMARY MODEL FOR DYNAMIC CONDITIONS

$$\frac{1}{N(t)}\frac{dN(t)}{dt} = EGR \cdot \alpha(t) \cdot f(t)$$

Initial guess

Distribution DB

$$\frac{1}{N(t)}\frac{dN(t)}{dt} = EGR \cdot \alpha(t) \cdot f(t)$$

N(0) is a distribution (1-4 log)

MODIFIED TERM FOR MICROBIAL INTERACTION

$$f(t) = \left(1 - \frac{N_{list}(t)}{N_{\max\ list}}\right)\left(1 - \frac{N_{LAB}(t)}{N_{\max\ LAB}}\right)$$

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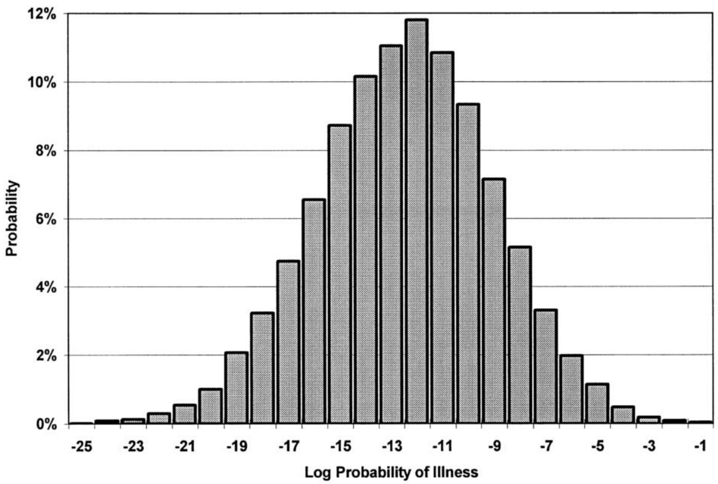
Outline

- ❑ Introduction and model scope
- ❑ Selection of D-R models
- ❑ Exposure assessment
- ❑ **Simulation and output**
- ❑ An easy-to-use framework: Excel Add-in "Lis-RA"
- ❑ Conclusions

Risk characterization: risk simulation

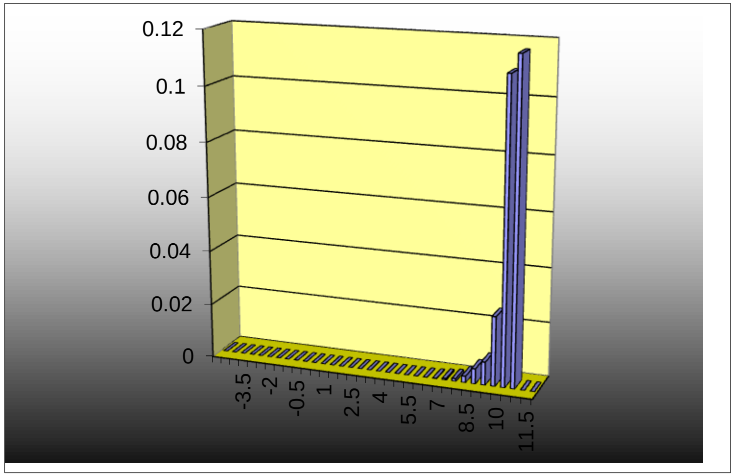
Individual risk:



Probability distribution for probability of illness from a single hamburger meal predicted by the *E. coli* O157:H7 Process Risk (Adapted from Cassin et al. 1998)

$Pill_i$ = $Prevalence_i$ x $Dose_i$ x r-value

Population risk:



Integration of probability distribution for the probability of illness for the whole population (total number of exposures)

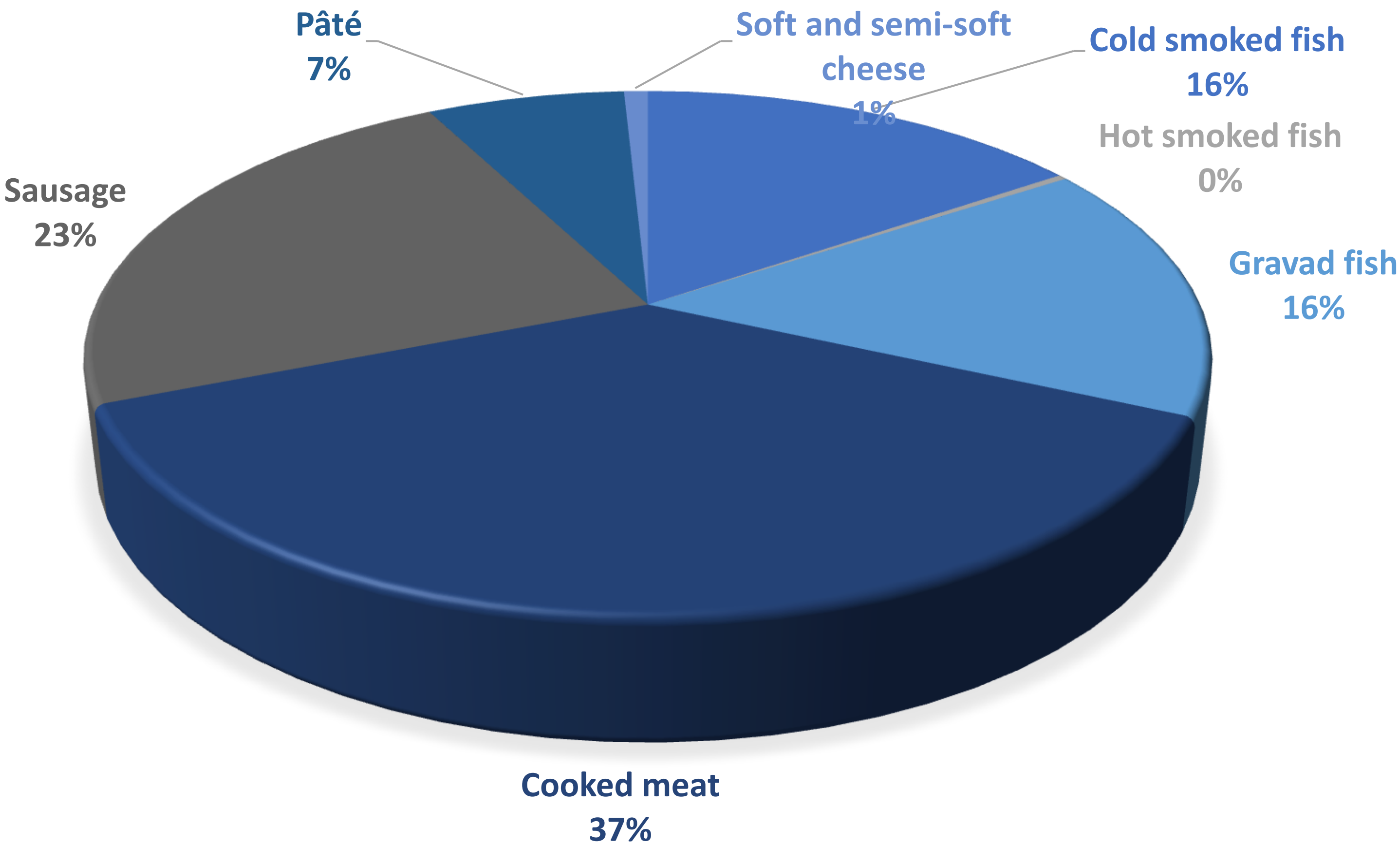
$$cases = \sum_{n=1}^{23} \pi_n \cdot P \cdot N \cdot f_n = \sum_{n=1}^{23} D_n \cdot r \cdot P \cdot N \cdot f_n = P \cdot N \cdot r \sum_{n=1}^{23} D_n \cdot$$

$$\sum_{i=1}^{n} Pill_i$$

Risk characterization: cases/year

Population risk

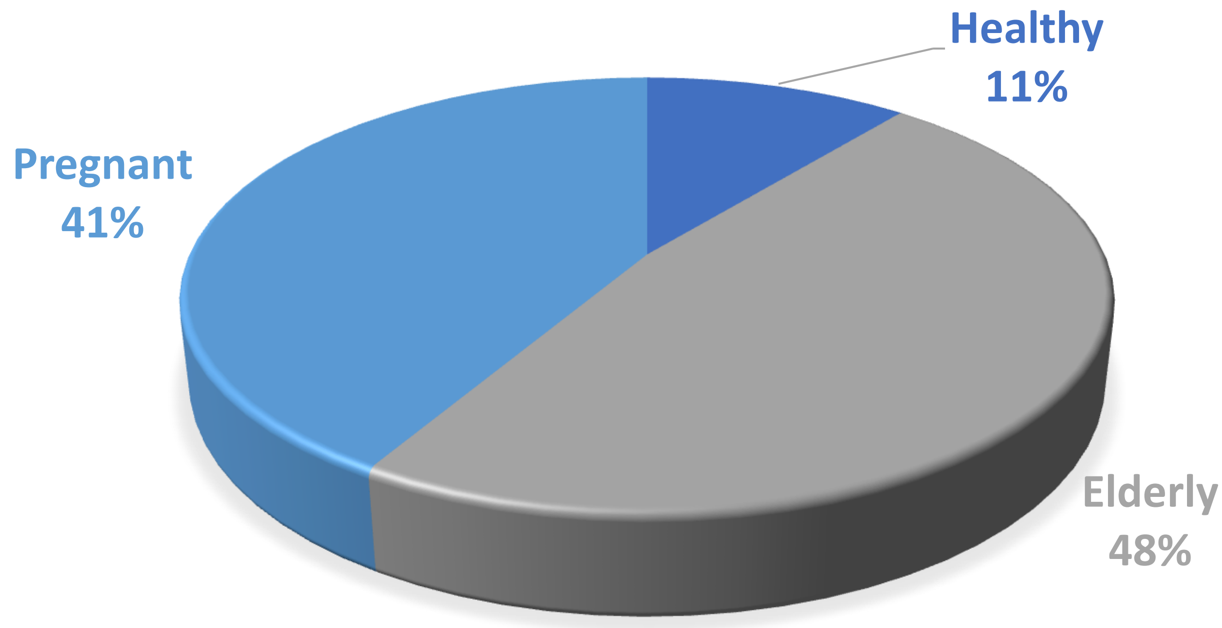
BASELINE MODEL



Risk characterization: cases/year

Population risk

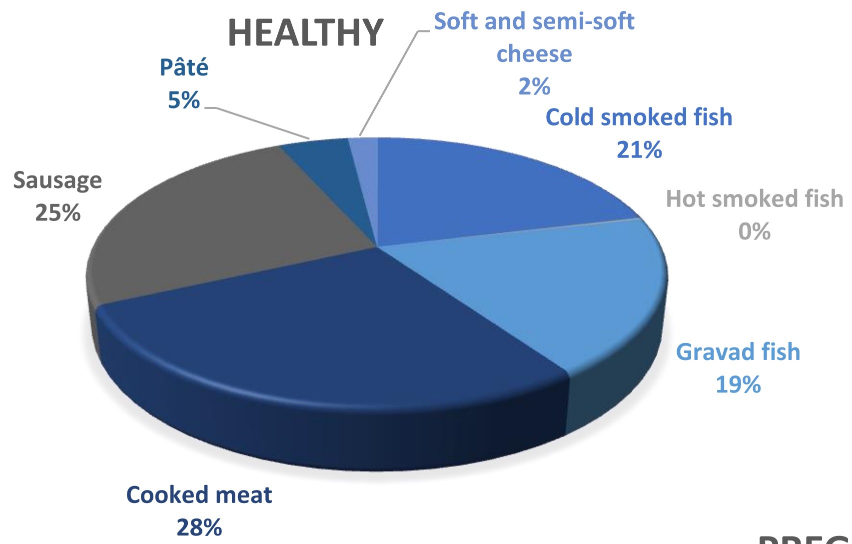
BASELINE MODEL

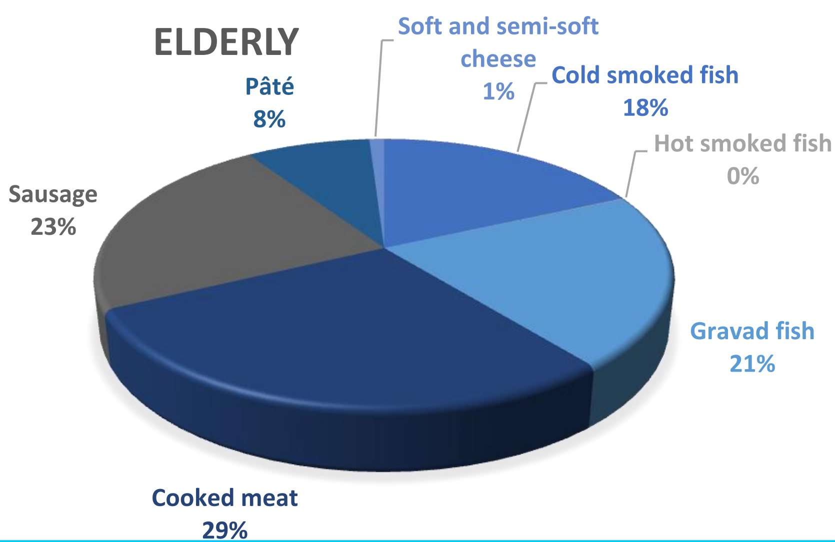


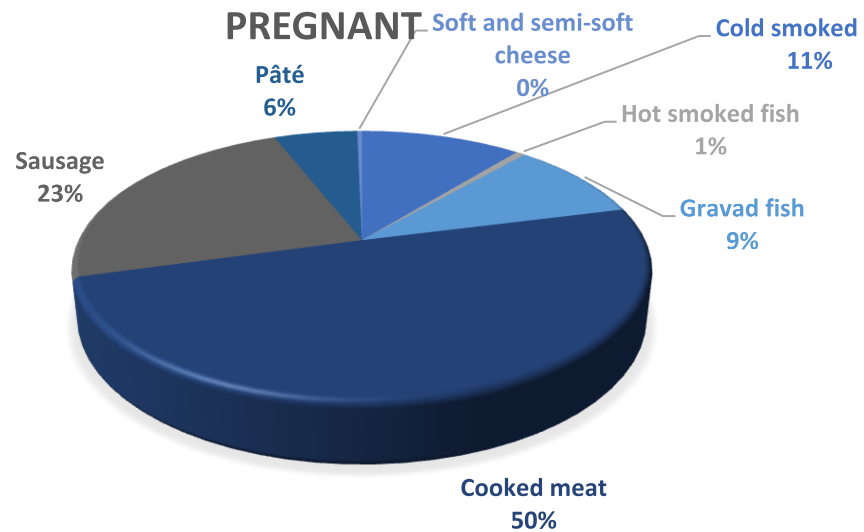
Risk characterization: cases/year

Population risk

BASELINE MODEL



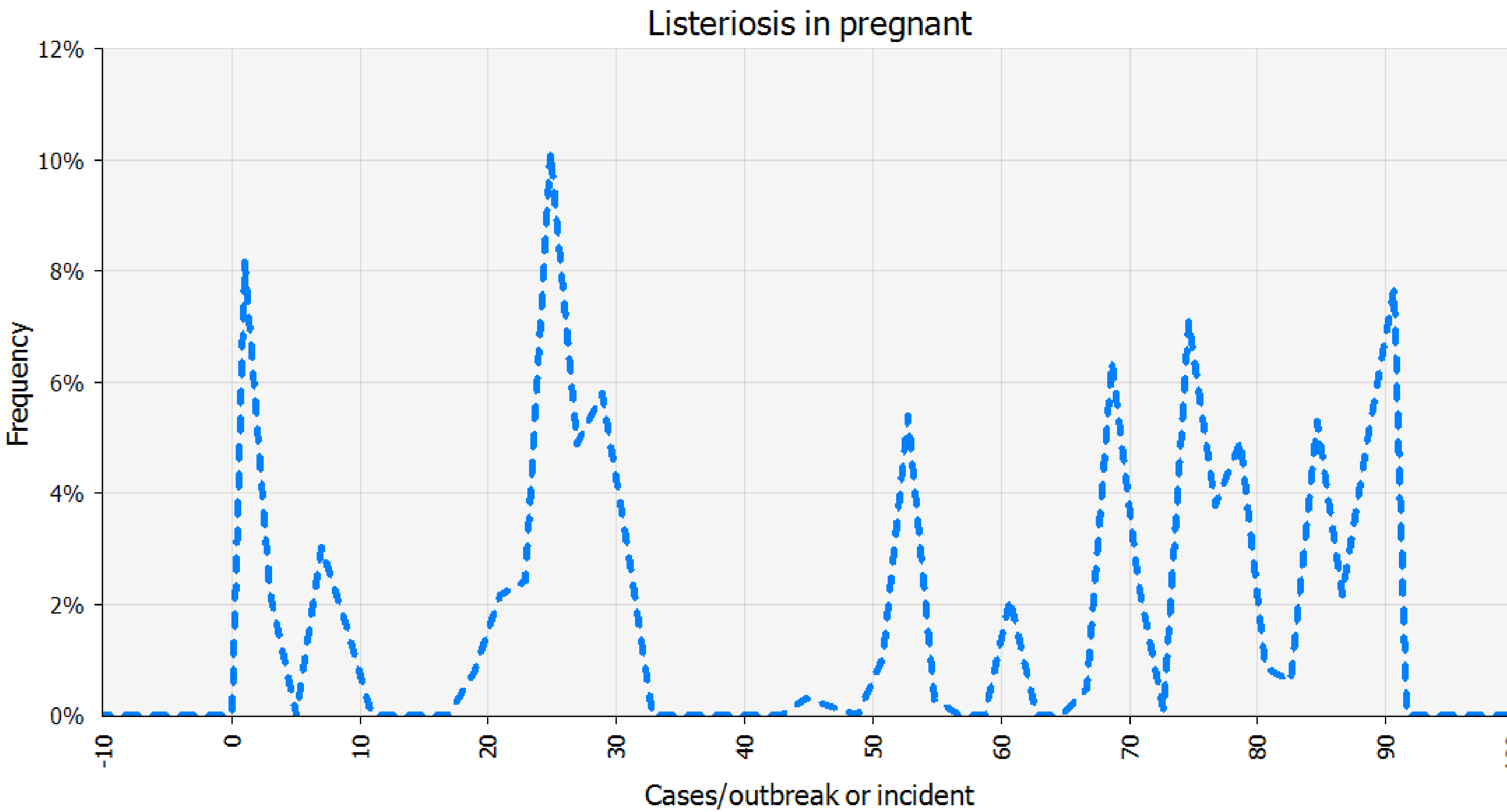




Risk characterization: cases/year

Population risk

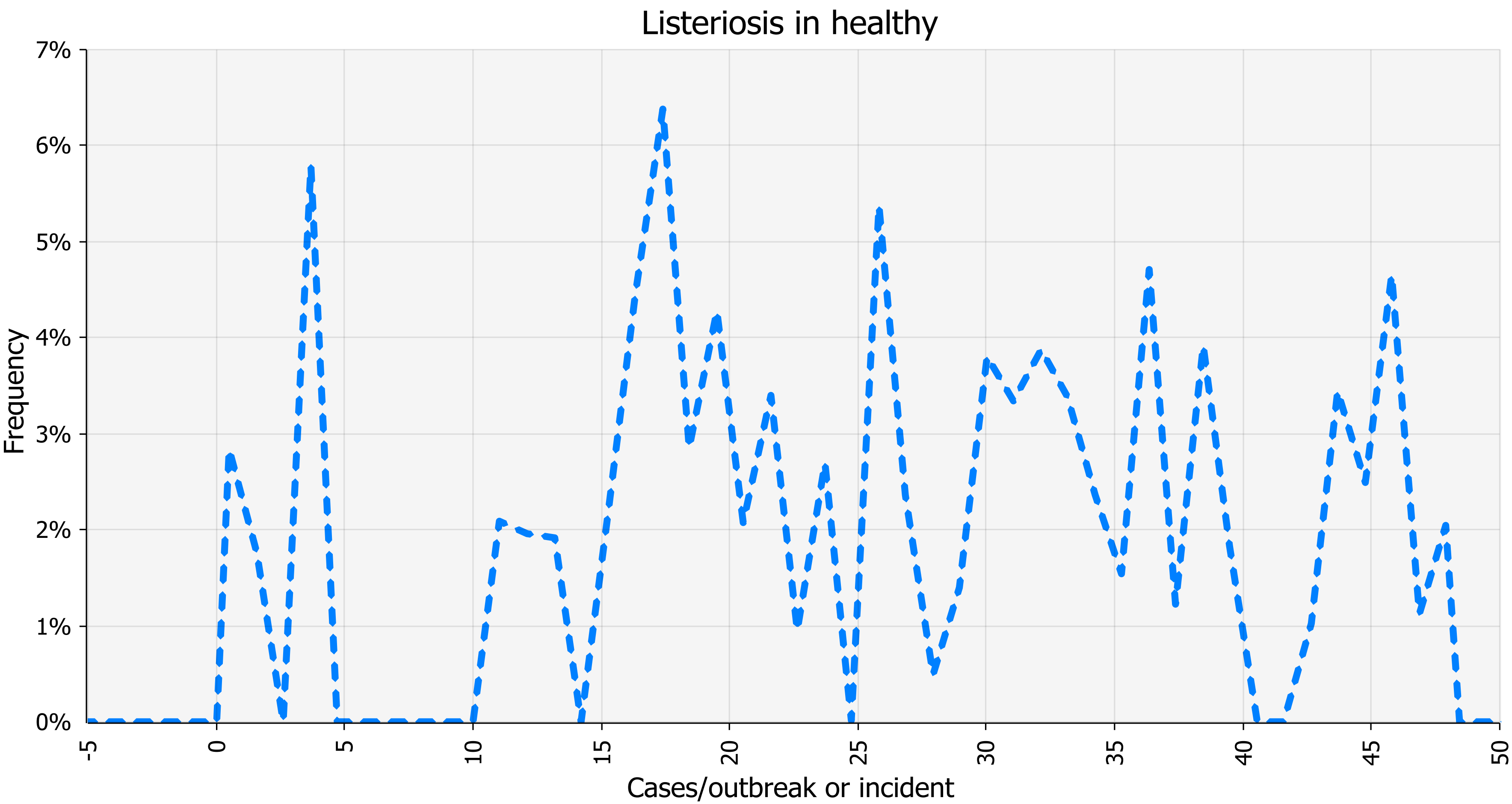
BASELINE MODEL



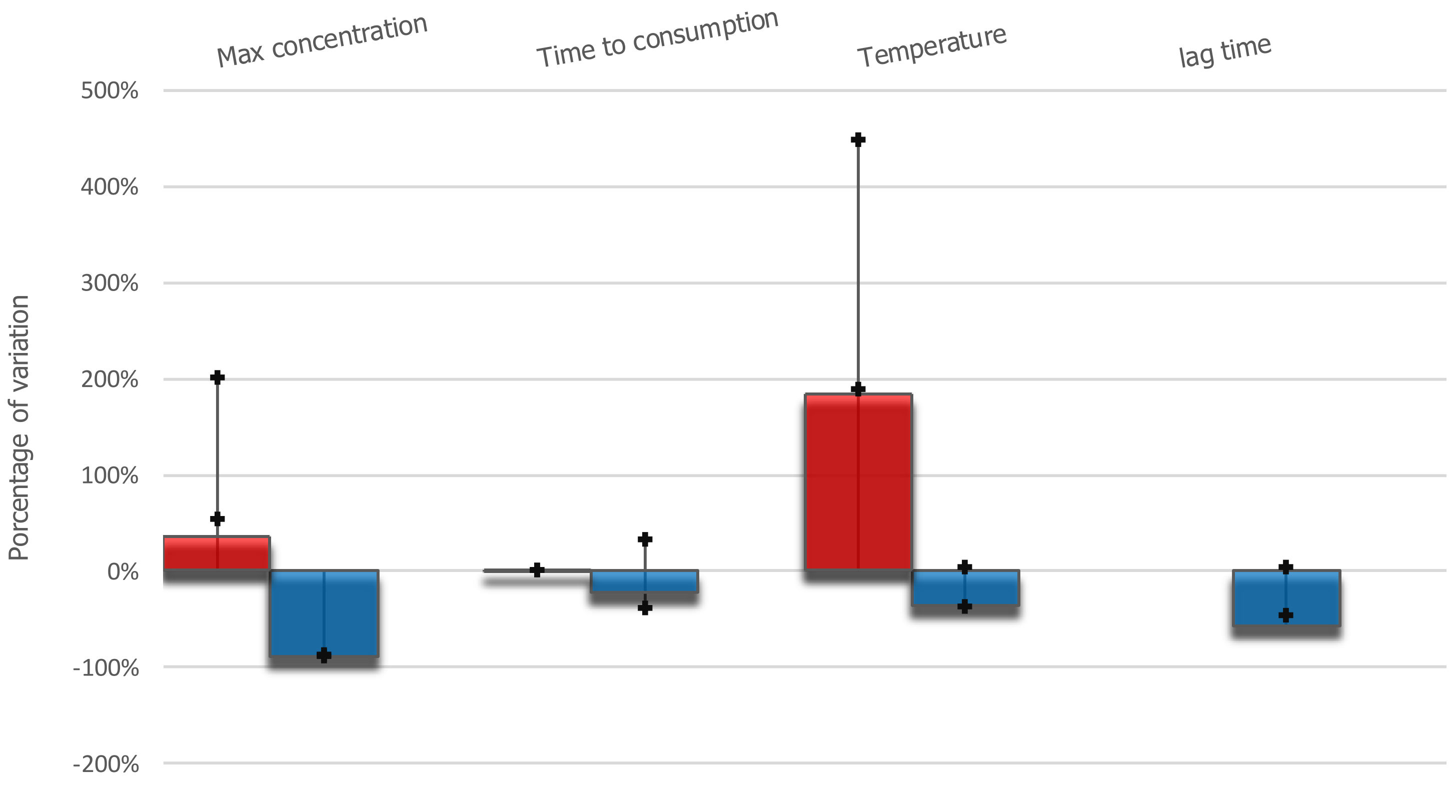
Risk characterization: cases/year

Population risk

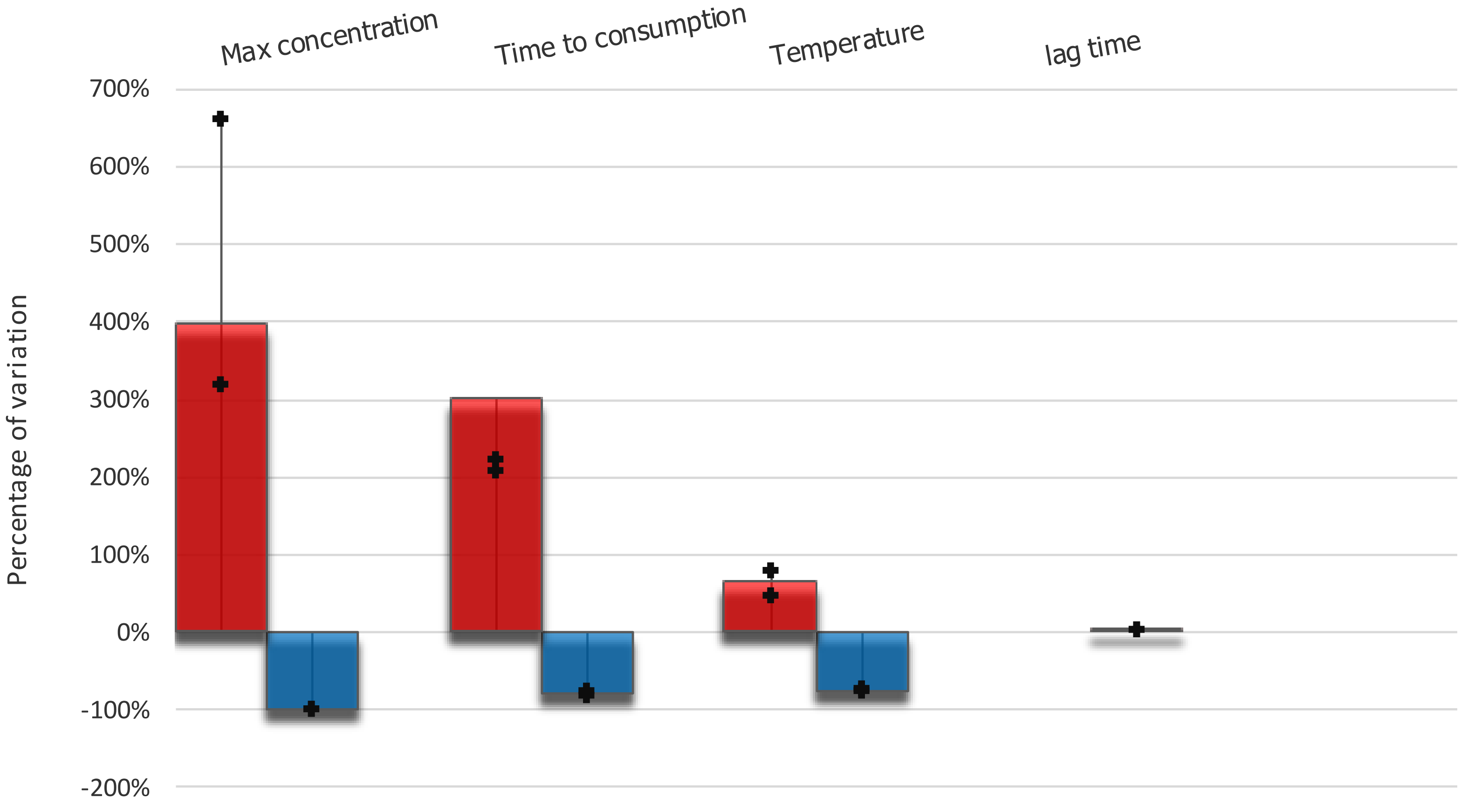
BASELINE MODEL



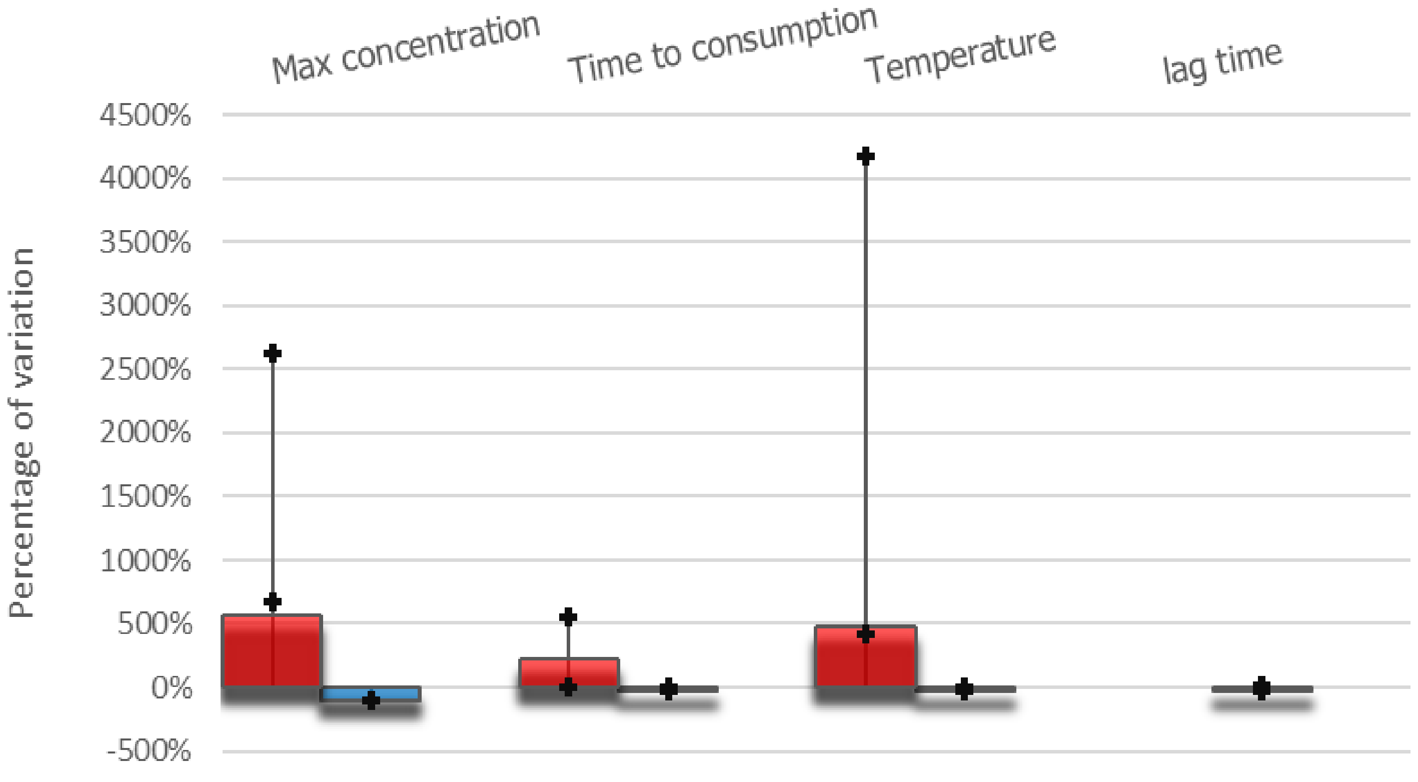
Scenario analysis: heat-treated meat



Scenario analysis: smoked and gravad fish



Scenario analysis: soft and semi-soft cheese



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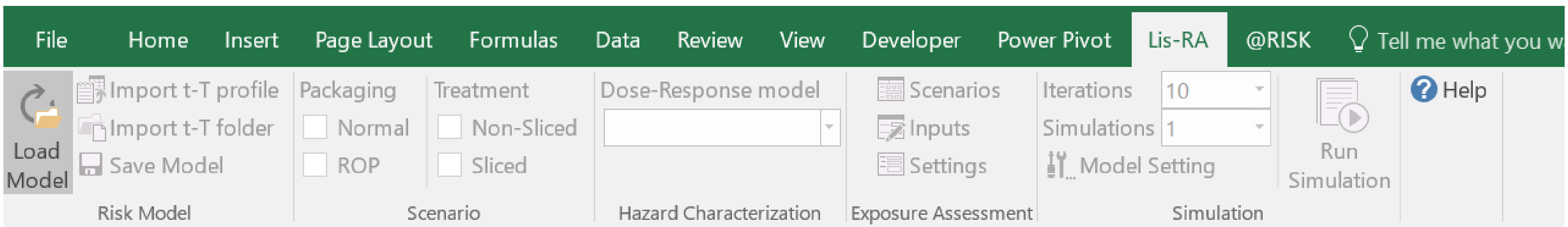
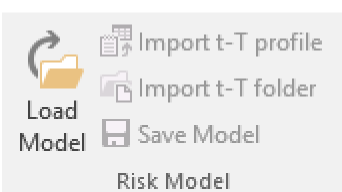
- **An easy-to-use framework: Excel Add-in "Lis-RA"**
- Conclusions

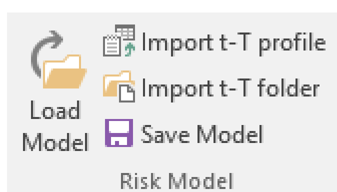
An Excel Add-in, "Lis-RA", for listeriosis risk model simulation

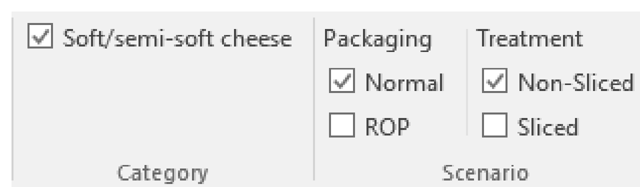
Lis-RA, a customized Ribbon-based system, was developed in VBA using libraries from @Risk software



Lis-RA allows users to select/upload models, time-temperature profiles sand scenarios.

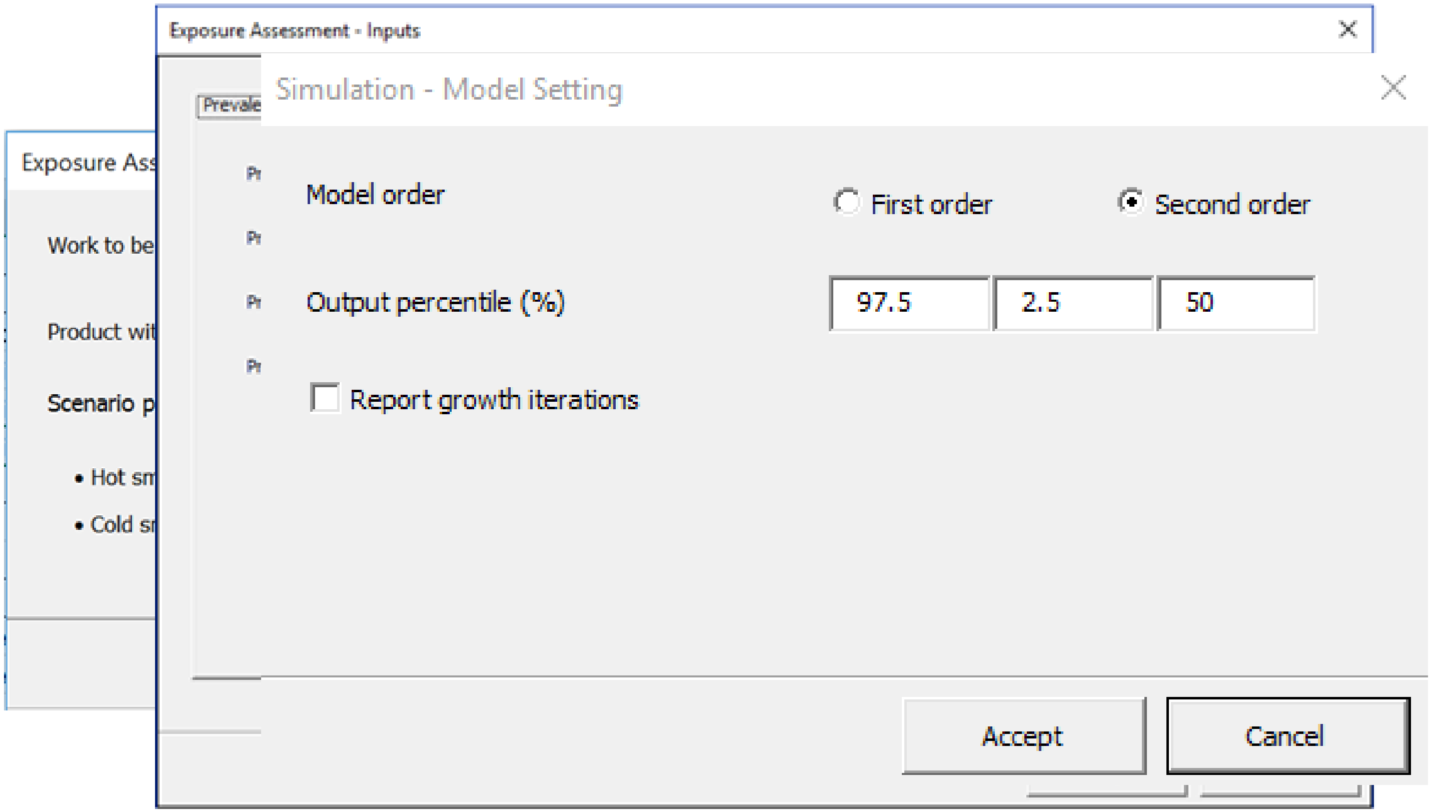






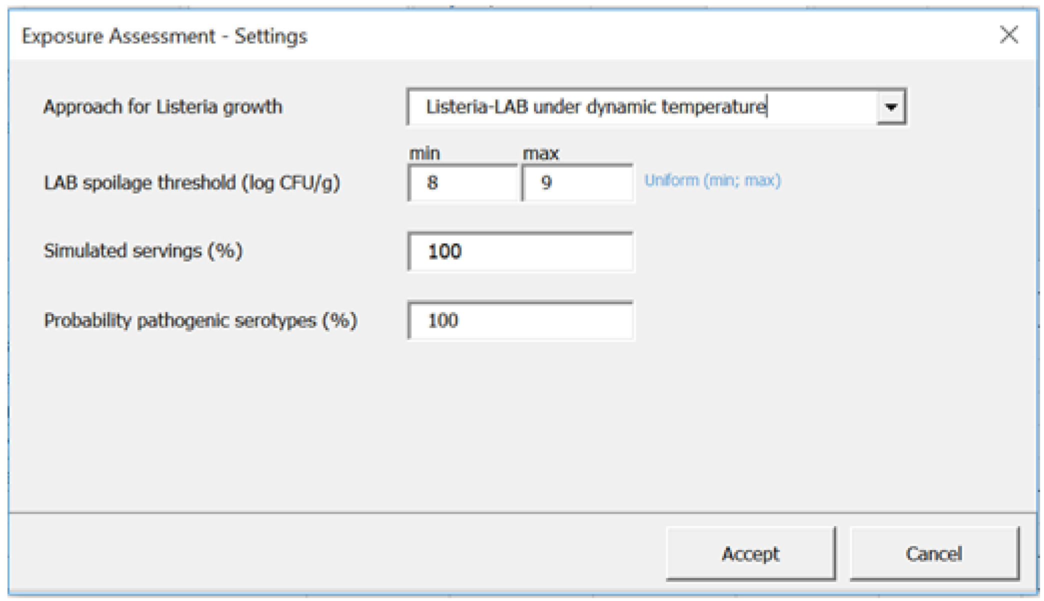
An Excel Add-in, “Lis-RA”, for listeriosis risk model simulation

Users can introduce scenario probabilities, input values and select the model order (first order or second order)



An Excel Add-in, “Lis-RA”, for listeriosis risk model simulation

Model simulation settings and selection of the type of growth model

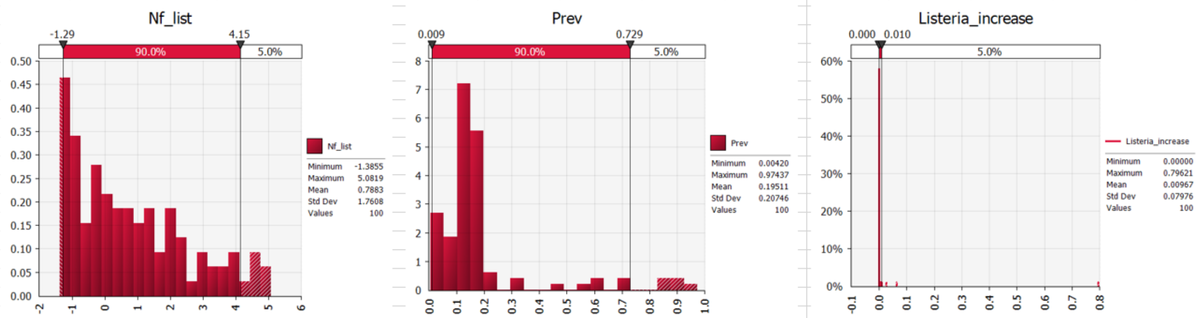


An Excel Add-in, "Lis-RA", for listeriosis risk model simulation

Graphical and numerical output are automatically reported after simulation

		Mean	SD	min	max	5.0th	50.0th	95.0th
Nf_list	Final concentration (log CFU/g)	0.788336923	1.760824319	-1.38548908	5.081900883	-1.29	0.34	4.15
Prev	Listeria prevalence	0.195109523	0.207462019	0.004196994	0.974372797	0.01	0.14	0.73
Listeria_increase	Listeria log increase (log CFU/g)	0.009672077	0.079760405	0	0.796213426	0	0	0.01
%gr_list	Listeria relative growth (%)	0.001494353	0.011907727	0	0.118734793	0	0	0
Temperature_mean	Mean temperature from the t-T profile	3.026090751	1.872262082	0.129511064	10.38483488	0.67	2.86	7.16

		Mean	SD	min	max	50.0th	2.5th	97.5th
Pill_healthy	Pill/serving in healthy					3.135E-14	2.5254E-14	3.81777E-14
Pill_susceptible	Pill/serving in susceptible/elderly					1.66623E-12	1.34224E-12	2.02912E-12
Pill_pregnant	Pill/serving in pregnant					1.98796E-11	1.6014E-11	2.42092E-11
Illness_healthy	Cases/year in healthy					0.450750923	0.331228284	0.594650393
Illness_susceptible	Cases/year in susceptible/elderly					360.0444977	282.7253866	448.1795183
Illness_pregnant	Cases/year in pregnant					2784.933075	1952.413911	3774.820551
	Total cases/year	0	0	0	0	3145.428323	2235.470526	4223.59472



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Conclusions

Similar values to those reported by the surveillance system, confirming higher incidence in elderly population

Heat treated meat was the RTE product with highest overall risk of listeriosis specifically for the subcategory cooked meat

Semi-soft cheese and hot smoked fish were the subcategories resulting in the lowest estimated risk

Aspects related to the consumption patterns, shelf-life and processing were key in the differences found between these subcategories

Concerning specific scenarios, the *highest risk* was obtained for normal packaged and sliced Pâté in pregnant population. The lowest risk values were observed for non-sliced hot smoked fish and soft and semi-soft cheese.

Maximum concentration at retail and temperature were the most relevant variables for listeriosis risk

Acknowledgement

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Anna Jofres
Margarita Garriga

European Food Safety Authority

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