

Predictive Microbiology (applications)

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**Summer School
“In Silico Methods for Food Safety”**

Predictive Microbiology

Tertiary models-Software (free access)

Pathogen Modeling Program

<http://www.ars.usda.gov/Services/docs.htm?docid=6796>

ComBase (database) (<http://www.combase.cc>)

ComBase Predictor (models) (<http://www.combase.cc>)

Food spoilage and safety predictor-FSSP

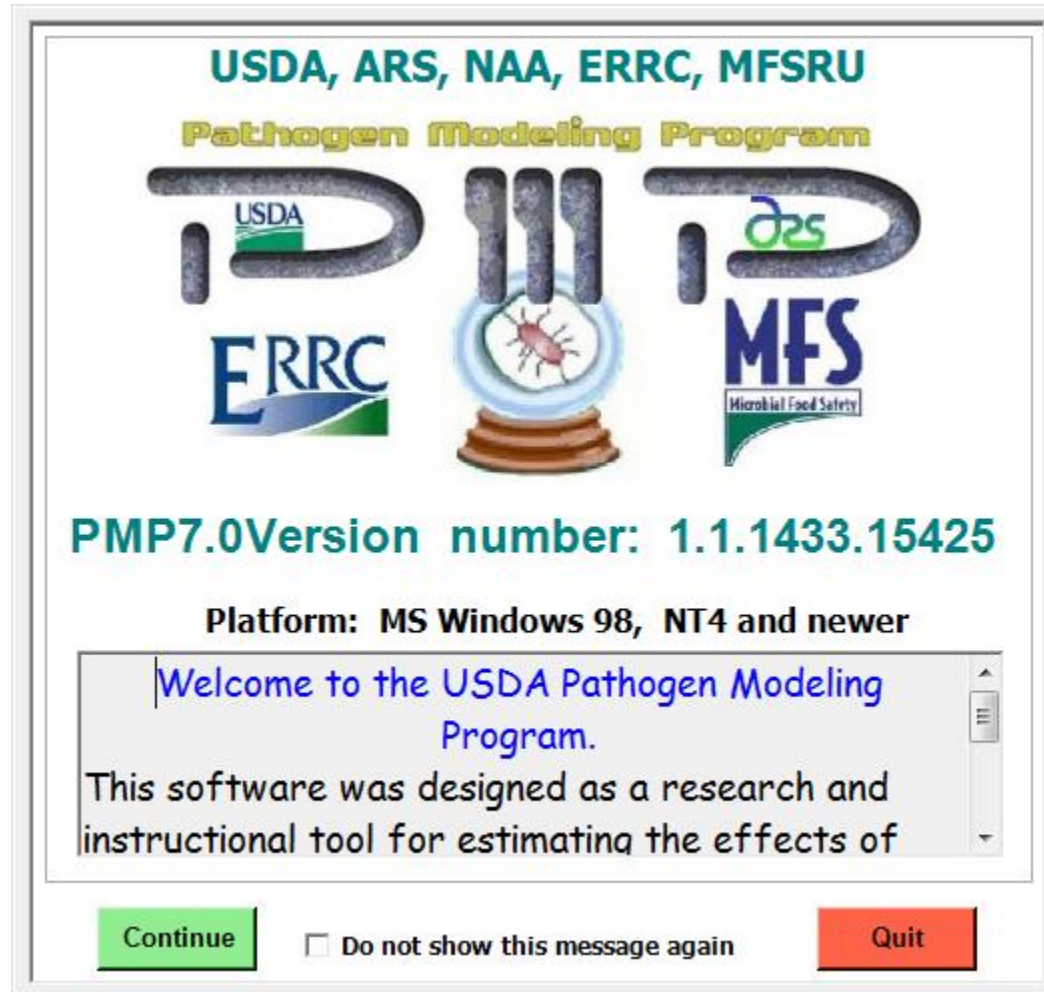
(<http://www.dfu.min.dk/micro/sssp/Home/Home.aspx>)

Refrigeration Index

(ijenson@mla.com.au; <http://www.mla.com.au>)

Predictive Microbiology

<http://www.ars.usda.gov/Services/docs.htm?docid=6796>



Predictive Microbiology

Tertiary models-Software

www.combase.cc

- large, searchable, database of microbiological raw data
- still growing, users can add data
- web-based, free access
- integrates “Food Micromodel” and “Pathogen Modeling Program” data, and many more
- includes new models in “ComBase Predictor”

Predictive Microbiology

Tertiary models-Software

www.combase.cc

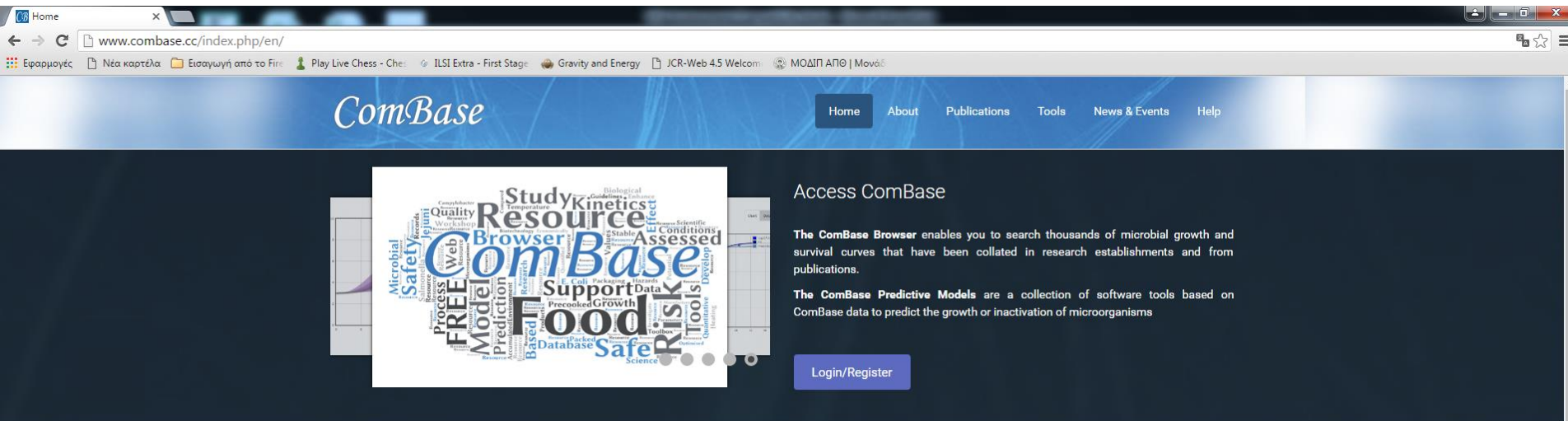
35,000 records on growth and survival of pathogens and spoilage organisms

- ~28,000 records on pathogens
- ~4,000 on spoilage organisms, including
- ‘total spoilage bacteria’ (346)
- ‘*bacillus* spoilage bacteria’ (65)
- *Brocothrix thermosphacta* (741)
- *enterobacteriaceae* (338)
- lactic acid bacteria (701)
- *Shewanella putrefasciens* (57)
- “spoilage yeast” (44)

Predictive Microbiology

Tertiary models-Software

www.combase.cc



Home About Publications Tools News & Events Help

Access ComBase

The **ComBase Browser** enables you to search thousands of microbial growth and survival curves that have been collated in research establishments and from publications.

The **ComBase Predictive Models** are a collection of software tools based on ComBase data to predict the growth or inactivation of microorganisms

[Login/Register](#)

A Web Resource for Quantitative and Predictive Food Microbiology

It includes:

- ✓ A systematically formatted database of quantified microbial responses to the food environment with more than 50,000 records
- ✓ The ComBase Predictive Models – based on ComBase data to predict the growth or inactivation of microorganisms in food.

It can be used for:

- ✓ Predicting and improving the microbiological safety and quality of foods
- ✓ Designing, producing and storing foods economically
- ✓ Assessing microbiological risk in foods.

Introducing ComBase

ComBase users say ...

I find ComBase absolutely essential to my teaching, research and outreach activities. I seldom go for more than a week without consulting it for solving a variety of food safety problems

Donald Schaffner
Distinguished Professor and Extension Specialist

Predictive Microbiology and Risk Assessment News

Predicting the Kinetics of *Listeria monocytogenes* and *Yersinia enterocolitica* Under Dynamic Growth/Death-Inducing Conditions, in Italian Style Fresh

- Browser
- ComBase Predictor
- Predictive Models
- Resources
- Help

Search

Responses Sources

Organism: Escherichia coli

Temperature: 0.0 - 40.0

pH: 4.0 - 7.5

Matrix: Beef

Aw/NaCl: 0.75 - 1.00

Include where unspecified

+Add another field

Environmental conditions

- Any
- Static
- Dynamic

Search

← Back to search

Search results [29 records] Export ?

Organism (Ascending)

1/3

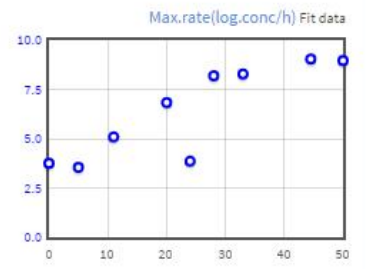
1. **Escherichia coli** in roast beef

Matrix: Beef
 Temp (°C): 3
 Aw: 0.994(assumed)
 pH: 5.6
 Conditions: Cut (minced, chopped, ground, etc), Inoculation in/on previously heated (cooked, baked, pasteurized, etc) but not sterilised food/medium, Sodium chloride in the environment: 1.1 %, Vacuum-packed
 Source: Michel (et al.), 1991: Pathogen survival in precooked beef products and determination of critical control points in processing. *Journal of Food Protection* 54(10): 767 - 772



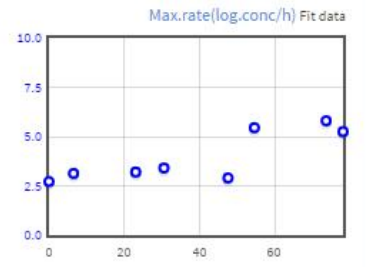
2. **Escherichia coli** in Beef carcass

Matrix: Beef
 Temp (°C): 11.1
 Aw: 0.987
 pH: 5.76
 Conditions: Other species also deliberately inoculated (but only one organism counted here)
 Source: Tasmanian Institute of Agriculture, University of Tasmania, Australia



3. **Escherichia coli** in Beef carcass

Matrix: Beef
 Temp (°C): 11.1
 Aw: 0.987
 pH: 5.76
 Conditions: Other species also deliberately inoculated (but only one organism counted here)
 Source: Tasmanian Institute of Agriculture, University of Tasmania, Australia



- Browser
- ComBase Predictor
 - Growth
 - Thermal Inactivation
 - Non-thermal Survival
 - Predictive Models
 - Resources
 - Help

Growth Model

Prediction Uncertainty

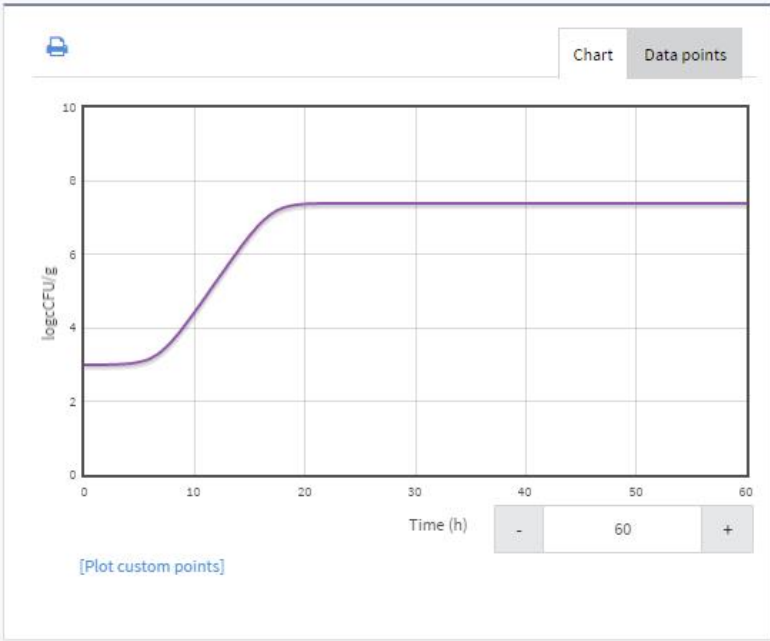
[Static | Dynamic] [Aw | NaCl]

Aeromonas hydrophila

Init. level	3	0	7
Phys.state	1.2e-3	0	1
Temp (°C)	20	2	37
pH	7	4.6	7.5
Aw	0.997	0.974	1

Max.rate (log.conc/h) 0.433 Dbl.time(Hours) 0.696

[Add prediction]

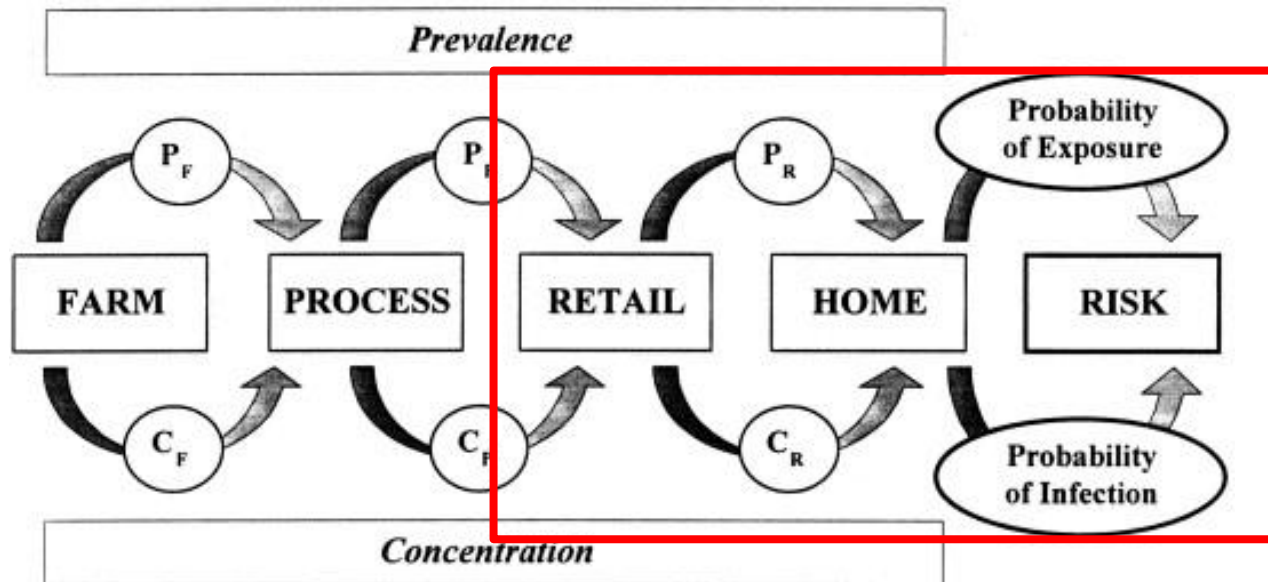


Case Study 1

Deterministic Exposure Assessment of *Listeria monocytogenes* in RTE Foods

For

Evaluating the compliance of RTE foods with the new safety criteria for *Listeria monocytogenes*



Case Study 1

For a vacuum packed, sliced turkey breast product with pH=5.5, aw=0.971 and sodium nitrite concentration of 50 ppm we want to determine a shelf life leading to compliance with the EU reg. 2073/2005 safety criteria for *L. monocytogenes*.

α) what is the maximum shelf life in days for compliance with the EU reg. 2073/2005

β) How we can increase the shelf life to 25 days

***(assume a mean chill chain temperature of 7 °C)**

The new EC regulation 2073/2005 on microbiological criteria for foodstuffs

Safety Criteria for *L. monocytogenes*

Food category	Sampling plan		Limits		Stage where the criterion applies
	n	c	m	M	
1.2 Ready-to-eat foods able to support the growth of <i>L. monocytogenes</i>	5	0	100 cfu/g ⁴		Products ready to be placed on the market and during their shelf-life
	5	0	Absence in 25 g ⁵		Before the food has left the immediate control of the food business operator, who has produced it
1.3 Ready-to-eat foods unable to support the growth of <i>L. monocytogenes</i>	5	0	100 cfu/g		Products ready to be placed on the market and during their shelf-life

Case study 1

Product: cooked ham

(pH=5.49, $a_w=0.971$, N=50ppm, SL=60 days)

(allows growth of Lm)

Requirement (technical) for the Food Industry for compliance with the new safety criteria for LM in RTE foods



Assure that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

Requirements (technical)

Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

Before the food has left the immediate control of the food business operator, who has produced it	Absence in 25 g or <math><-1,4 \log \text{ cfu/g}</math>
Products ready to be placed on the market and during their shelf-life	<math><100 \text{ cfu/g}</math> or <math><2 \log \text{ cfu/g}</math>

Assure that total growth of *L. monocytogenes* will not exceed 3,4 logs cfu/g during the shelf life

Case study 1

PMP70 - [Anaerobic Growth Models: Listeria monocytogenes in Broth Culture (aW)]

File View Models >> Bacterium Bacteria >> Model References Window Help

Microorganism: *Listeria monocytogenes (Broth Culture) (aW)*

Input Conditions

Aerobic Anaerobic

Temperature: Range: 4 to 37
7.0 °C 44.6 °F

pH: Range: 4.5 to 8
5.5

Water Activity: Range: 0.97 to 0.997
0.971

Sodium Nitrite (ppm): Range: 0 to 150
50 (ppm)

Calculate Growth Data

Initial Level
3.0 log(CFU/ml) 1000 CFU/ml

Level of Concern
6.4 log(CFU/ml) 2,511,886.4 CFU/ml

Source and/or Relevant Publications
R.L. Buchanan, H.G. Stahl and R.C. Whiting, Effects and Interactions of Temperature, pH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the Growth of *Listeria monocytogenes*: Journal of Food Protection (1989) 52(12):844-851 - <http://www.zis.gov/MES/HTML/ERRCPubs/5435.pdf>

Calculate Model with:

Time Scale:

Display Format:

Modeled Growth Parameters:

	Days
Lag Phase Duration:	8.19
Lower Confidence Limit:	5.89
Upper Confidence Limit:	11.40
Generation Time:	0.62
Lower Confidence Limit:	0.47
Upper Confidence Limit:	0.83
Time to Increase 3.4 logs:	15.21
Lower Confidence Limit:	11.16
Upper Confidence Limit:	20.74

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Case study 1

PMP70 - [Anaerobic Growth Models: *Listeria monocytogenes* in Broth Culture (aW)]

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Microorganism: *Listeria monocytogenes (Broth Culture) (aW)*

Input Conditions

Aerobic Anaerobic

Temperature: Range: 4 to 37
7.0 °C 44.6 °F

pH: Range: 4.5 to 8
5.5

Water Activity: Range: 0.97 to 0.997
0.971

Sodium Nitrite (ppm): Range: 0 to 150
125 (ppm)

Calculate Growth Data

Initial Level
3.0 log(CFU/ml) 1000 CFU/ml

Level of Concern
6.4 log(CFU/ml) 2,511,886.4 CFU/ml

Source and/or Relevant Publications
R.L. Buchanan, H.G. Stahl and R.C. Whiting, Effects and Interactions of Temperature, pH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the Growth of *Listeria monocytogenes*: Journal of Food Protection (1989) 52(12):844-851 - <http://www.zenon.com/MES/HTML/ERRCPubs/5435.pdf>

Calculate Model with: Lag No Lag

Time Scale: Days Hours

Display Format: Show Table Show Chart

Modeled Growth Parameters:

Days	
Lag Phase Duration:	13.61
Lower Confidence Limit:	9.24
Upper Confidence Limit:	20.05
Generation Time:	1.02
Lower Confidence Limit:	0.73
Upper Confidence Limit:	1.42
Time to Increase 3.4 logs:	25.11
Lower Confidence Limit:	17.47
Upper Confidence Limit:	36.14

Listeria monocytogenes in Broth Culture (aW)

log(CFU/ml)

Days

— log(CFU/ml) — LCL — UCL

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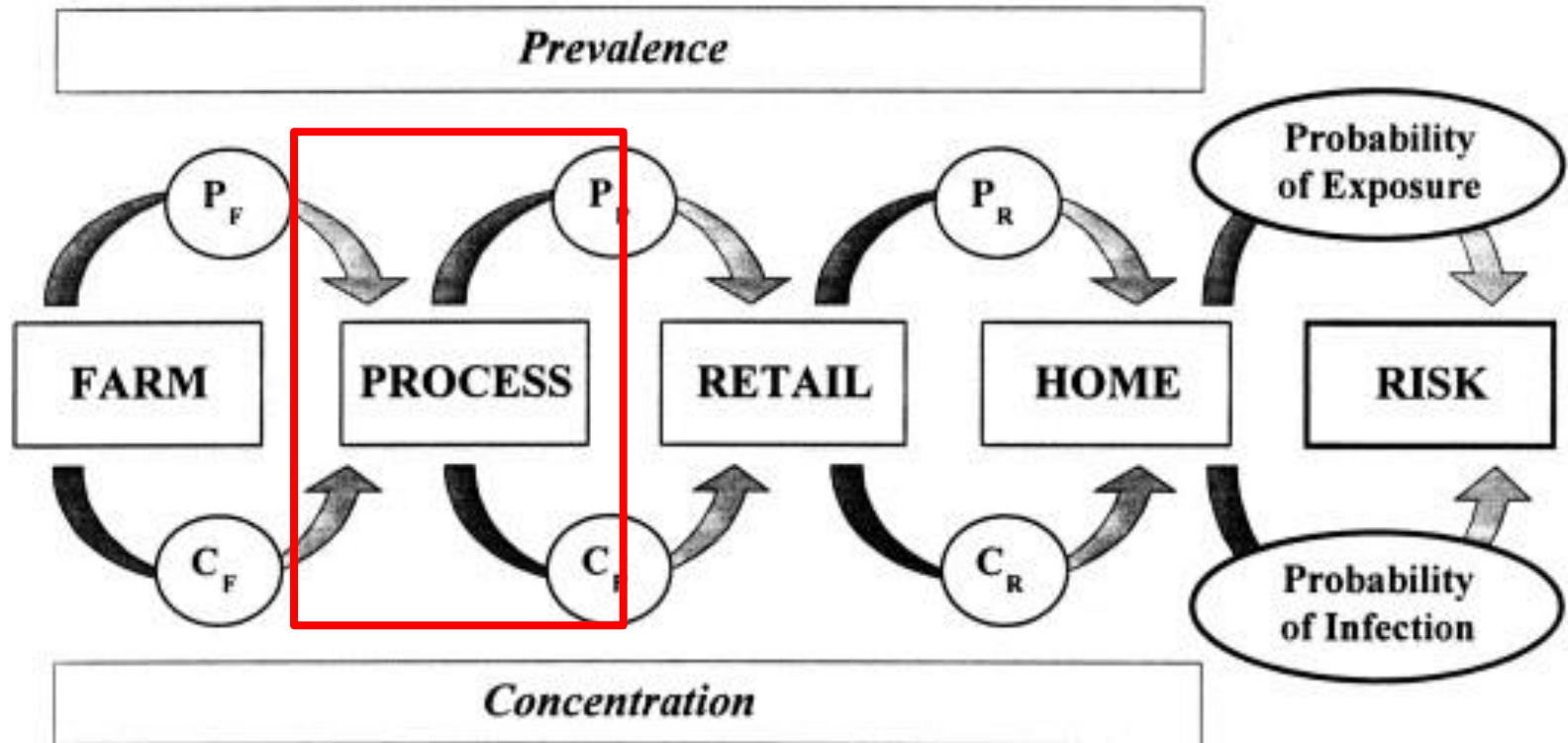
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Case Study 2

Microbial Inactivation in Exposure Assessment



Case Study 2

A raw beef with (pH=5.7 and aw=0.989) batch is contaminated with 10.000 cfu/g E. coli O157:H7

What is the concentration of the pathogen after thermal processing at 62 °C for 3.5 min

Case Study 3

Stochastic Exposure Assessment of *Listeria monocytogenes* in RTE Foods

2. Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

PMP70 - [Anaerobic Growth Models: *Listeria monocytogenes* in Broth Culture (aW)]

File View Models>>Bacterium Bacteria>>Model References Window Help

Microorganism: *Listeria monocytogenes* (Broth Culture) (aW)

Input Conditions

Aerobic Anaerobic

Temperature: Range: 4 to 37
7.0 °C 44.6 °F

pH: Range: 4.5 to 8
5.5

Water Activity: Range: 0.97 to 0.997
0.971

Sodium Nitrite (ppm): Range: 0 to 150
50 (ppm)

Calculate Growth Data

Initial Level
3.0 log(CFU/ml) 1000 CFU/ml

Level of Concern
6.4 log(CFU/ml) 2,511,886.4 CFU/ml

Source and/or Relevant Publications

R.L. Buchanan, H.G. Stahl and R.C. Whiting, Effects and Interactions of Temperature, pH, Atmosphere, Sodium Chloride, and Sodium Nitrite on the Growth of *Listeria monocytogenes*: Journal of Food Protection (1989) 52(12):844-851 - <http://www.aee.org/MES/HTML/ERRCPub/5435.pdf>

Calculate Model with: Lag No Lag

Time Scale: Days Hours

Display Format: Show Table Show Chart

Modeled Growth Parameters:

Days	
Lag Phase Duration:	8.19
Lower Confidence Limit:	5.89
Upper Confidence Limit:	11.40
Generation Time:	0.62
Lower Confidence Limit:	0.47
Upper Confidence Limit:	0.83
Time to Increase 3.4 logs:	15.21
Lower Confidence Limit:	11.16
Upper Confidence Limit:	20.74

Listeria monocytogenes in Broth Culture (aW)

log(CFU/ml)

Days

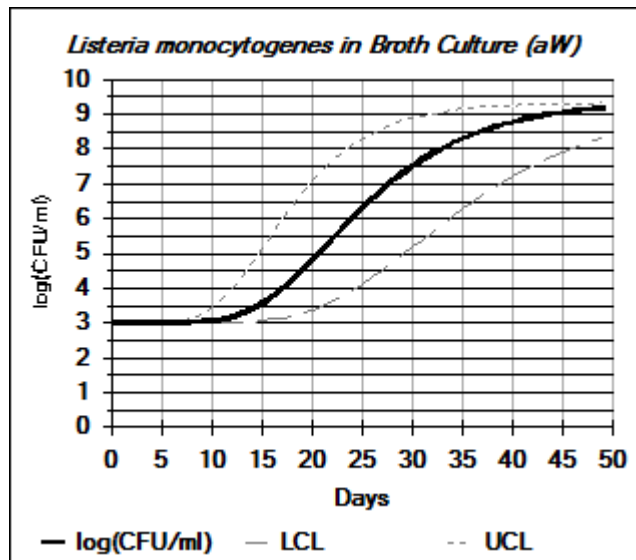
Legend: — log(CFU/ml) - - LCL - - UCL

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2. Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

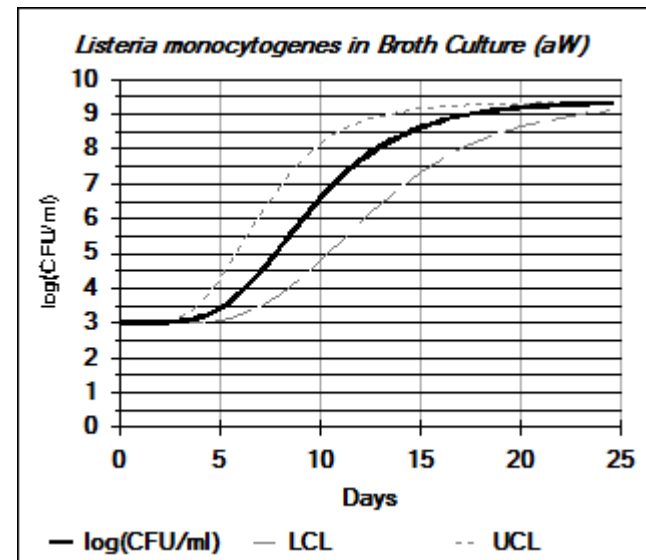
Use of available predictive modeling software (i.e PMP)

4 °C



25 days for 3 logs increase

10 °C



10 days for 3 logs increase

2. Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

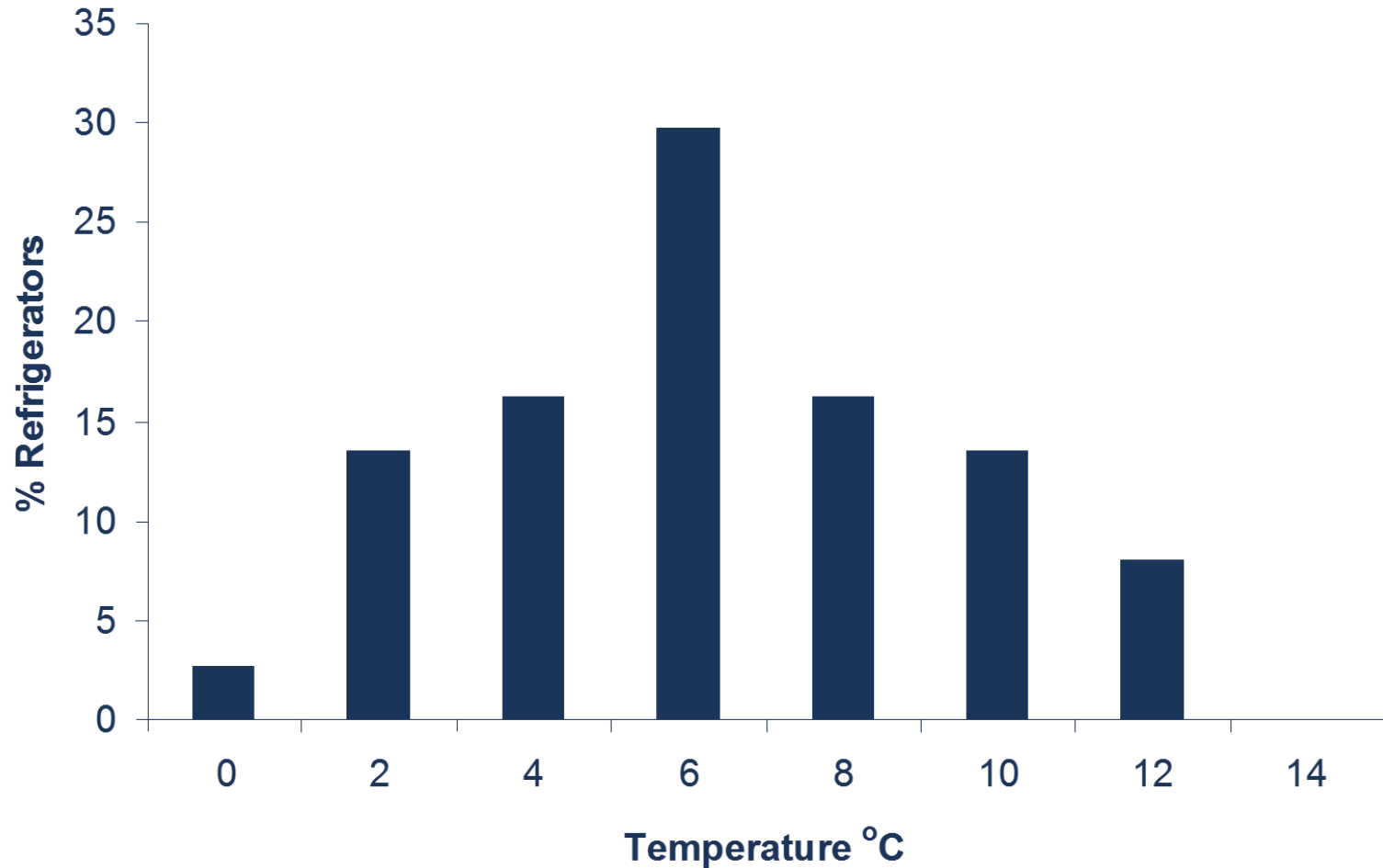
At which temperature ?

General Requirements in Reg. 2073/2005

the food safety criteria applicable throughout the shelf-life of the products can be met under reasonably, foreseeable conditions of distribution, storage and use.

Chill Chain Conditions

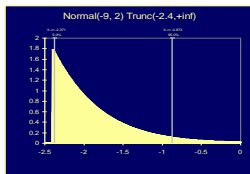
Temperature distribution in retail refrigerators (survey in Greece)



Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

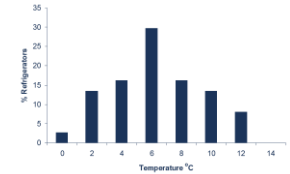
Probabilistic approach

Initial contamination



Product Characteristics (pH, aw, Shelf Life...)

Chill chain characteristics

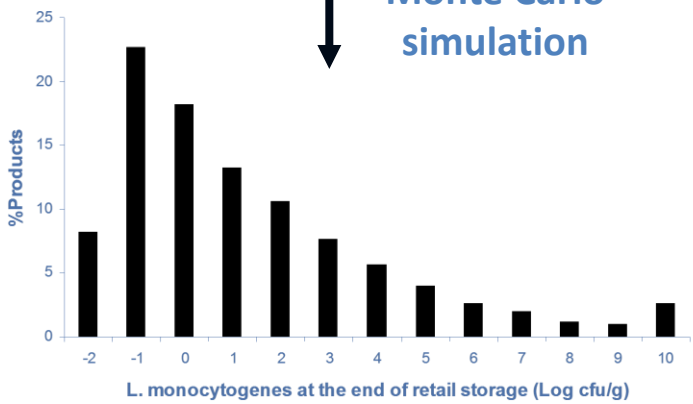


G/NG and Kinetic model*

Biological variability

Monte Carlo simulation

Distribution of *L. monocytogenes* concentration at the end of shelf life

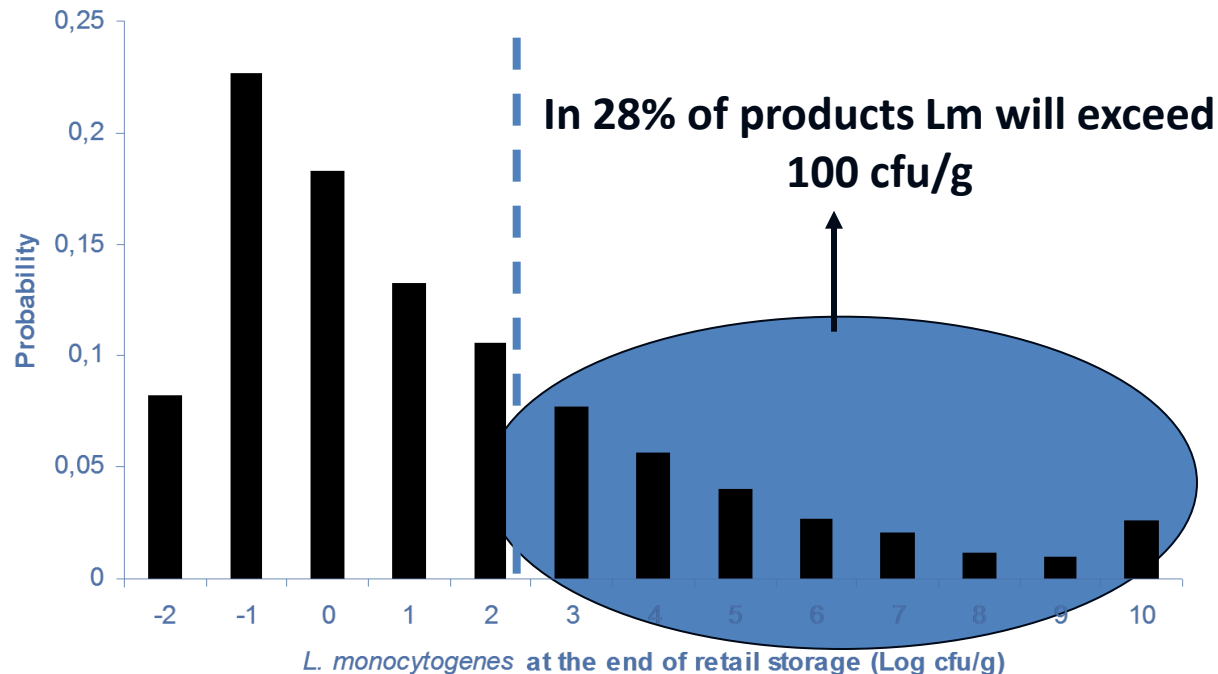


Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

Case study

Product: cooked ham (pH=5.49, $a_w=0.971$, N=50ppm, SL=60 days)

Distribution of *L. monocytogenes* at the end of shelf life in retail



Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

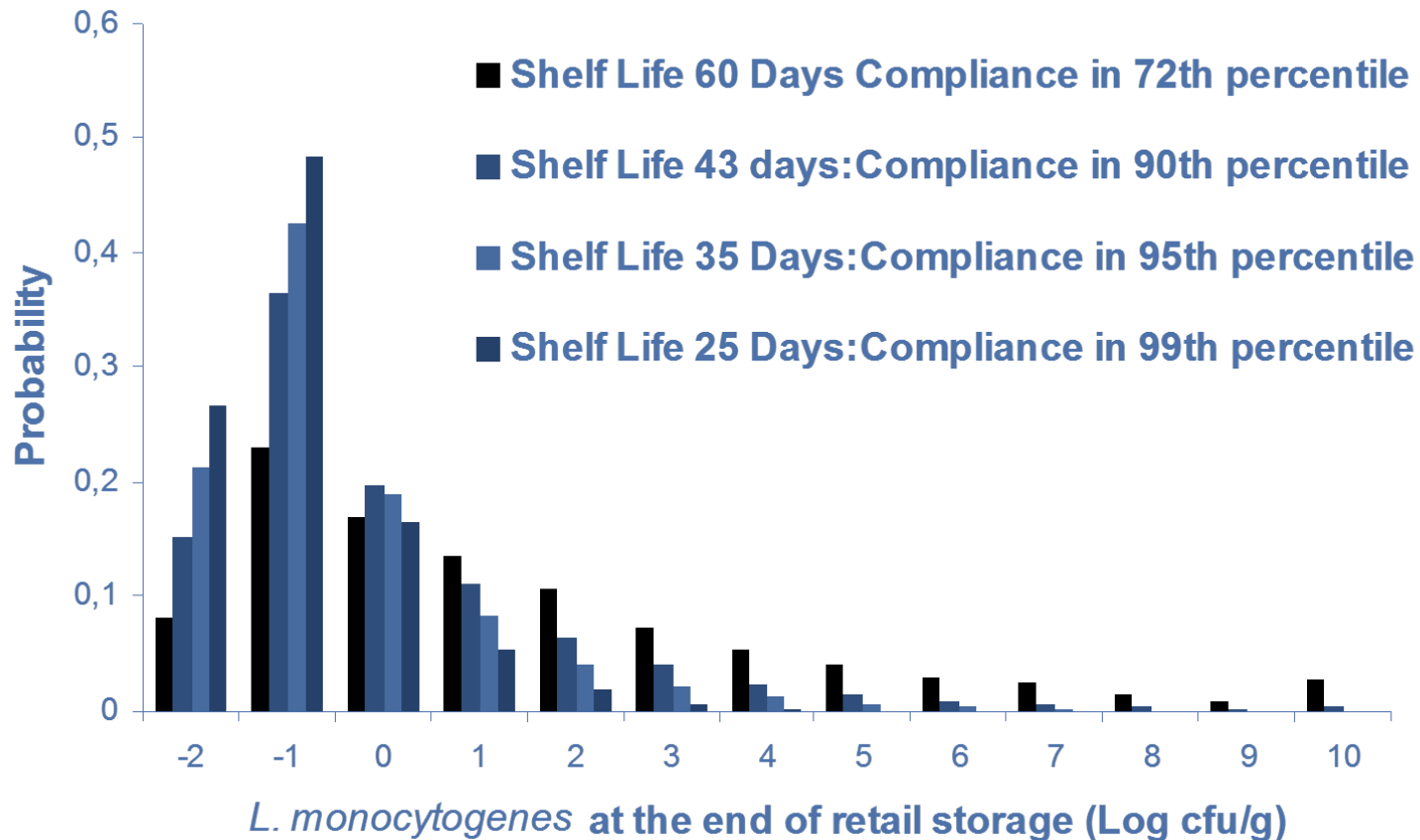
Potential actions for achieving the desired level of compliance to the Safety criteria

- **Adjust the shelf life of the product**
- **Modify the formulation of the product**

Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

Potential actions for meeting the Safety criteria

Adjust the shelf life of the product



Prove that the concentration of the pathogen will not exceed 100 cfu/g at the end of shelf life in retail

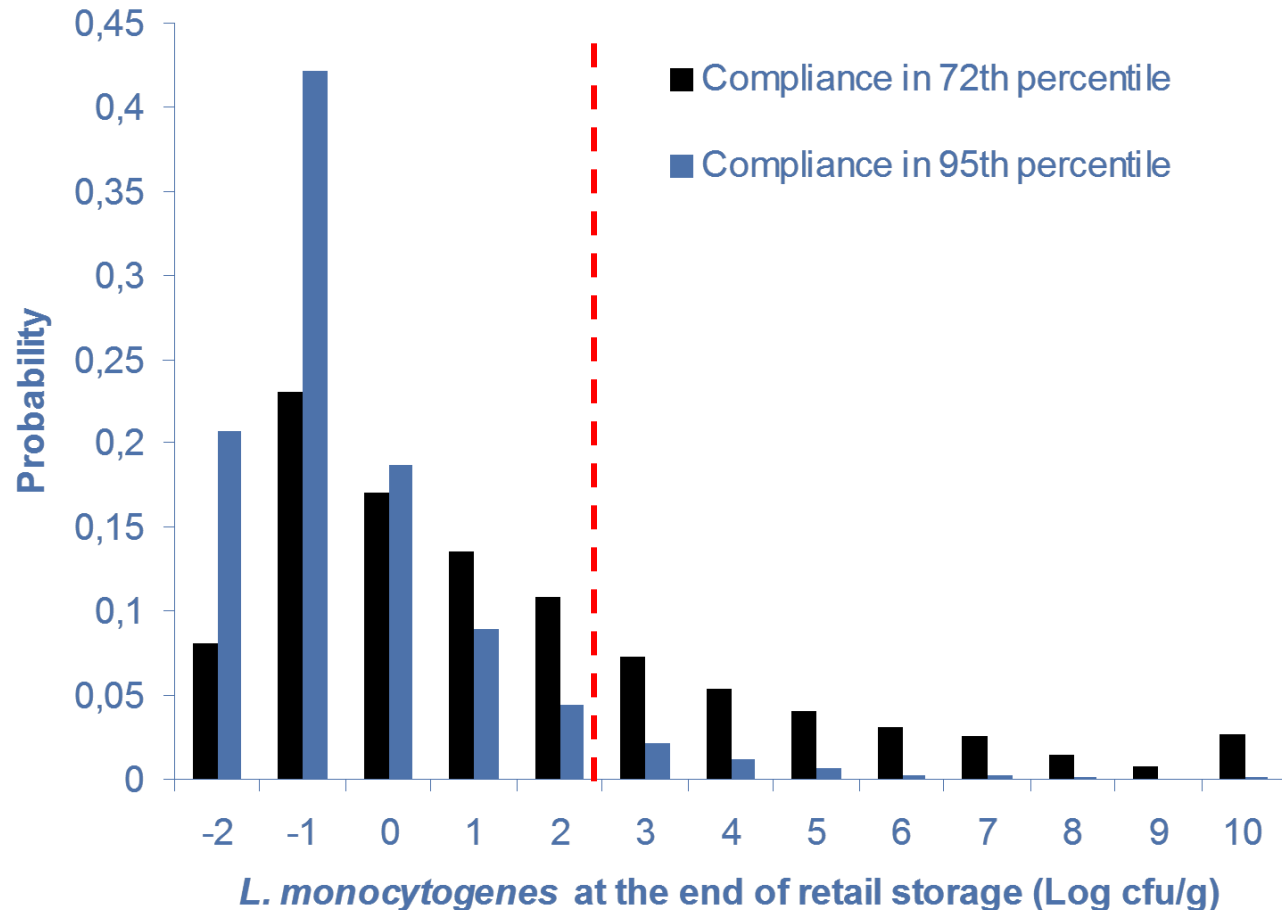
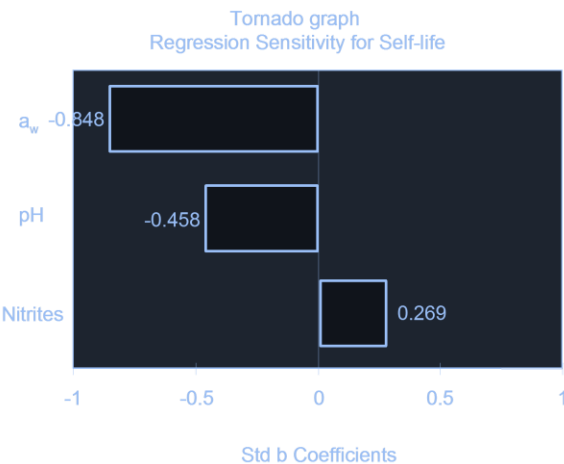
Potential actions for meeting the Safety criteria

Modify the formulation of the product

Cooked ham

a_w 0.971 → 0.96

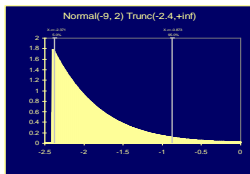
N 50ppm → 80ppm



Using the probabilistic approach for both safety and spoilage

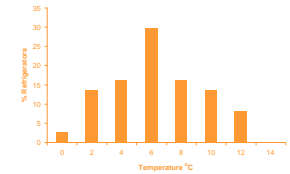
Probabilistic approach

Initial contamination



Product Characteristics (pH, a_w, Shelf Life...)

Chill chain characteristics



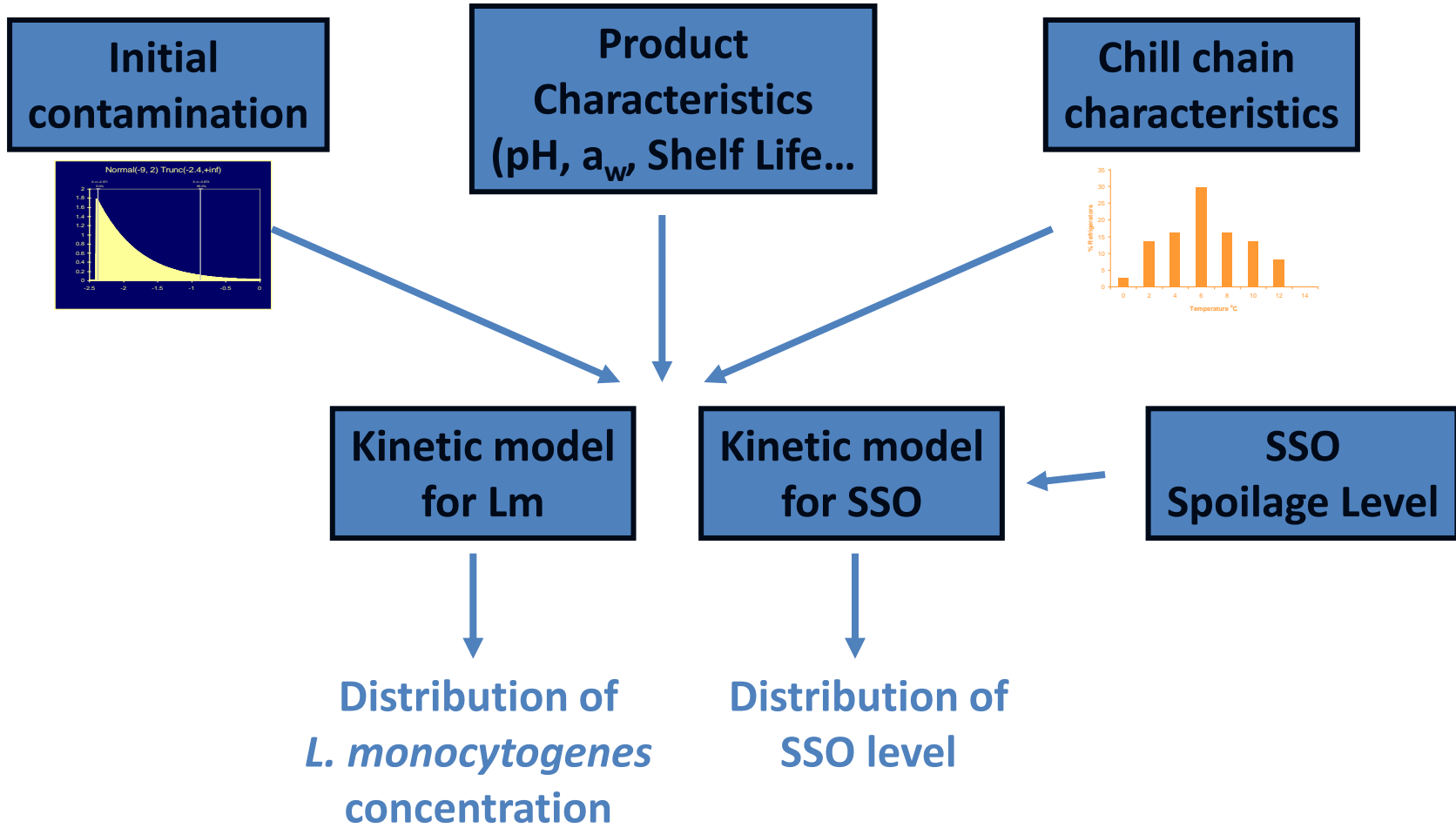
Kinetic model for Lm

Kinetic model for SSO

SSO Spoilage Level

Distribution of *L. monocytogenes* concentration

Distribution of SSO level



Using the probabilistic approach for both safety and spoilage

Case study

Product: cooked ham

pH=5.49, $a_w=0.971$, N=50ppm, SL=60 days

SSO: *L. sakei*

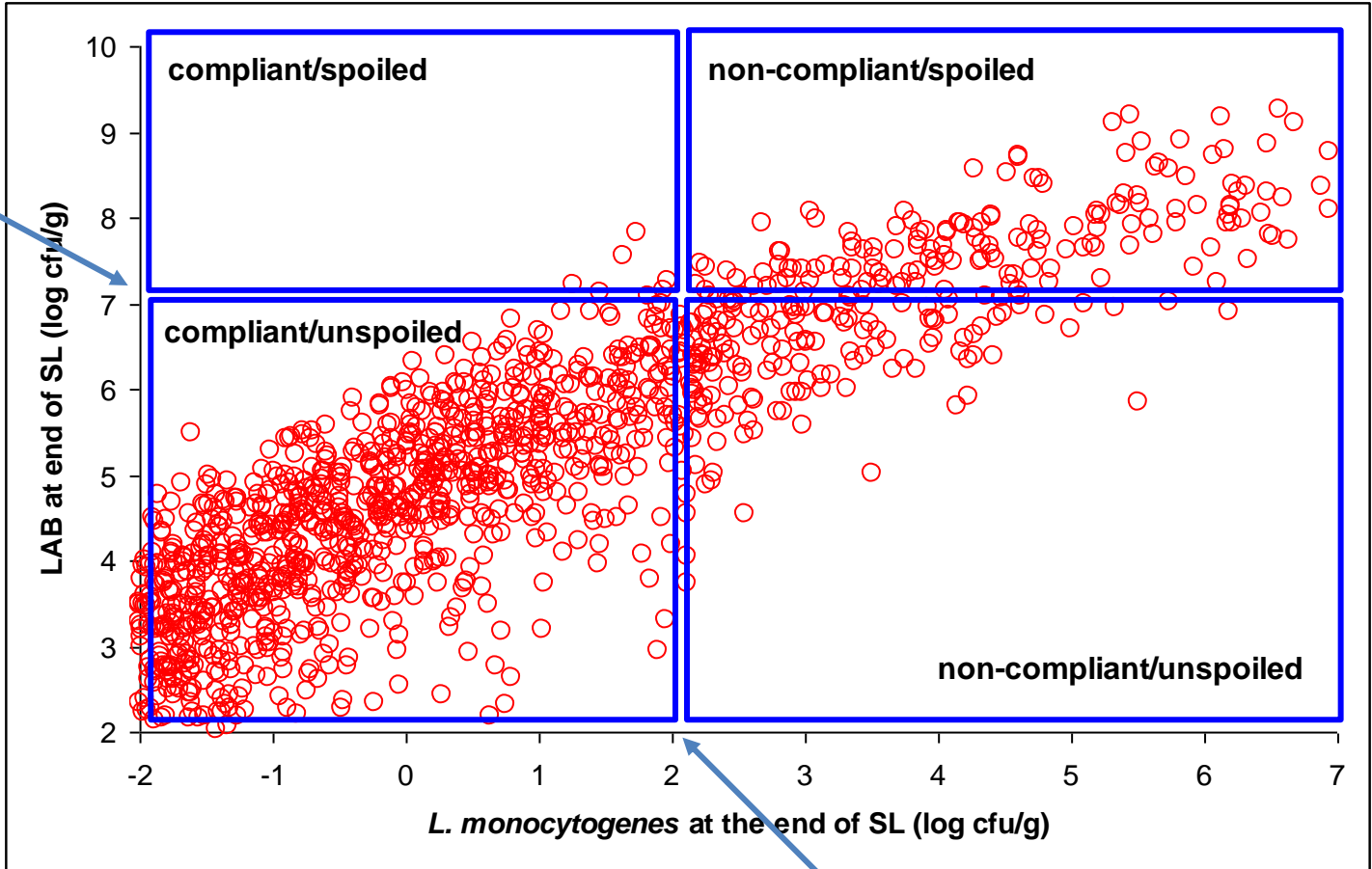
Spoilage level 10^7 cfu/g

Using the probabilistic approach for both safety and spoilage

Case study

Product: cooked ham SL 60 days

Spoilage
Level



Safety criterion

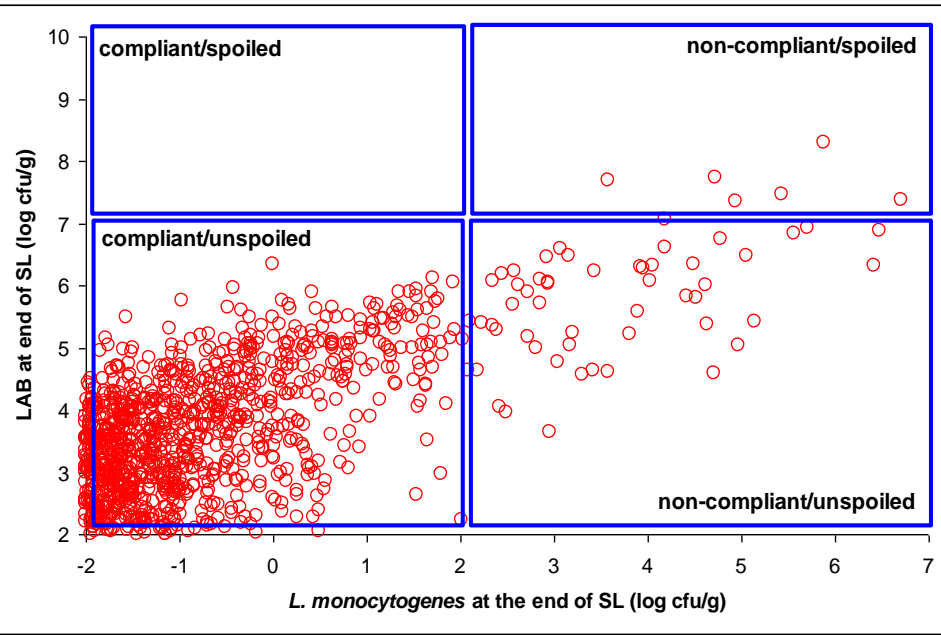
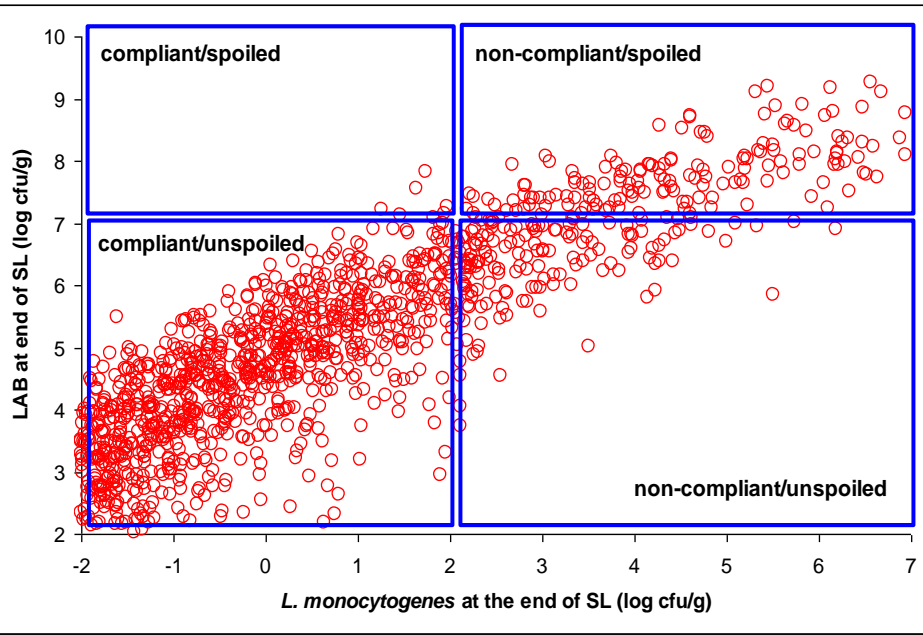
Using the probabilistic approach for both safety and spoilage

Case study

Product: cooked ham

SL 60 days

SL 25 days



72% compliance

99% compliance

17% spoiled before end of Shelf life

0.1% spoiled before end of Shelf life

Predictive Microbiology (applications)

Questions?

For future questions you can contact me
kkoutsou@agro.auth.gr



Summer School
“In Silico Methods for Food Safety”