International Regulation for *Listeria monocytogenes* Control in RTE Foods

Canadian Meat Council 2009 TECHNICAL SYMPOSIUM
"Advanced *Listeria monocytogenes* Control Measures in RTE Meats and Poultry Products"
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Overview of the Presentation

- Surveillance of the disease and extent of RTE food contamination
- New standards
- Risk assessments
- Modelling
- Challenge studies
- Research
- Case-control studies and expert opinion
- Future directions

Surveillance and Monitoring

Listeriosis Around the World

- *Listeria* is widely distributed in the natural environment and some strains can persist in processing plants with opportunities to contaminate and survive in RTE food
- In developed countries where listeriosis has been documented and regulations for its control have been in place over many years, the incidence rate of human infections seems very similar (0.3 – 0.7 per 100,000 population) with slightly higher rates in northern European countries
- Many developed countries have established regulations, industry guidance documents, and consumer educational strategies but levels are still unacceptably high
- Most developing countries do not considered listeriosis as a major pathogen because the cold chain is not widespread

Incidence of Listeriosis in Selected Countries (0.18-1.3/100,000)

Country	Period	Rate /100,000	Reference
Australia	2006	0.3	CDI (2009)
Canada	1990-1998	0.18-0.34	Alberta Health and Wellness (2005)
Denmark	2007	1.1	EFSA (2009a)
European Union	2007	0.3	EFSA (2009a)
Finland	2007	0.8	EFSA (2009a)
France	1999	0.45	Goulet et al. (2008)
France	2007	0.46	Goulet et al. (2008)
Germany	2007	0.4	EFSA (2009a)
Iceland	2007	1.3	EFSA (2009a)
Luxembourg	2007	0.6	EFSA (2009a)
New Zealand	2006	0.5	ESR (2008)
New Zealand	2004, 2007	0.6	ESR (2008)
Norway	2007	1.0	EFSA (2009a)
Sweden	2007	0.6	EFSA (2009a)
England & Wales	2001	0.27	de Valk et al., 2005
Scotland	2001	0.29	de Valk et al. (2005)
UK	2007	0.4	EFSA (2009a)
USA	2000	0.4	Healthy People 2010 (2009)
USA	2008	0.29	MMWR (2009)

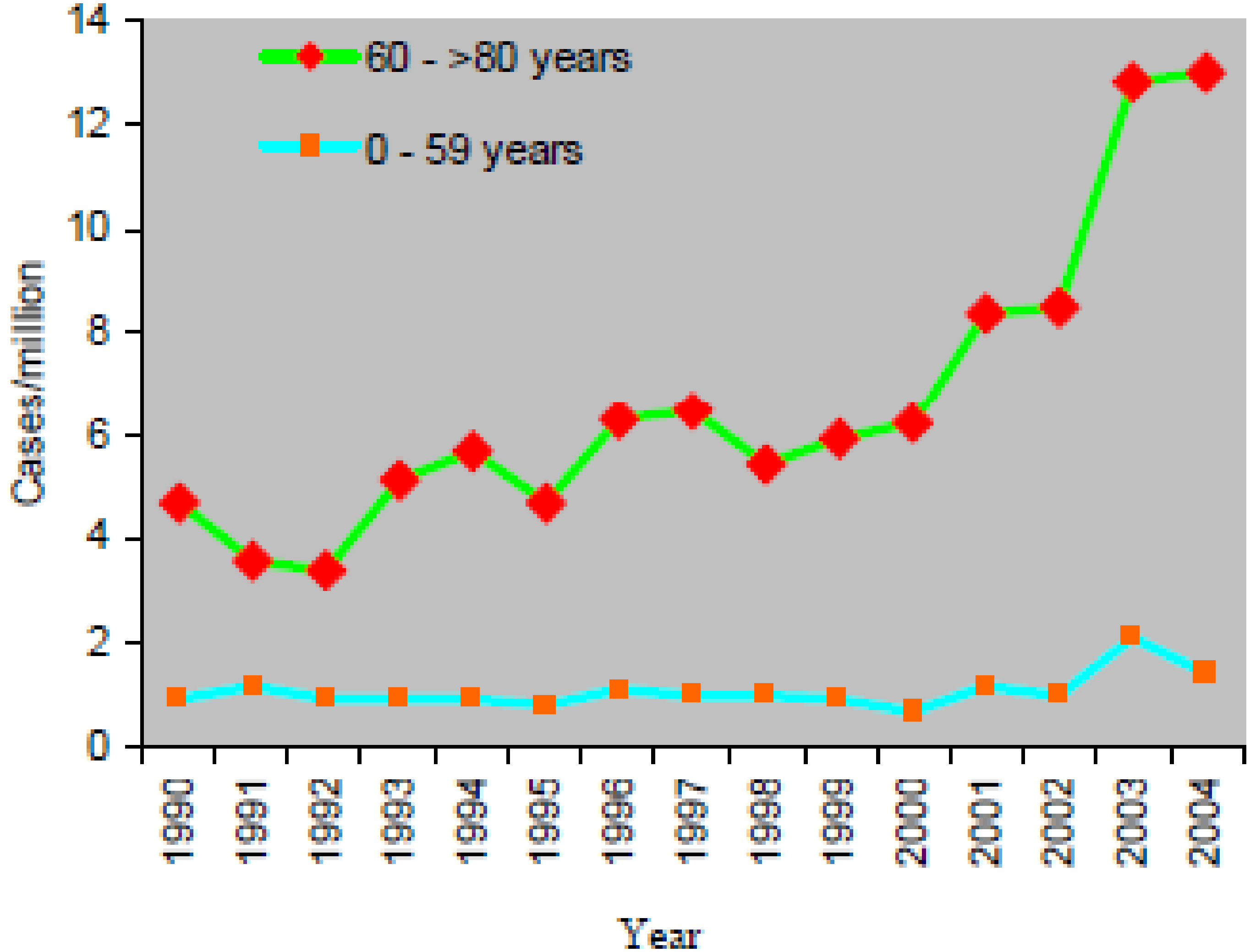
Healthy People 2010 Goals in U.S.

- Reduce foodborne listeriosis by 50% by the end of the year 2005 (0.25/100,000)
- FoodNet data indicated that the incidence of *Listeria monocytogenes* infection decreased between 1996 and 2001 from 0.5 to 0.3 cases per 100,000 people per year
- The level then reached a plateau, and that goal was not achieved, as it remained at 0.29/100,000 in 2008 (MMWR, 2009)
- In order to reduce further the incidence to a level of 0.25 cases per 100,000, it became evident that additional targeted measures were needed
- Risk assessments, food attribution studies and use of growth inhibitors on food were initiated in support of this goal

European Cases of Listeriosis

- In Europe increases of cases since 2000 are not caused by large foodborne disease outbreaks or pregancy-associated cases
- Increases have occurred mainly among persons $\geq$ 60 years of age and appear to be most pronounced for persons $\geq$ 70 years of age.
- The cause of this selective increased incidence is unknown
- Scandinavian countries of Iceland (1.3), Denmark (1.1), Norway (1.0) and Sweden (0.6) have consistently higher rates than other European countries (0.6-1.1/100,000), and probably relates to the higher consumption of smoked fish in these countries

Age-specific Rates for Listeriosis in England/Wales (Gillespie et al., 2006)



Foodborne Outbreaks Caused by *Listeria monocyogenes* in 2005 – 2008

Year	Country	Vehicle	Location	No. of cases (deaths)	References
2005	Spain	Unspecified meat		2 outbreaks	EFSA (2007)
2005	Poland	?	Sanatorium	9 (0)	EFSA (2007)
2005	Norway	Unspecified meat			EFSA (2007)
2005	Germany	?			EFSA (2007)
2005	USA (Texas)	Raw milk Hispanic soft cheese		12 (?)	CDC (2009)
2005	USA (Maryland)	Turkey deli meat		13 (?)	CDC (2009)
2005	USA (New York)	?		6 (?)	CDC (2009)
2005	USA (New York)	Grilled chicken		3(?)	CDC (2009)
2005	Australia (South Australia)	Deli meats	Processor	2 (2), both cancer patients	ABC (2005)
2006	Switzerland	Dairy product	Dairy	? (?)	EFSA (2007)
2006	Czech Republic	Soft cheese		78 (13) in 3 outbreaks	EFSA (2007)
2006	Germany	Harz cheese		6 (1)	EFSA (2007)
2006	USA (Ohio)	Ham	Home	3 (0)	CDC (2009)
2007	Norway	Raw milk soft cheese	Farm	21 (5)	EFSA (2009b)
2008	Canada (5 provinces)	Deli meats	Processor	58 (20)	Anonymous (2009)
2008	Canada (Quebec)	Raw milk soft cheese	Small processor(s)	21 (1)	Anonymous (2008)

Levels of *L. monocytogenes* in Outbreaks (Todd and Notermans, 2009)

- In 2006, Germany reported a *L. monocytogenes* outbreak affecting at least 6 persons, one of whom died. The source was a contaminated Harz cheese with cheese samples containing 52,000-120,000 *L. monocytogenes* cfu/g
- An outbreak in Norway in 2007 was caused by a soft cheese produced on a small dairy farm with very high numbers of *L. monocytogenes* in the cheese as well as in the production facilities

Zoonoses and Zoonotic Agents in the European Union in 2007(EFSA, 2009)

- In 2007 in the EU overall, *L. monocytogenes* was found rarely in various meat products; pork, 2.2%; red, mixed or unspecified meat, 2.5%; 3.0% in RTE broiler meat, with < 1% of samples exceeding the 100 cfu/g limit
- However, some countries did have samples much more frequently contaminated: Germany, Greece, Italy, Poland, and Slovenia reported presence of *L. monocytogenes* in samples of 25 g in 11.0%, 20.7%, 13.6%, 62.9, and 16.7%, respectively

Standards

The Changing World of Listeria Standards

- *Listeria*: Risk Assessment, Regulatory Control and Economic Impact (Todd, E., in "Listeria, Listeriosis, and Food Safety", 3rd Edit. edited by Ryser and Marth, 2007; 767-812)
  - "Currently there is no international agreement on what numbers of *L. monocytogenes* in foods are acceptable to protect the consumer. In several countries, different criteria or recommendations for tolerable levels of *L. monocytogenes* in RTE foods have been established
  - Some countries like USA, Austria, Australia, New Zealand and Italy require absence of *L. monocytogenes* in 25 g of foods (referred to as zero-tolerance). Other European countries e.g., Germany, Netherlands and France, have a tolerance of < 100 cfu/g at the point of consumption"
- All changing or about to change because of Codex Alimentarius Commission decision in 2009

2005 EU Criteria for *L. monocytogenes* in Foods

Category of Food	Sampling plan	Limits	Where criterion applies
RTE foods intended for infants and SMP	n=10 c= 0	Absence in 25 g	Products in the market
RTE foods able to support the growth of Lm, other than those intended for infants and SMP	n=5 c=0 n=5 c=0	100 cfu/g Absence in 25 g	Products in the market Before it has left the processor
RTE foods unable to support the growth of Lm, other than those intended for infants and SMP	n=5 c=0	100 cfu/g	Products in the market

SMP = special medical purposes

Codex Guidelines (CAC, 2009)

- The primary purpose of the Guidelines on the Application of General Principles of Food Hygiene to the Control of *L. monocytogenes* in RTE Foods
  - providing advice to governments on a framework for the control of the pathogen in these types of food, to:
  - 1) protect the health of consumers from listeriosis and
  - 2) ensure fair practices in food trade

Codex Listeria Standard, July 2009

- A maximum level was set for certain foods where the bacteria cannot grow (100 cfu/g), while in RTE products where growth is possible, no *Listeria monocytogenes* will be allowed (zero tolerance)
- For practical purposes, a food in which growth of *L. monocytogenes* will not occur defined as one having no observable increase in Lm levels > 0.5 $\log_{10}$ cfu/g
  - 1) for the expected shelf life as labeled by the manufacturer;
  - 2) under reasonably foreseeable conditions of distribution, storage and use; and
  - 3) includes a safety margin, e.g., 1.3 times the period specified

Criterion for RTE Foods in Which Growth of *L. monocytogenes* Will **Not** Occur (CAC, 2009)

Point of application	Microorganism	n	c	m	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported products), to the point of sale	*Listeria monocytogenes*	5 [a]	0	100 cfu/g [b]	2 [c]

[c]Assuming a log normal distribution, this sampling plan would provide 95% confidence that a lot of food containing SD of 0.25 log cfu/g would be detected and rejected based on any of the five samples >100 cfu/g *L. monocytogenes.*

Such a lot may consist of 55% of the samples <100 cfu/g and up to 45% of the samples >100 cfu/g, whereas 0.002% of all the samples from this lot could be >1000 cfu/g

Criterion for RTE Foods in Which Growth of *L. monocytogenes* **Can** Occur (CAC, 2009)

Point of application	Microorganism	n	c	m	Class Plan
Ready-to-eat foods from the end of manufacture or port of entry (for imported products), to the point of sale	*Listeria monocytogenes*	5 [a]	0	Absence in 25 g (< 0.04 cfu/g) [b]	2 [c]

[c]Assuming a log normal distribution, this sampling plan would provide 95% confidence that a lot of food containing a geometric mean concentration of 0.023 cfu/g and a SD of 0.25 log cfu/g would be detected and rejected if any of the five samples are positive for *L. monocytogenes.*

Such a lot may consist of 55% of the 25 g samples being negative and up to 45% of the 25 g samples being positive. 0.5 % of this lot could have concentrations >0.1 cfu/g

Annex II: “Microbiological Criteria for *Listeria monocytogenes* in RTE Foods (CAC, 2009)

- “...authorities may choose to establish and implement other limits for the *L. monocytogenes* concentrations at the point of consumption or at other points that provide an acceptable level of consumer protection …”
  - Codex criteria similar to the 2007 EU Lm regulation and needs to tolerate <100 cfu/g throughout the shelf life with the manufacturer able to demonstrate that the product will not exceed this limit throughout the shelf life
- CAC does not prescribe any sampling/testing frequencies to leave both the flexibility and possible inconsistency in testing by industry and government (Andersen and Nørrung, 2009)

Annex II: "Microbiological Criteria for *Listeria monocytogenes* in RTE Foods (CAC, 2009)

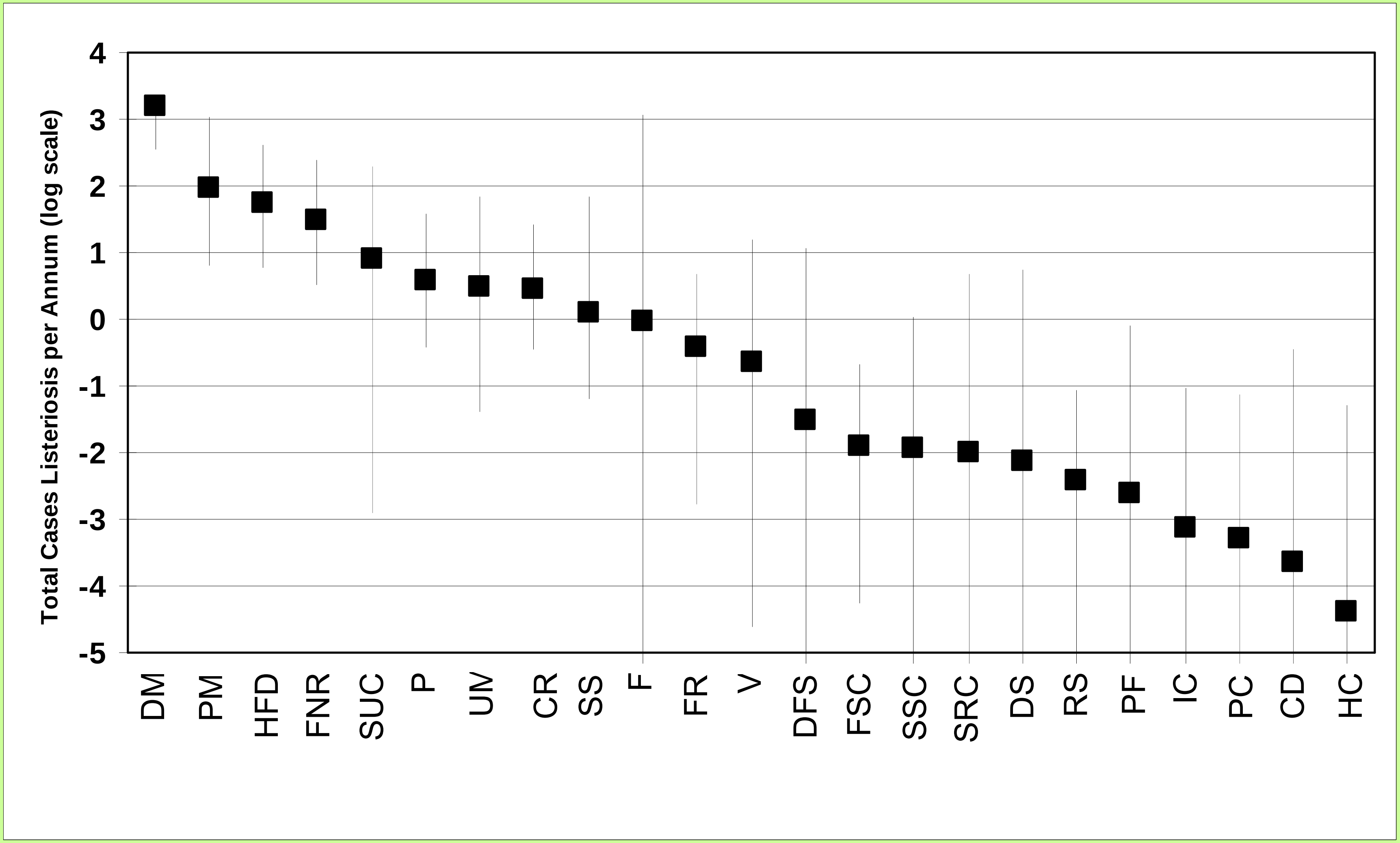
- Information needed by competent authorities
- ■ product criteria: 1) specification for physicochemical characteristics of the products, such as pH, a_w, salt, preservatives, 2) packaging system, 3) storage and processing conditions, 4) the possibilities for contamination, 5) the foreseen shelf life including a safety margin, and 6) consultations of available scientific literature and research data regarding the growth and survival of Lm
- ■ predictive mathematical modelling
- ■ challenge tests

Risk Assessments

Risk Assessments for Lm in Ready-to-eat Foods

- FDA/FSIS (2003) has done a risk ranking of 23 RTE foods: risk per serving vs. risk per population
- USDA (2003) has a QRA for Lm in meats and poultry; (2009) QRA for processed and retailed deli meats
- FAO/WHO (2004) has determined the risks of listeriosis from consumption of 4 RTE foods internationally and used “what if” scenarios

Predicted Cases of Listeriosis Associated with Foods for USA on a Yearly Basis (FDA/FSIS, 2003)



USDA QRA for *L. monocytogenes* in Meat and Poultry (2003) - Some Conclusions

- Increased frequency of food contact surface testing and sanitation is estimated to lead to a proportionally lower risk of listeriosis
- Combinations of interventions (e.g., micro testing and sanitation of food contact surfaces, pre-and post-packaging interventions, and the use of growth inhibitors/product reformulation) appear to be much more effective than any single intervention (multiple hurdles)
  - Example: no. of annual deaths in elderly would drop from 250 to <100 (median prediction) if industry used growth inhibitors and used post-packaging pasteurization

Final Rule on *L. monocytogenes* in RTE Meat and Poultry Products (FSIS, 2003)

ALTERNATIVE 1. Use a post-lethality treatment that reduces or eliminates Lm **AND** an antimicrobial agent or process that suppresses or limits LM growth throughout shelf-life

ALTERNATIVE 2. Use either a post-lethality treatment that reduces or eliminates Lm **OR** an antimicrobial agent or process that suppresses or limits Lm growth throughout shelf-life

- program of testing food contact surfaces in the post-lethality processing environment for Lm or indicator organisms

ALTERNATIVE 3. Use only sanitation measures to prevent Lm contamination

- program of testing food contact surfaces in the post-lethality processing environment for Lm or indicator organisms
- plants using Alternative 3 will get the most frequent verification testing attention from government regulators

- Lactate is permitted in meat and poultry products not >4.8%, and diacetate not >0.25%, to inhibit microbial growth

FSIS QRA of Deli Meats (Quesenberry et al., 2009)

- FSIS revised its risk assessment on deli meats with newer data, 1) retail contamination information for deli meat (Draughon, 2006) and 2) consumer behaviour data for deli meats (Cates et al., 2006)
- Of those listeriosis cases and deaths attributed to deli meats, approximately 83% were associated with deli meats sliced at retail (estimated mean of 919.6 cases and 166.9 deaths per year)
- This compares with the estimated mean of 188.6 cases and 34.1 deaths per year from pre-packaged deli meats
- Storage time (median): retail-sliced product, 6.4 days (maximum 22 days); pre-packaged product is 13.3 days (maximum 100 days)
- Fewer illnesses associated with consumption of deli meats with a growth inhibitor (retail-sliced 146.4; pre-packaged, 58.2) compared with those without any inhibitor added (retail-sliced 773.2; pre-packaged, 130.4); deaths also were lower

The Effect of Defective Servings on a Number of Listeriosis Cases Using Two Standards (FAO/WHO, 2004)

Assumed percentage of defective servings	Predicted no. of listeriosis cases at 0.04 cfu/g	Predicted no. of listeriosis cases at 100 cfu/g
0	0.5	5.7
0.00001	1.7	6.9
0.0001	12.3	17.4
0.001	119	124
0.01	1185	1191
0.018	2133	2133
0.1	11,837	11,848
1	117,300	117,363

Defective servings assumed to contain 10^6 cfu/g

Modelling

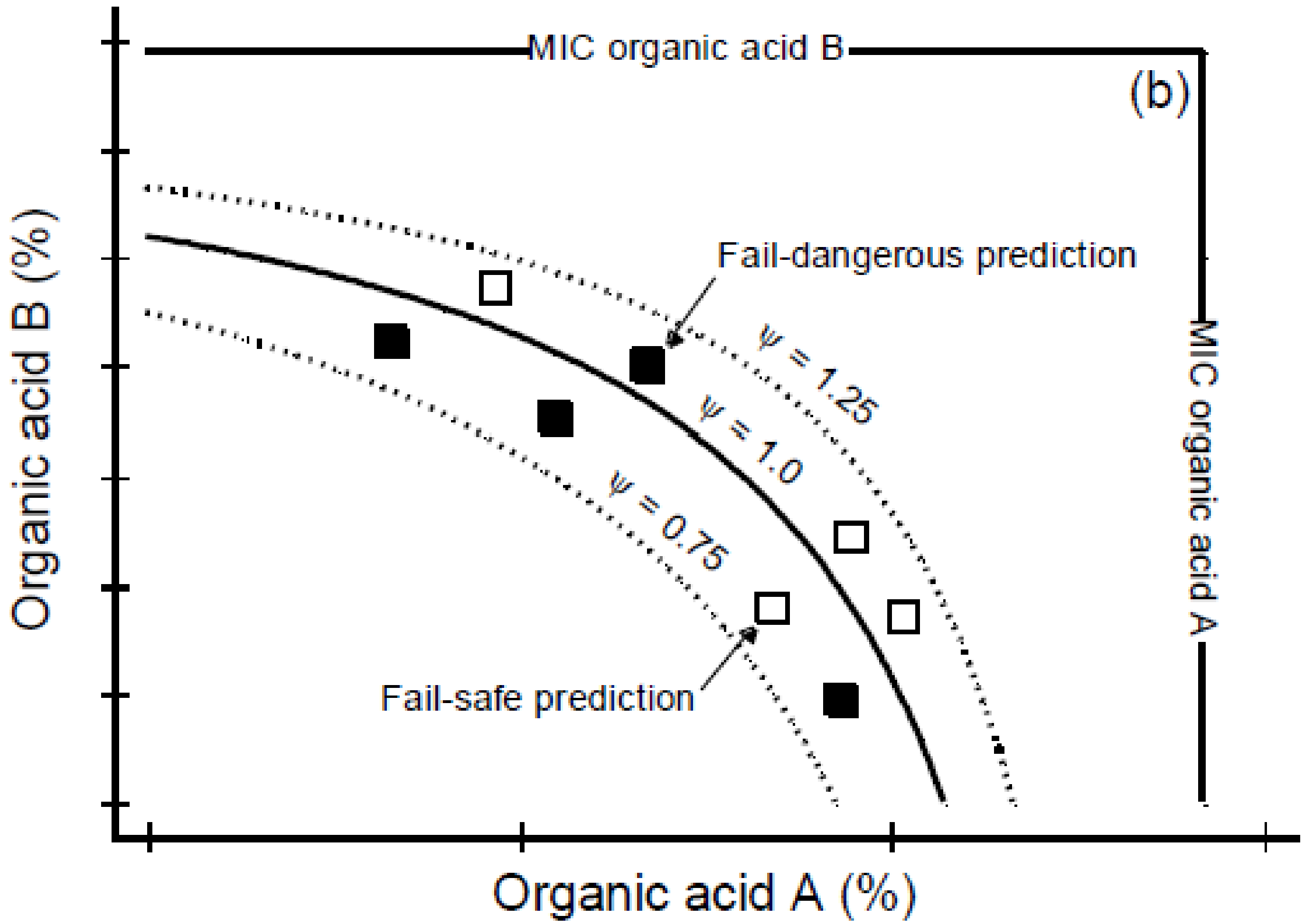
Environmental Parameters in Predictive Models for *L. monocytogenes* (Mejlholm and Dalgaard, 2009)

Models	Temp.	NaCl/a_w	pH	CO_2	Smoke	Nitrite	Acetic acid /diacetate	Lactic acid
Pouillot et al. (2007)	+	-	-	-	-	-	-	-
Zuliani et al. (2007)	+	+	+	-	-	-	+	+
Augustin et al. (2005)	+	+	+	+	+	+	-	-
PURAC (2007)[a]	+	+	+	-	-	(+)	+	+
Gunvig et al. (2007)[b]	+	+	+	+	-	+	+	+
Mejlholm and Dalgaard (2009)[c]	+	+	+	+	+	+	+	+

Comparison of Predicted and Observed Growth/No-growth Reponses for *L. monocytogenes* (Mejlholm and Dalgaard, 2009)

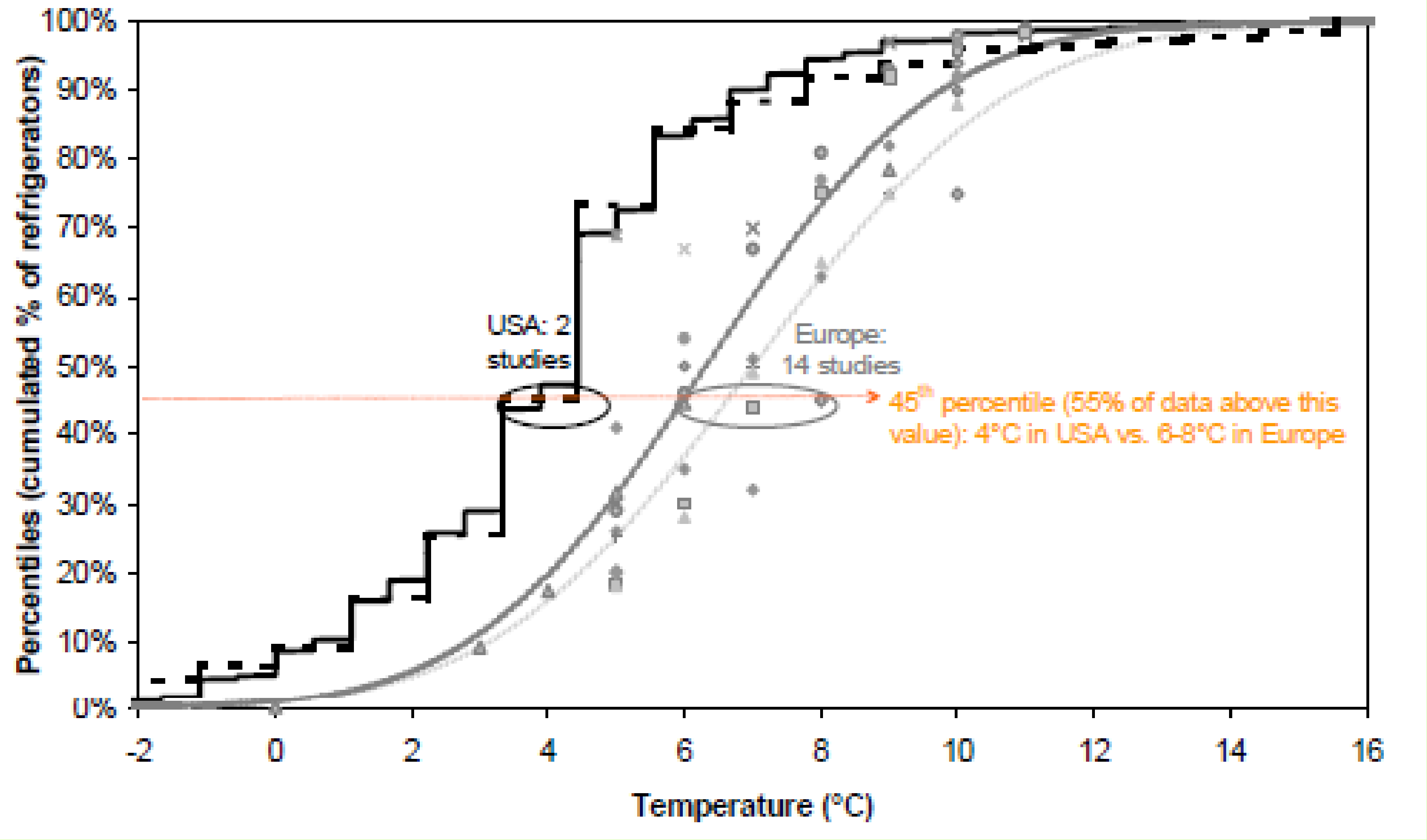
		Number of fail-dangerous/fail-safe predictions					
Products	n	Pouillot et al. (2007)	Zuliani et al. (2007)	Augustin et al. (2005)	PURAC (2007)	Gunvig et al. (2007)	Mejlholm and Dalgaard (2009)
Meat							
Pork loin	100	0/64	8/0	6/29	8/2	4/14	6/1
Ham/cold-cuts	154	0/52	27/13	17/26	10/30	6/29	8/22
Sausages	448	0/144	50/20	28/66	102/25	25/57	29/29
Seafood	193	0/33	12/9	39/18	21/6	0/27	1/6
Poultry	64	0/14	10/0	1/13	5/1	1/2	1/1
Dairy	12	0/0	0/0	0/0	0/0	0/0	0/0
All data	**971**	**0/307**	**107/42**	**91/152**	**146/64**	**36/129**	**45/59**
Correct predictions (%)		68	85	75	78	83	89
Fail-dangerous (%)		0	11	9	15	3	5
Fail-safe (%)		32	4	16	7	14	6

Predicted Growth Boundary (Solid Line) and Interfaces (Dotted Lines) of *L. monocytogenes* Depending on Organic Acid A and B Concentrations (Meijholm et al., 2009)

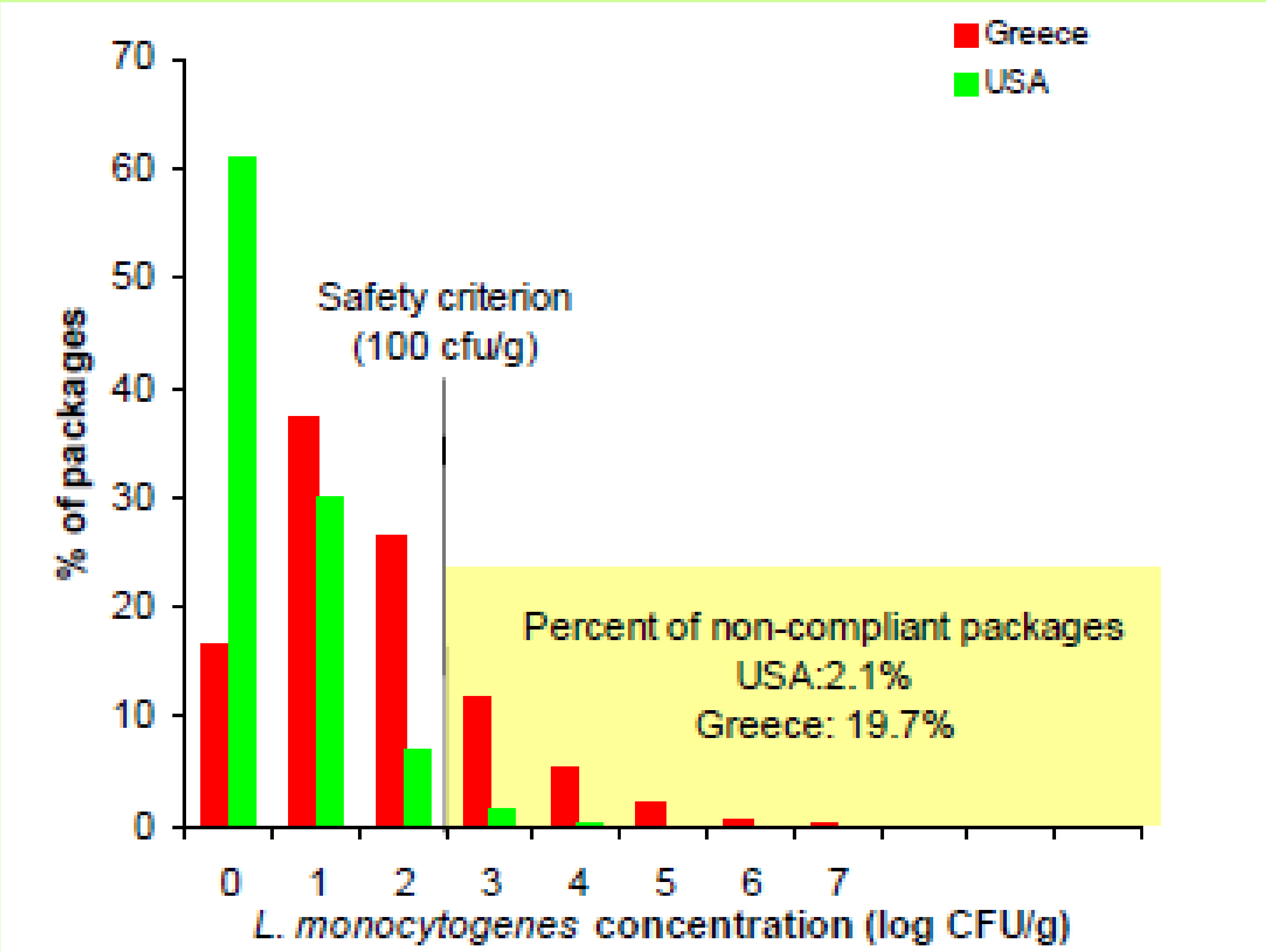


Theoretical growth (■) and no-growth (□) responses of *L. monocytogenes*

Temperature Distribution in Domestic Refrigerators in the 2000s in Europe (14 Studies) and US (2 Studies) (EFSA, 2007)



L. monocytogenes in Meat at pH 5.5, a_W 0.95, Nitrites 50 ppm, Starting with 1 cfu/g, After 10 Days Storage in US and Greece (EFSA, 2007)



Challenge Tests

Challenge Tests for *L. monocytogenes* in RTE Food (EU Community Reference Laboratory)

- The procedure for the determination of the growth potential assigns a RTE food:
  - 1) into a growth or no growth category, and
  - 2) if the food is able to support the growth of *L. monocytogenes*, to quantify its behavior in a food between production and consumption in a food
- Parameters of the test
  - 1) product characteristics, 2) shelf-life of the product, 3) number of batches, 4) choice of the strain(s), 5) preparation of the inoculum, 6) preparation and inoculation of the test units, 7) storage conditions, 8) measurement of physico-chemical characteristics of the food, 9) followed by quantitative microbiological analysis to measure any increase in the pathogen load

EU Challenge Tests – Example of Growth Potential for *L. monocyogenes* in a RTE Food

Batches	Day	*L. monocytogenes* concⁿ (cfu/g)	Concⁿ (log_{10} cfu/g) **median**	Difference between the median concentration at "day end" and the median concentration at "day 0" (log_{10} cfu/g)	Growth potential (δ)
1	"day 0"	25	**1.40**	2.28-1.40 = 0.88	**0.88**
		20	1.30		
		55	1.74		
	"day end"	100	2.00		
		210	2.33		
		190	**2.28**		
2	"day 0"	60	1.78	2.54-1.70 = 0.84	
		30	1.48		
		50	**1.70**		
	"day end"	250	2.40		
		350	**2.54**		
		390	2.59		
3	"day 0"	20	1.30	1.72-1.30 = 0.42	
		25	1.40		
		20	**1.30**		
	"day end"	43	1.63		
		52	**1.72**		
		76	1.88		

Challenge Tests for *L. monocytogenes* in RTE Food (EU Community Reference Laboratory)

- If δ is higher than 0.5 $\log_{10}$, then it is assumed that the food is able to support the growth of *L. monocytogenes* (example 0.88)
- Where growth of *L. monocytogenes* may occur (δ = > 0.5 $\log_{10}$) we can predict the concentration at the end of the shelf-life, if the concentration at the beginning of the shelf-life is known:
  - final concentration = initial concentration + δ
- It may also be used to set up the concentration at the beginning of the shelf-life in order to respect the limit of 100 cfu/g:
  - initial concentration = 100 cfu/g - δ

Research

Filling a Data Gap –Transfer Coefficients for Lm in Deli Meats

- Develop a series of *Listeria* transfer coefficients that can be incorporated into risk assessment calculations to determine the likelihood of cross-contamination between foods marketed by retail foodservice establishments and delicatessens

- Transfer coefficient = $\dfrac{\text{CFUs recovered from contact surfaces}}{\text{CFUs from RTE food}} \times 100$

Lm in Deli Meat Transfer Coefficient Findings (MSU)

- Slicing a contaminated product will lead to contamination of all slicer components
- > 90% of *Listeria* transfer from the blade to the product occurs during the first 10-15 slices of delicatessen meats after mechanical or knife slicing
- Deli meats will “clean” the slicer blade, but with varying effectiveness
- Depending on the original contamination load and the likelihood of growth in the newly contaminated product, this may increase the risk of listeriosis

Case-Control Studies and Expert Opinion

Case–Control Studies (Todd and Notermans, 2009)

- Three somewhat dated case-control studies of sporadic infection implicated frankfurters and chicken (U.S.), milk and pâté (Denmark), and soft cheese, food purchased from delicatessens and, in immunosuppressed patients, chicken (U.S.)(Schuchat et al., 1992; Schwartz et al., 1998; Jensen et al. 1994)
- Also, from 1997 through 2001, listeriosis disproportionately affected persons of Hispanic ethnicity in the U.S., mostly among pregnancy-associated cases

Expert Opinion Studies (Hoffman et al., 2007)

In expert opinion, the top ranking for deaths were:

- *L. monocytogenes*/luncheon meats
- *Salmonella*/poultry
- *Toxoplasma*/pork
- *Salmonella*/eggs
- *L. monocytogenes*/dairy

Expert Opinion Studies (Hoffman et al., 2007)

The highest ranking pathogen-food combinations for illnesses were:

- Norovirus/produce
- Norovirus/seafood
- *Campylobacter*/poultry
- Norovirus/ luncheon meats
- Norovirus/breads and bakery
- *Salmonella*/poultry
- Norovirus/beverages
- Any *Listeria*-food combination was much lower ranked (≥68)

Food Attribution and Listeria (Todd and Notermans, 2009)

- Although some foods have been identified as consistent links to outbreaks, e.g., deli meats and soft cheese, others may play a role that has yet to be fully elucidated, e. g., hummus and melons (Varma et al., 2007), as well as the role of the consumer in permitting cross-contamination and growth in the home

- Thus, food attribution studies are critical to determine where to focus control and prevention strategies

Future Directions

Scientific Opinion of the Panel on Biological Hazards (EFSA, 2007)

- Monitoring surveys revealed contamination associations with food packaging type, preparation
- Poor practices (e.g. the use of slicing machines for meat products), storage temperatures, the stage of sampling with respect to shelf life, the lack of an effective HACCP system, and lack of education and training of food handlers
- Growth of *L. monocytogenes* is a function of the type of food, the storage time and the storage temperature
- Storage temperature at retail and domestic refrigerators can vary significantly, especially for the domestic refrigerators

Investigation of Recalls in New Zealand in 2006/2007 (Crerar et al., 2009)

- 1) Inadequate environmental monitoring programs (sample sites, frequency of monitoring, inappropriate swabbing techniques, and a failure to analyze trends and respond correctly to environmental positives)
- 2) Improper design of the production line that did not sufficiently separate the raw ingredients from processed product, potentially permitting cross-contamination
- 3) Inadequate cleaning and sanitation as a result of poor building and equipment design or not following the correct procedures
- 4) Lack of validated scientific evidence to justify the shelf-life set by the food operator to have fewer pathogen hurdles for growth than expected

2009 UK Advisory Committee Report on Increase of Listeriosis in the Elderly - Causes

4 possible theories for rise in listeriosis in compromised people >60 years of age:

- **1)** Disease associated with improved case recognition
- 2) Lm has become more virulent and 'new' strains are better able to cause bactremia
- 3) The population predominantly affected by the recent increase has become more susceptible to infection with Lm
- 4) Levels of Lm exposure to the population have increased

2009 UK Advisory Committee Report on Increase of Listeriosis in the Elderly - Recommendations

- Create pan-European surveillance, epidemiological and microbiological investigations
- Investigate differences in virulence of Lm
- Target active surveillance (monitoring) for *Listeria* spp. in foods to inform control of this organism
- Identify food consumption patterns of the over 60s (including vulnerable groups)

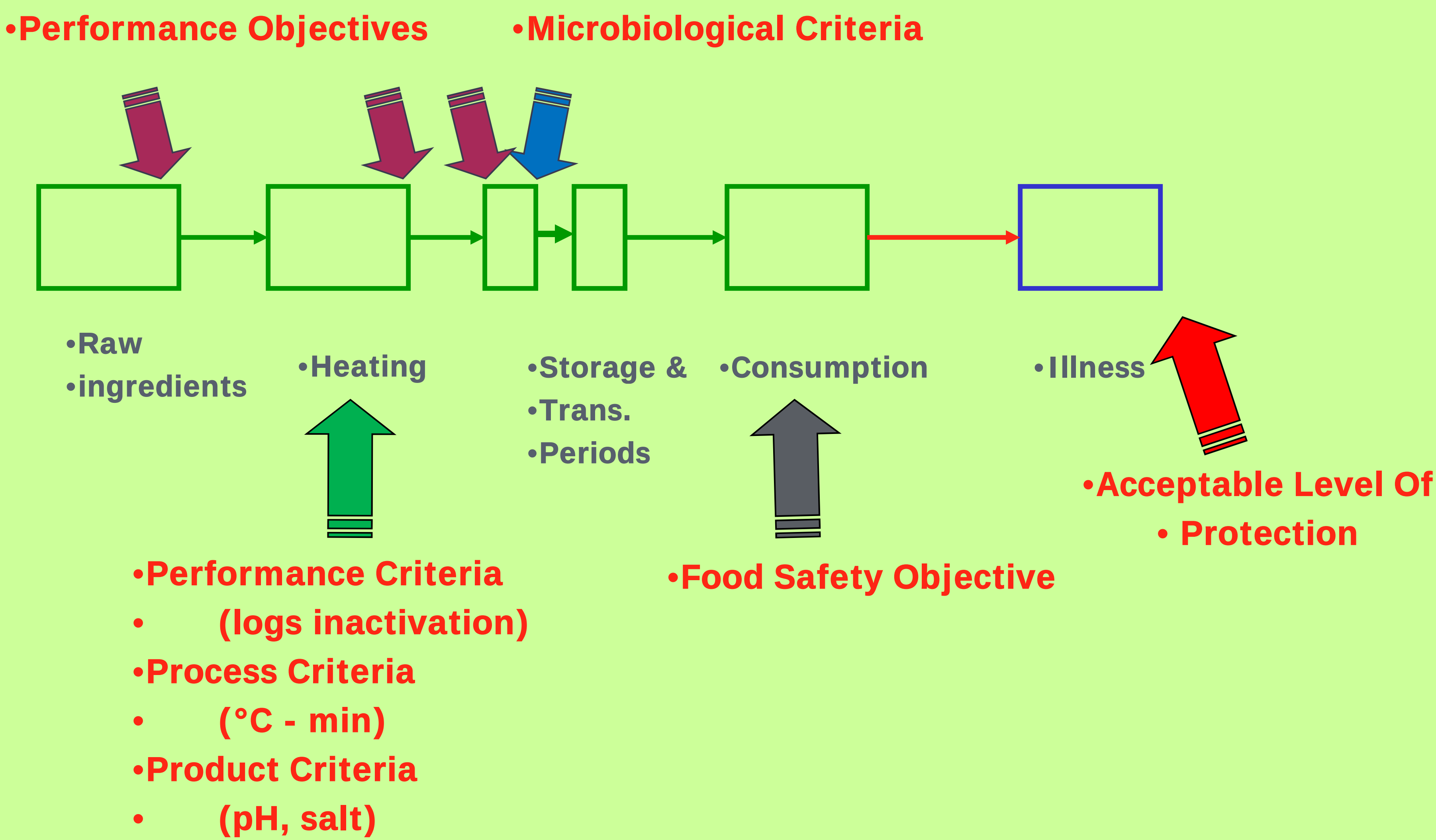
2009 UK Advisory Committee Report on Increase of Listeriosis in the Elderly - Recommendations

- Communicate general food safety advice to the over 60s, as well as to those involved in their care and preparation of their food

- Consider food storage and handling practices of older people

- Stress importance of temperature and shelf life control, hygiene/cleaning and formulation of food

Generic Process Risk Assessment Model (Whiting, 2009)



Summary: How Do We Reduce Listeriosis Around the World?

- 1) Improve outbreak and sporadic case surveillance, with a focus on the growing elderly population by 10-year intervals
- 2) Evaluate the burden of gastrointestinal *Listeria* infection
- 3) Monitor more foods for *Listeria* spp. and *L. monocytogenes* and conduct case-control studies to identify risk factors and foods that are contaminated
- 4) Based on the above, conduct food attribution studies for focus on control
- 5) Determine which products support the growth of *L. monocytogenes* under processing, transportation, retailing and consumer storage scenarios
- 6) Design equipment that reduces the opportunities for food build-up on machinery with effective cleaning and sanitization

Summary: How Do We Reduce Listeriosis Around the World?

- 8) Identify more inadvisable consumer practices for targeted educational strategies (e.g., time-temperature)
- 9) Adopt the Codex standard as national regulations and have manufacturers and retailers determine which of their products support growth of the pathogen during their shelf-lives
- 10) Have governments work with industry to consider ALOPs, FSOs and setting performance, process and product criteria for RTE foods
- 11) Based on Codex standards countries can agree on export/import surveillance and avoid trade barriers
- 12) Continue research/surveys into transfer, modelling, risk assessment, case-control studies, expert opinion, Lm virulence, and consumption patterns by populations

Thank you for your attention

Questions?