



Hypothetical examples of FSO-derived microbiological criteria:

Listeria monocytogenes in smoked fish

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Outline

- The disease: Listeriosis
- The organism: *Listeria monocytogenes*
- The product: cold-smoked salmon

- Risk assessment → ALOP
- ALOP → FSO
- FSO → PO
- PO → MC

Listeriosis

- Listeriosis affects people in risk groups
 - elderly, immunosuppressed, neonates
- Listeriosis is a food-borne disease
- Listeriosis is caused by ready-to-eat-products (long shelf life)
- Listeriosis is caused by "high numbers" of *Listeria monocytogenes*

Listeriosis?

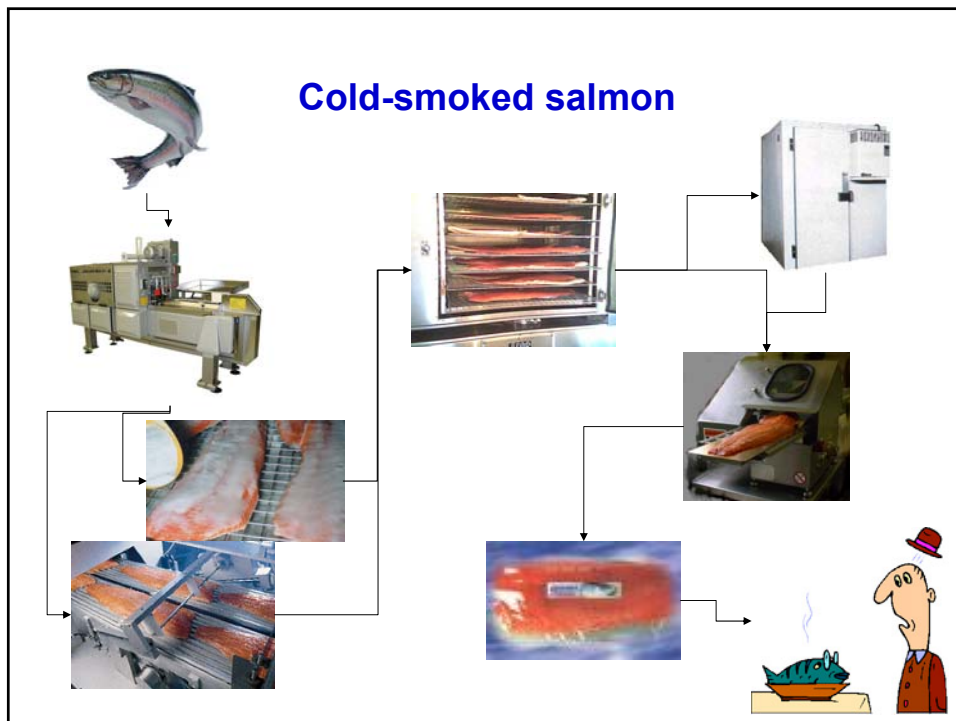
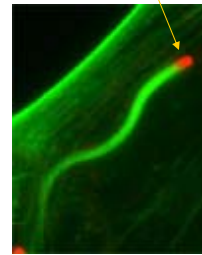
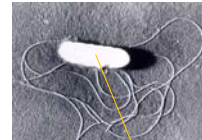
Causes of foodborne disease in UK and Wales 1992-2000

Bacterium	Cases	% of cases	Deaths	% of deaths	dead/cases
<i>Yersinia</i> spp.	293,436	12.4	8	1	"0"
<i>Campylobacter</i>	281,826	11.9	67	9	0.02
<i>Cl. perfringens</i>	117,370	5.0	124	17	0.1
<i>Salmonella</i>	93,651	4.0	268	37	0.3
<i>Staphylococcus aureus</i>	13,429	0.6	0	0	0
<i>E. coli</i> O157	879	0.04	19	3	2.2
<i>Listeria monocytogenes</i>	168	0.01	67	9	35
Un-known	1,375,981	58	139	19	0.01
Total	2,365,909	100	718	100	-

Adak et al. 2002

Listeria monocytogenes

- is an environmental bacterium
- is an intracellular pathogen
 - not all sub-types can be linked to cases or out-breaks
- often persists in food processing environments
- can grow in salt and at low temperature



Cold smoked salmon

- is a heterogenous product group
 - raw material (lactate between 65 and 130 mM)
 - salting: dry salting, brine injection, brining, combinations
 - smoking: automatic ovens, old-fashioned smoking, liquid smoke (2 – 11 ppm phenol)
 - freezing steps
 - storage conditions (+/- lactic acid bacteria)
- all of which decides survival and subsequent growth potential of *L. monocytogenes*

Listeria contamination

fish → slaughter → smoke house → consumer.....

Samples	# of samples	Positive for Lm	% positive for Lm
Fish from freshwater	24	2	9
Fish from marine water	22	0	0
Fish at slaughter	24	6	25
Fish in smoke house	12	5	40
Process environment	lots!	some	4-30

Vogel et al. 2001, Wulff et al. 2005, Hansen 2005

Listeria monocytogenes sub-types by RAPD

Hansen et al. 2005. in prep

RAPD-type	No isolates of particular RAPD-type						Total	
	Fish farming		Fish slaughter			Smoke		
	Fresh	Marine	In	Bled	Out	In		Out
AAA	1						1	
BCB	8				2		10	
CBC	3						3	
DAA	1						1	
DCA					1		1	
DCB	1						1	
DCC	1			2	1	16	1	21
DCJ			7					7
DEC						3		3
EDD							1	1
FEC						1		1
FJC						1		1
GAA					1			1
HFE				1				1
ICI			2					2
JFF		1						1
KCC	2			2	1			5
KIA			1					1
LGG					2			2
MHH					1			1
No RAPD-types	7	1	3	3	7	4	2	

Listeria monocytogenes sub-types by RAPD

Wulff et al. 2005 Submitted

RAPD-type	Number	Symbol	# <i>L. monocytogenes</i> strains of RAPD type at fish plants								Total		
			Smokehouse				Fish slaughter house						
			1	2	3	4	A	B	C	D			
1	1	▲	1									1	
2	1	●	1									1	
3	1	▼	1					3				4	
4	4	◆	4									4	
5	8	●	8									8	
6	1	▲		1								1	
7	1	▼		1								1	
8	1	●		1								1	
9	1	▲		1								1	
10	3	■	3	25		10		30		18		86	
11	1	●	1					4	1			6	
12	1	●	1									1	
13	2	●	2									2	
14	2	●	2									2	
15	5	◆	5									5	
16	1	▲	1	17								18	
17	5	▲	5	11	12							28	
18	2	■	2									2	
19	1	●	1									1	
20	1	▲	1									1	
21	1	▲	1									1	
22	1	●	1									1	
23	1	▼	1									1	
24	4	●			4							4	
25	1	▲			1							1	
26	1	◆			1							1	
27	5	▲						5				5	
28	2	●						2				2	
29	1	●						1				1	
30	1	◆						1				1	
31	5	▼							5			5	
32	1	■							1			1	
33	7	▲								7		7	
34	1	●							1			1	
35	5	▲									5	5	
36	1	●									1	1	
37	1	▲									1	1	
X ¹	X	X										25	
Total			5	2	11	4		46	7	29	3	7	238

1. Number of *L. monocytogenes* positive samples detected only by PCR and from which no strains were recovered

Listeria growth

<http://www.foodsafety.gov/~dms/lmr2-toc.html>

Product	Total samples	Lm pos	Number of samples with indicated level (cfu/g)					
			0.04-0.1	0.1-1	1-10	10-10 ²	10 ² -10 ⁴	10 ⁴ -10 ⁶
Soft cheeses (white)	2,931	5	2	0	0	3	0	0
Blue cheese	1,623	23	18	3	1	1	0	0
Other soft cheeses	1,347	14	12	0	20	0	0	0
Packed salads	2,966	22	17	1	1	2	1	0
Meat, luncheon	9,199	82	42	20	10	2	8	0
Deli salads	8,549	202	162	28	9	2	1	0
Fish salads	2,446	115	82	19	10	2	2	0
Smoked fish	2,644	114	67	11	19	8	7	2
Total	31,705	577	402	82	52	20	19	2

Gombas et al. 2003 JFP 66:559-569

Listeriosis - risk from smoked fish

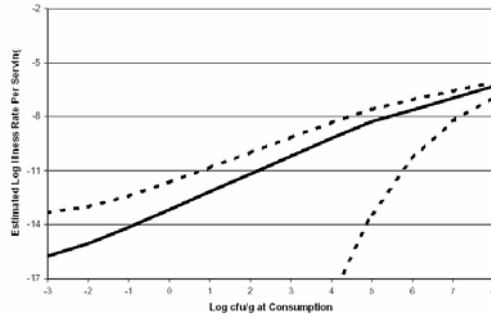
Risk assessment	Product	Listeriosis cases per 100,000 consumers	Listeriosis cases per 10 ⁶ servings
WHO/FAO	Milk	0.091	0.005
	Ice cream	0.00012	0.000014
	Cold-smoked fish	0.016	0.053
FSIS/FDA	Milk	3.24	0.001
	Ice cream	<0.003	0.000000049
	Smoked fish	0.046	0.0062
	Deli meats	57	0.077

An ALOP for *Lm* ?

- Dose-response and risk estimate curves have been developed for product – consumer – combinations

Based on deli-meat
 Elderly population
 Risk estimate of 3×10^{-7}
 Roughly equal to 5×10^6 cfu/g
 Roughly equal to 850 cases
 (in US/year)

<http://www.foodsafety.gov/~dms/lmr2-toc.html>



An ALOP for *Lm*

Predicted annual number of listeriosis cases in the susceptible (US) population when the level of *Listeria monocytogenes* was assumed not to exceed a specified maximum level in the food (based on distribution as indicated)

Level (cfu/g)	Maximum dose	% servings with max dose	Estimated # listeriosis cases per year
0.04	1	100	0.5
0.1	3	3.6	0.5
1	32	1.7	0.7
10	316	0.8	1.6
100	3,160	0.4	5.7
1000	31,600	0.2	25.4
Baseline level			~ 2,100

Table 5.3 from WHO/FAO 2004 Risk assessment of *Listeria monocytogenes* in ready-to-eat foods

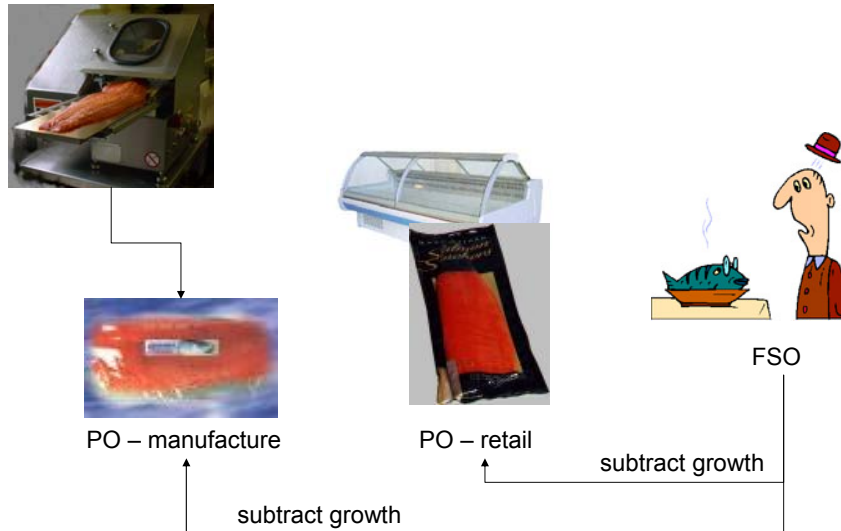
Assumptions

- Serving size for this product is 50 g
- Shelf life of the product is 3 weeks.: 1 week between manufacture and retail; all product consumed by the end of three weeks.
- At retail, contamination is 2 to 6% with less than 100 CFU/g
- Constant storage temperature 4-5°C – growth potential:
 - a) 1.0 log cycles per week
 - b) 0.3 log cycles per week.
- The primary source of *L. monocytogenes* contamination is post smoking - no further bactericidal treatments

An FSO(s) for Lm

FSO cfu/50 g serving	FSO Log(cfu/ 50 g serving)	FSO Log(cfu/g)
10 ⁷	7	5.3
10 ⁶	6	4.3
10 ⁵	5	3.3
10 ⁴	4	2.3
10 ³	3	1.3
10 ²	2	0.3
10	1	-0.7
1	0	-1.7

FSO → PO



POs

- Two POs were selected as likely inspection points
 - Product after final packaging (manufacturer) = P_{Om}
 - Product at point of retail = P_{Or}
- Given a specified FSO expressed as a log number:
 - $P_{Om} = FSO - \text{expected growth between production and consumption} = FSO - (\text{growth rate} \times 3 \text{ (weeks)})$
 - $P_{Or} = FSO - \text{expected growth between sale and consumption} = FSO - (\text{growth rate} \times 2 \text{ (weeks)})$
- Point estimates were used

POs - Example 1: growth of 1 log per week

FSO Log(cfu/g)	POr Log(cfu/g)	POm Log(cfu/g)
5.3	3.3	2.3
4.3	2.3	1.3
3.3	1.3	0.3
2.3	0.3	-0.7
1.3	-0.7	-1.7
0.3	-1.7	-2.7
-0.7	-2.7	-3.7
-1.7	-3.7	-4.7
-2.7	-4.7	-5.7

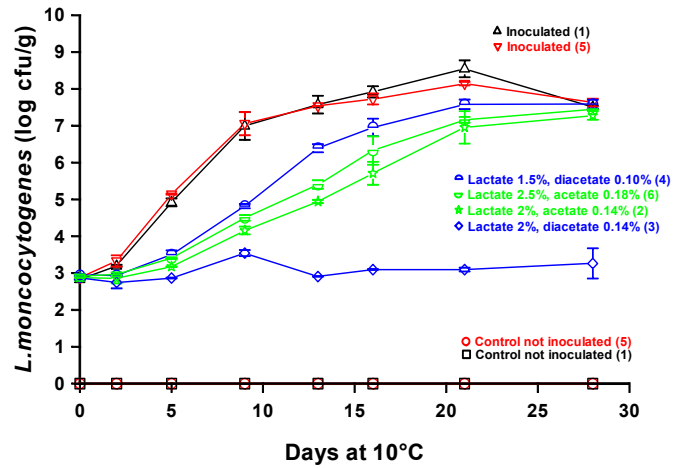
POs - Example 2: growth of 0.3 log per week

FSO Log(cfu/g)	POr Log(cfu/g)	POm Log(cfu/g)
5.3	4.7	4.4
4.3	3.7	3.4
3.3	2.7	2.4
2.3	1.7	1.4
1.3	-0.7	0.4
0.3	-0.3	-0.6
-0.7	-1.3	-1.6
-1.7	-2.3	-2.6
-2.7	-3.3	-3.6

POs - Example 3: no growth during storage

Growth of *Listeria monocytogenes* in VP cold-smoked salmon supplemented with lactate, diacetate or acetate

Vogel et al. 2005 in prep.



Microbiological criteria are not FSOs

FSO	MC
A goal	Defines acceptability of a lot
States maximum frequency and/or concentration of a hazard	Includes: microorganism, sampling plan, analytical unit, method, limits, number of units analysed

Microbiological criteria

- POs are the upper limit of a frequency distribution around a mean log count
- How far from the PO is the mean log count?
 - What is the standard deviation?
 - With what level of probability should a sampling plan reject non-conforming lots?
 - What method will be used – what can be detected?

Microbiological criteria - example

- FSO = 10^5 cfu/serving = 3.3 log cfu/g
- POr = 2.7 log cfu/g (assuming 0.3 log/week)

Mean log counts given the POr and given s.d.

- s.d. 0.2 → $\mu(\text{POr}) = 2.7 - 3 \times 0.2 = 2.1$ log cfu/g
- s.d. 0.4 → $\mu(\text{POr}) = 2.7 - 3 \times 0.4 = 1.5$ log cfu/g
- s.d. 0.8 → $\mu(\text{POr}) = 2.7 - 3 \times 0.8 = 0.3$ log cfu/g
- methods
 - presence/absence: 0.04 cfu/g = -1.39 log cfu/g
 - quantitative MPN: 0.3 cfu/g = -0.52 log cfu/g
 - quantitative plate: 10 cfu/g (2 x 0.5 ml) = 1 log cfu/g
 - quantitative plate: 100 cfu/g (1 x 0.1 ml) = 2 log cfu/g

Microbiological criteria - example

Number of samples needed to find with 95% probability at least one positive sample unit in a defective lot

log(cfu/g)		# of units (n) with m (detection) equalling			
FSO/PO	s.d.	μ (POr)	0.04	0.3	100
3.3 / 2.7	0.2	2.1	1	1	3
	0.4	1.5	1	1	27
	0.8	0.3	1	2	177

based on calculations by Dr. S. Dahms

Microbiological criteria - example

- If levels of *L. monocytogenes* are distributed in the sample with a standard deviation of 0.2 log units, then a single sample using presence/absence test would ensure with 95% probability that the PO of 2.7 log units is not exceeded.
- If the standard deviation is 0.8, then 2 negative presence/absence tests are required to give the same probability.
- If the standard deviation is 0.8, then a 177 negative 100 cfu/g tests would ensure with 95% probability that the PO of 2.7 log units is not exceeded

Microbiological criteria - example

Number of samples needed to find with 95% probability at least one positive sample unit in a defective lot

FSO/PO	log(cfu/g)		# of units (n) with m (detection) equalling		
	s.d.	μ (POr)	0.04	0.3	100
2.3 / 1.7	0.2	1.1	1	1	-
	0.4	0.5	1	1	-
	0.8	-0.7	2	6	-
3.3 / 2.7	0.2	2.1	1	1	3
	0.4	1.5	1	1	27
	0.8	0.3	1	2	177
4.3 / 3.7	0.2	3.1	1	1	1
	0.4	2.5	1	1	2
	0.8	1.3	1	1	15

based on calculations by Dr. S. Dahms

Microbiological criteria

- Only apply when
 - the criteria can accomplish what they purport to (e.g. ensure or improve safety of foods)
 - the criteria are technically feasible
 - the criteria are administratively feasible

Summary

- Listeriosis is a rare but very serious food-borne disease
- Low levels of the bacterium presents a low risk
- An ALOP – and hence an FSO – could be derived at from risk assessments
- POs can be developed going "back" from the FSO
 - Need a good understanding of microbial ecology of the particular product and processing
 - Different processors can justify different POs
- MCs can be developed based on POs
 - Need several risk management decisions (s.d., probability of rejection etc.)

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