



Enterobacter sakazakii **in Powdered Infant Formula**

Dr. Jeff Farber
Director
Bureau of Microbial Hazards
Food Directorate
Health Products and Food Branch
Health Canada



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Outline



- **The organism/disease:** *E. sakazakii*/ meningitis, septicemia
- **The product:** powdered-infant formula (PIF)
- **Risk assessment:**



ALOP? → FSO? → PO? → MC

Enterobacter sakazakii



- Gram-negative rod; Enterobacteriaceae family
- Non-spore forming, motile
- 1980, designated as a unique species based on differences from *E. cloacae* in DNA relatedness, pigment production and biochemical reactions (sorbitol, α -glucosidase)

Enterobacter sakazakii



- 14 or 15 Biogroups
- Biochemical reactions; motility
- Produces a capsule
- Genetically diverse (4 clusters; 2 lineages described to-date)

E. sakazakii infections



- About 90 cases worldwide to-date, 80% of them in infants < 1 y old
- Among infants, 66% of cases are 0-1 month of age and 14% > 1 month – 1 year
- **Conclusion:** Group at particular risk is infants (<1 year; 4-6 months)
- **Greatest risk:** neonates (<28d) and immunocompromised, especially those of LBW (<2500 g)

Hazard Characterization



• *Symptoms*

– Meningitis, septicemia, NEC, death possible, chronic sequelae

• *Dose response*

– Unknown

– Likely depends on age /weight of patient/health status

Environmental sources of *E. sakazakii*



- Dust
- Fruit flies, house/stable flies
- Rats
- Soil, rhizosphere
- Sediment, wetlands

Foods from which the organism has been isolated



- Cured meat, minced beef, sausages
- Lettuce, vegetables, alfalfa sprouts
- Tofu; bread, cheese; rice seed
- Herbs & spices
- Sous (licorice drink)
- Dried products (infant cereal, vegs., spices, whey, egg yolk/eggnog, flour/meal)
- Mother's milk

Occurrence in food production environments and households



Site	Samples positive for <i>E. sakazakii</i> (%)
Milk powder factory	14/68 (21)
Chocolate factory	2/8 (25)
Cereal factory	4/9 (44)
Potato flour factory	4/15 (27)
Pasta factory	6/25 (23)
Households	5/16 (31)

Powdered infant formula (PIF)



Formulations can contain:

- Milk ingredients and/or corn syrup solids
- Fats, sugars, starch
- Vitamins/minerals

PIF is given to:

- In general, infants under 6 months
- Low and very-low-birthweight newborns
- Newborns/infants with special nutritional needs
- Infants of mothers with nutritional or health problems (e.g., HIV positive)

Outbreaks linked to PIF



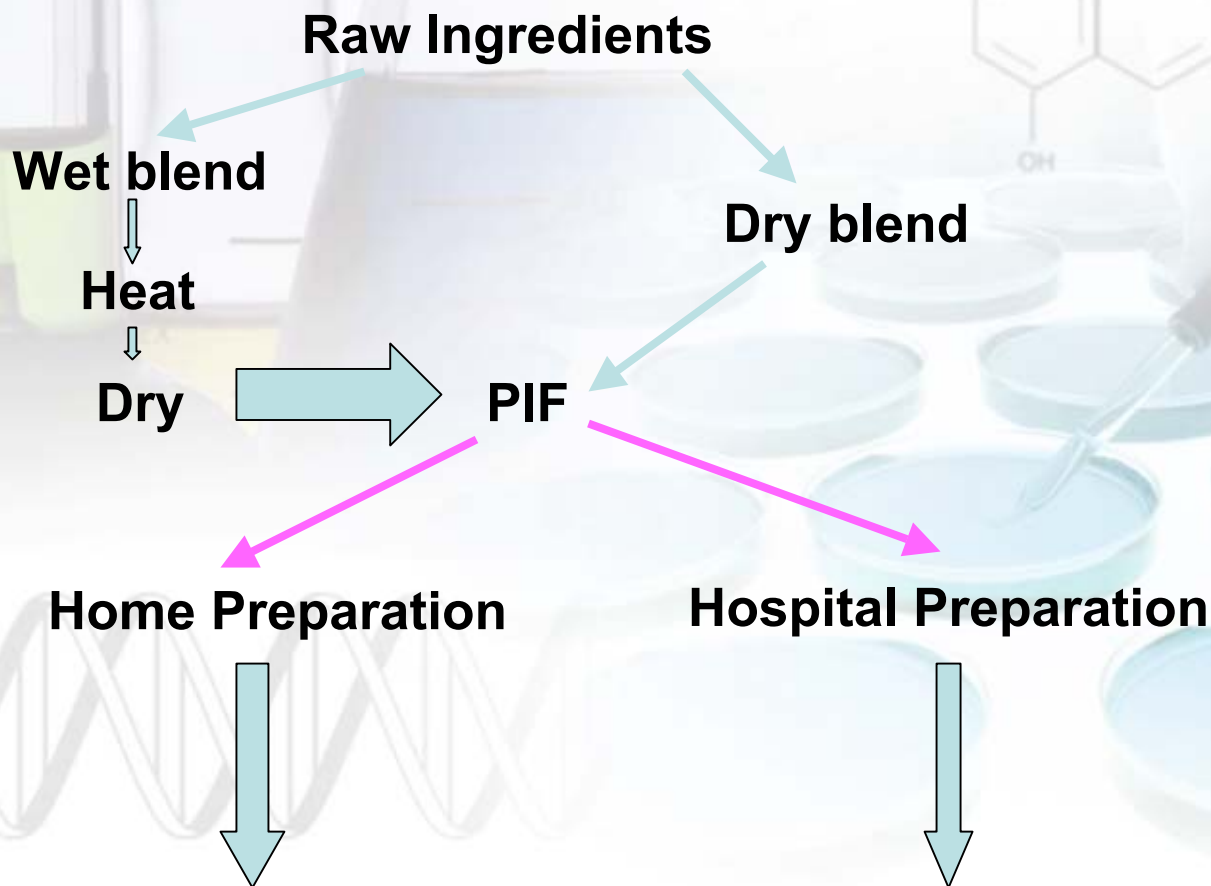
Location	Cases	Comments	Reference
Iceland	3 (1 death)	2 normal term infants; 1 Down's	Biering et al., 1989
Tennessee	4; 3 sepsis, 1 bloody diarrhea	Es; 8 cfu/100g	Simmons et al., 1989
Belgium	12	6/12 with NEC positive for Es	Van Acker et al., 2001
Tennessee	9	1 confirmed, 2 suspect, 6 colonized	Himmelright et al., 2001
Israel	5	3 colonized only	Bar-Oz et al., 2001
New Zealand	5 (1 death)	4 colonized	2004
France	9 (2 deaths)	5 colonized	AFSSA, 2005

Incidence of *E. sakazakii* in PIF



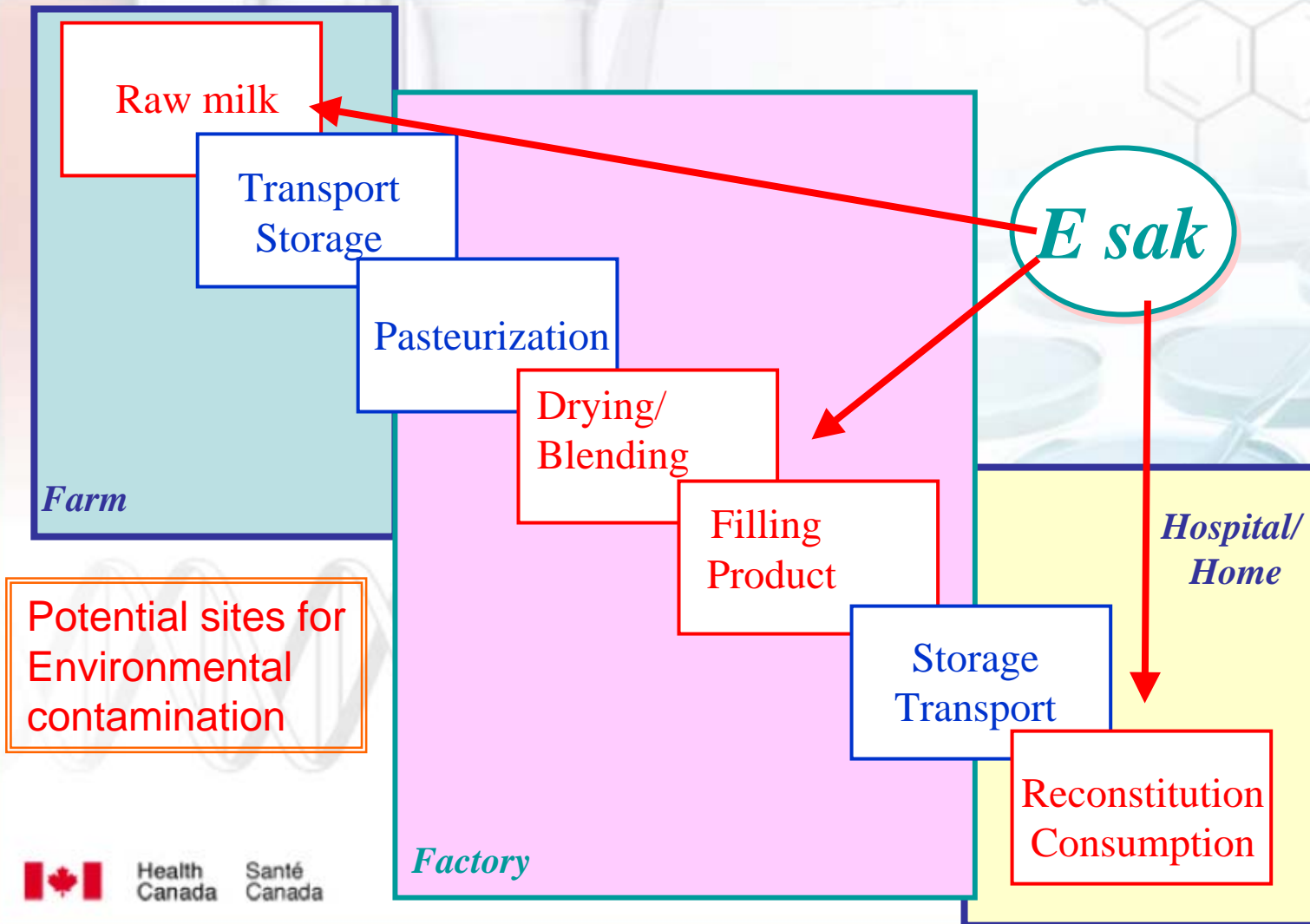
Samples positive (%)	Reference
20/141 (14)	Muytjens (1988)
8/120 (6.7)	N-White & Farber (1997)
8/210 (3.8)	Heuvelink et al. (2001)
3/141 (2.1)	Heuvelink et al. (2003)
8/58 (13.8)	Leuschner et al. (2004)
35/3,467 (1.0)	IFC (2004)
1/835 (0.12)	WHO (2004)

Production methods for PIF

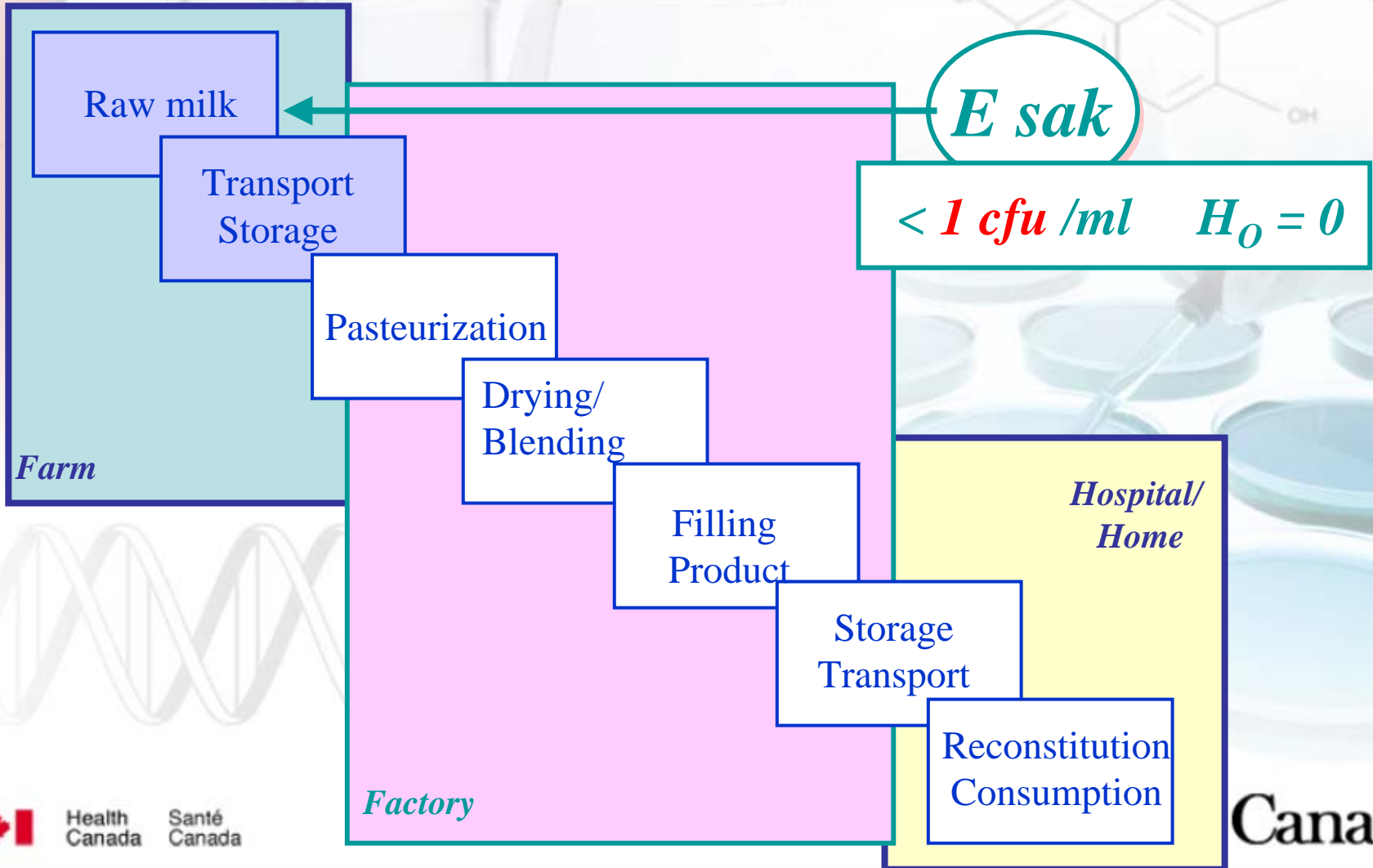


WHO, 2004

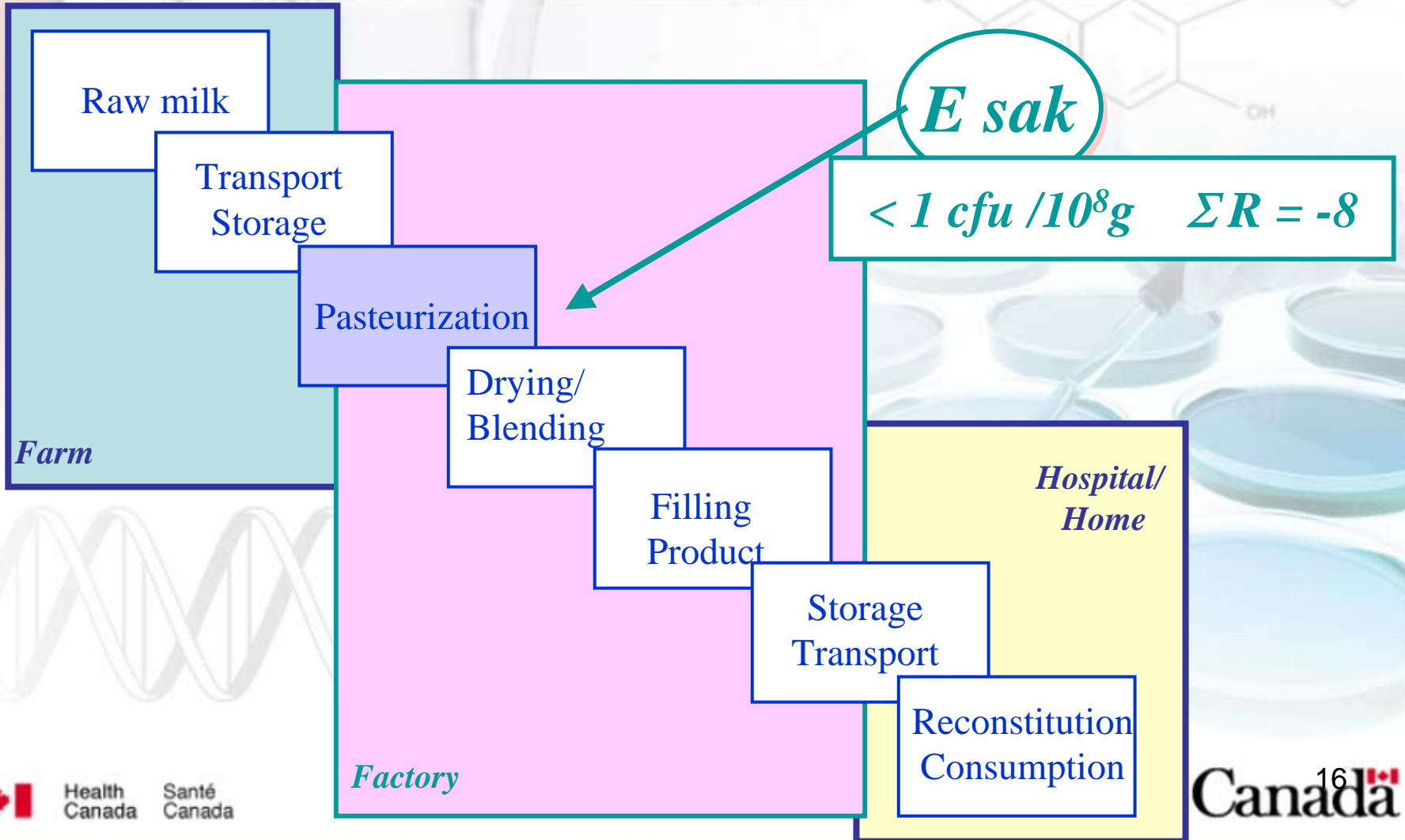
Sites of potential contamination



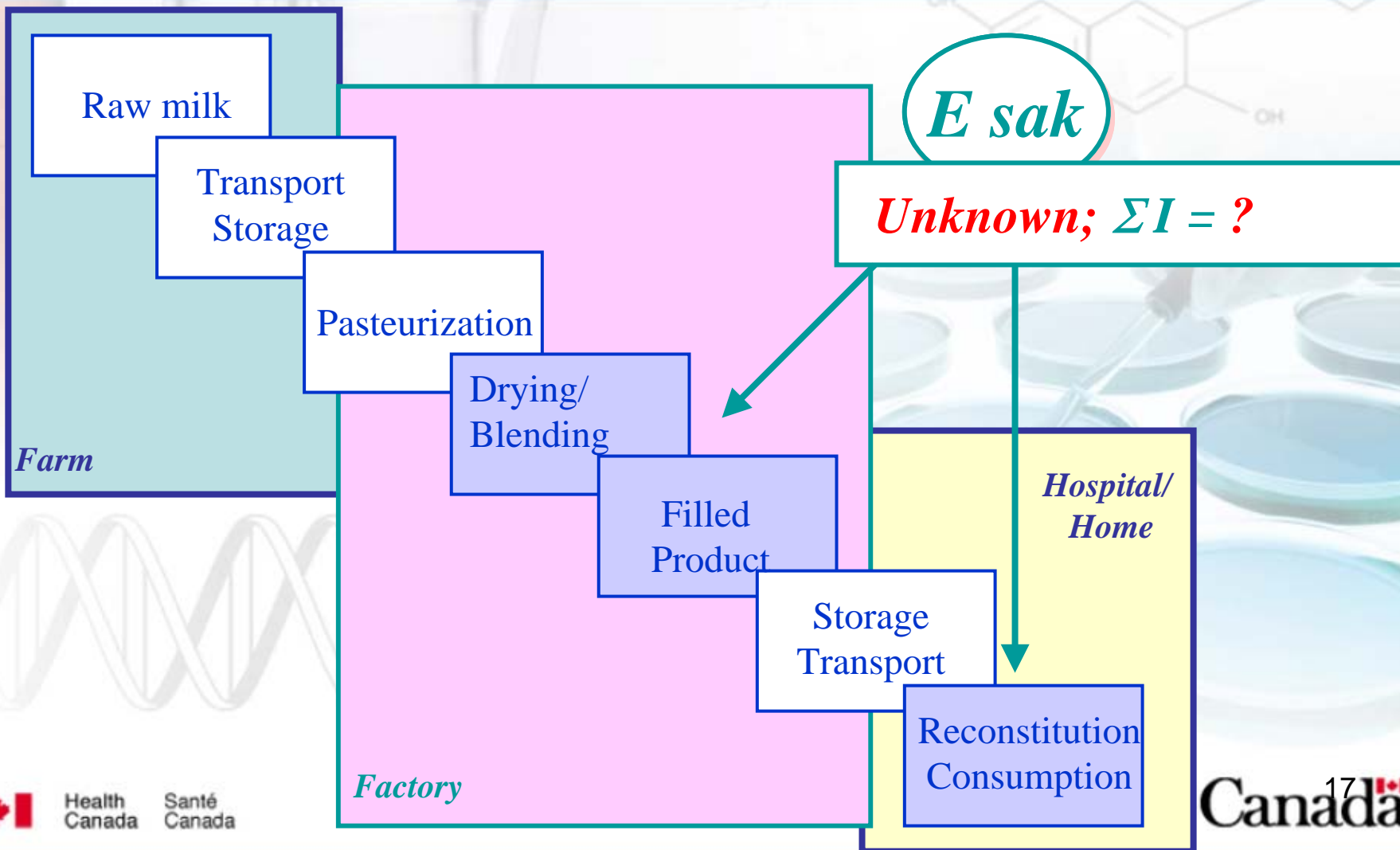
Initial Levels



Reduction



Increase through recontamination and growth



E. sakazakii – Growth in PIF



Temp.	Lag time	Gen. time	Reference
6°C	ND	13.7 h	Iversen et al. (2004)
10°C	19-47 h	4.2-5.5 h	N-White & Farber (1997)
21°C	ND	1.7 h	Iversen et al. (2004)
23°C	1.8-3.4 h	37-44 min	N-White & Farber (1997)
	3.9-4.7 h	43 min	Lenati (2005)
37°C	ND	19-21 min	Iversen et al. (2004)
	2.2-3.0 h	17.4 min	Lenati (2005)

ND=Not Determined

Key Factors Affecting the Risks Associated with PIF



- Initial level of contamination
- Level of hygiene in the preparation & delivery of the rehydrated formula
- Bactericidal treatment at time of preparation
- Duration of time to consumption and holding/storage temperature



Relative risk of *E. sakazakii* infection for specified holding time and temps*



Time (hours)	Room Temperature (°C) - liquid mixing temp 40°C		
	15°C	25°C	35°C
1	1.0	1.0	1.0
2	2.5	5.4	9.1
3	4.8	24.2	78.1
4	8.2	98.3	681.9
8	47.3	2.0x10 ⁴	3.9x10 ⁶

Source: Decisionalysis Risk Consultants, 2004

*Risk estimates relative to est. risk at 1 h of holding at each temp

Control measures for *E. sakazakii* in PIF



Reducing the level of contamination of the reconstituted PIF (e.g., heat) prior to use

$$H_0 - \Sigma R + \Sigma I \leq FSO$$



Reducing the concentration/prevalence of intrinsic contamination



Minimise the chance of contamination of reconstituted formula during preparation

+

Minimize the growth of *E. sakazakii* following reconstitution prior to consumption

Food Safety Objective (FSO) and Performance Objective (PO)



PO – at packaging



FSO

subtract growth

Reconstitution, Storage and/or Holding Times

Hypothetical example in establishing Performance Objective



- FSO of < 1 CFU/day
- LBW infants receive 150 mL liquid formula per kg bodyweight/day
- 0.216 g PIF / mL
- Example: 2.5 kg infant receives 81 g PIF / day and so FSO would be an absence in 81 g (-1.9 log CFU/g)

Selection and calculation of POs and other limits



Scenario 1: 12 h at 10°C; 3.3 h at RT

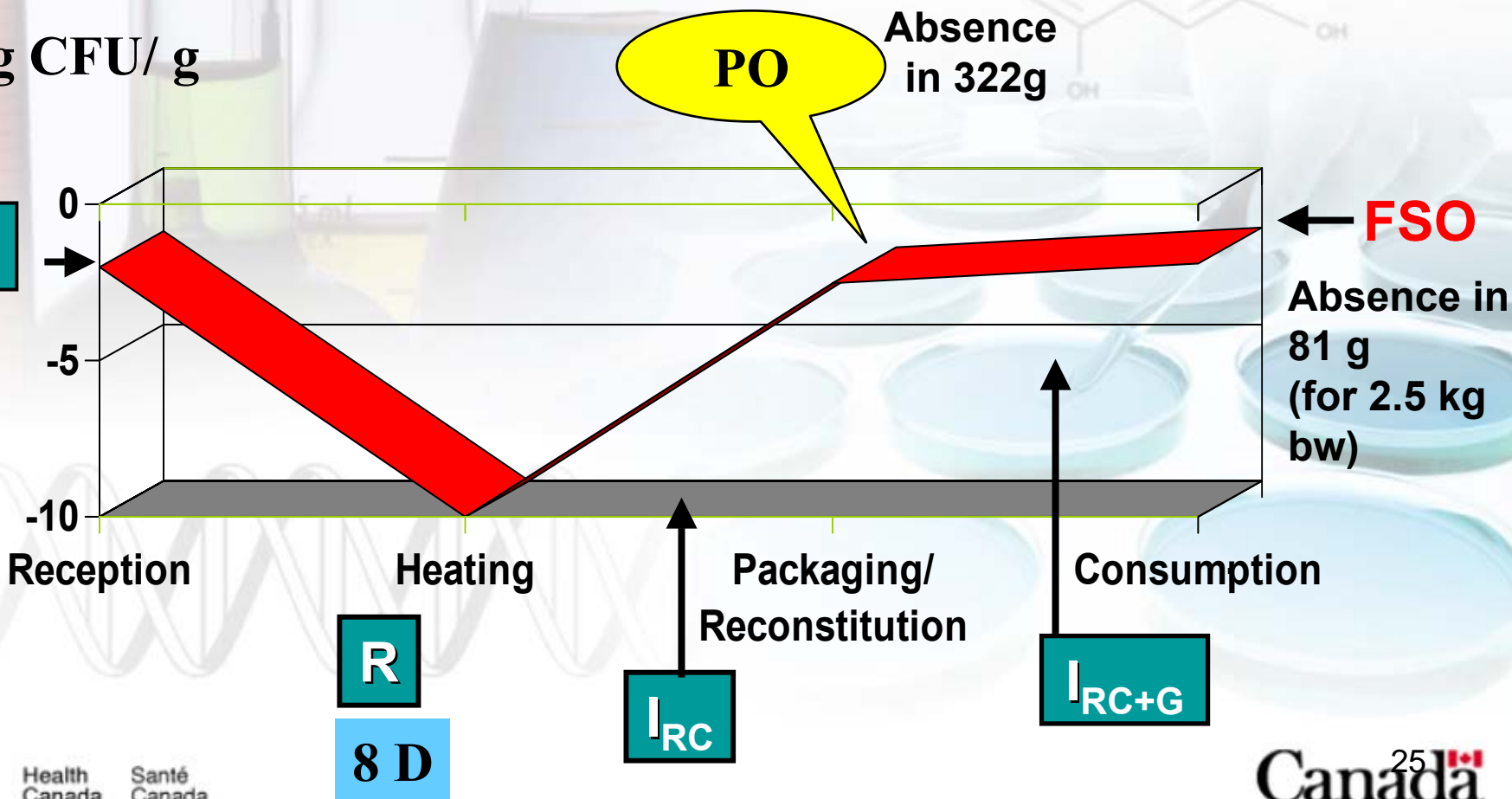
Birthweight (kg)	FSO (log cfu/g)	Predicted growth (log cfu/g)	PO (log cfu/g)	Absence in (g)
1.00	-1.51 (absence in 33g)		-2.11	129
1.50	-1.69	0.60	-2.29	193
2.00	-1.81		-2.41	258
2.50	-1.91 (absence in 81g)		-2.51	322

Product / Pathogen / Pathway of *E. sakazakii* in PIF



Log CFU/ g

H_0



Selection and calculation of POs and other limits



Scenario 2: 12 h at 10°C; 1 h at 37°C; 3.3 h at RT

Birthweight (kg)	FSO (log cfu/g)	Predicted Growth (log cfu/g)	PO (log cfu/g)	Absence in (g)
1.00	-1.51	1.51	-3.02	1048.4
1.50	-1.69		-3.20	1572.7
2.00	-1.81		-3.32	2096.9
2.50	-1.91		-3.42	2621.1

Selection and calculation of POs and other limits

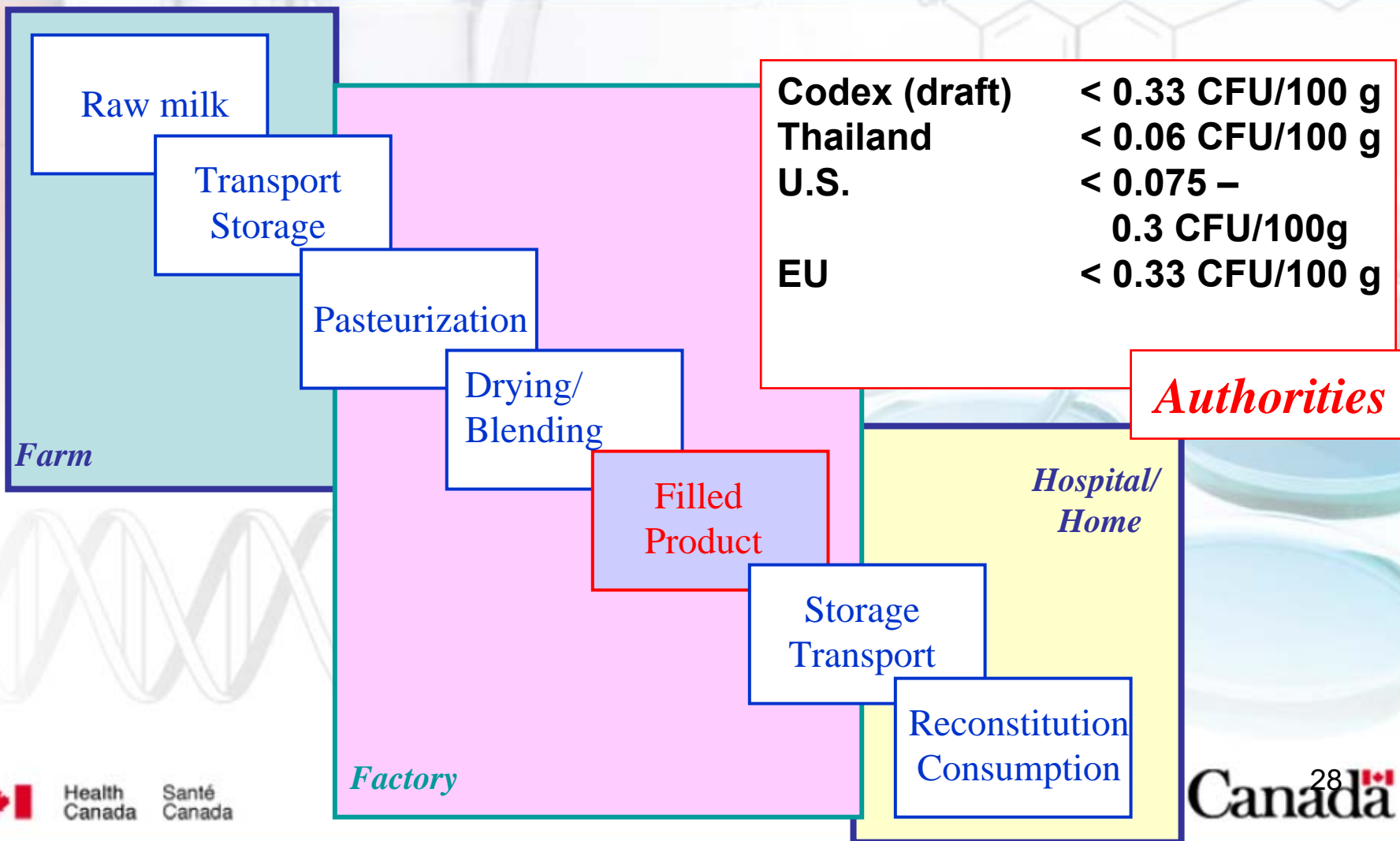


Scenario 5: 8 h at 30°C (e.g., developing country)

Birthweight (kg)	FSO (log cfu/g)	Predicted growth (log cfu/g)	PO (log cfu/g)	Absence in (kg)
1.00	-1.51	3.61	-5.12	132
1.50	-1.69		-5.30	198
2.00	-1.81		-5.42	264
2.50	-1.91		-5.52	330

Microbiological Criteria

- *E. sakazakii*

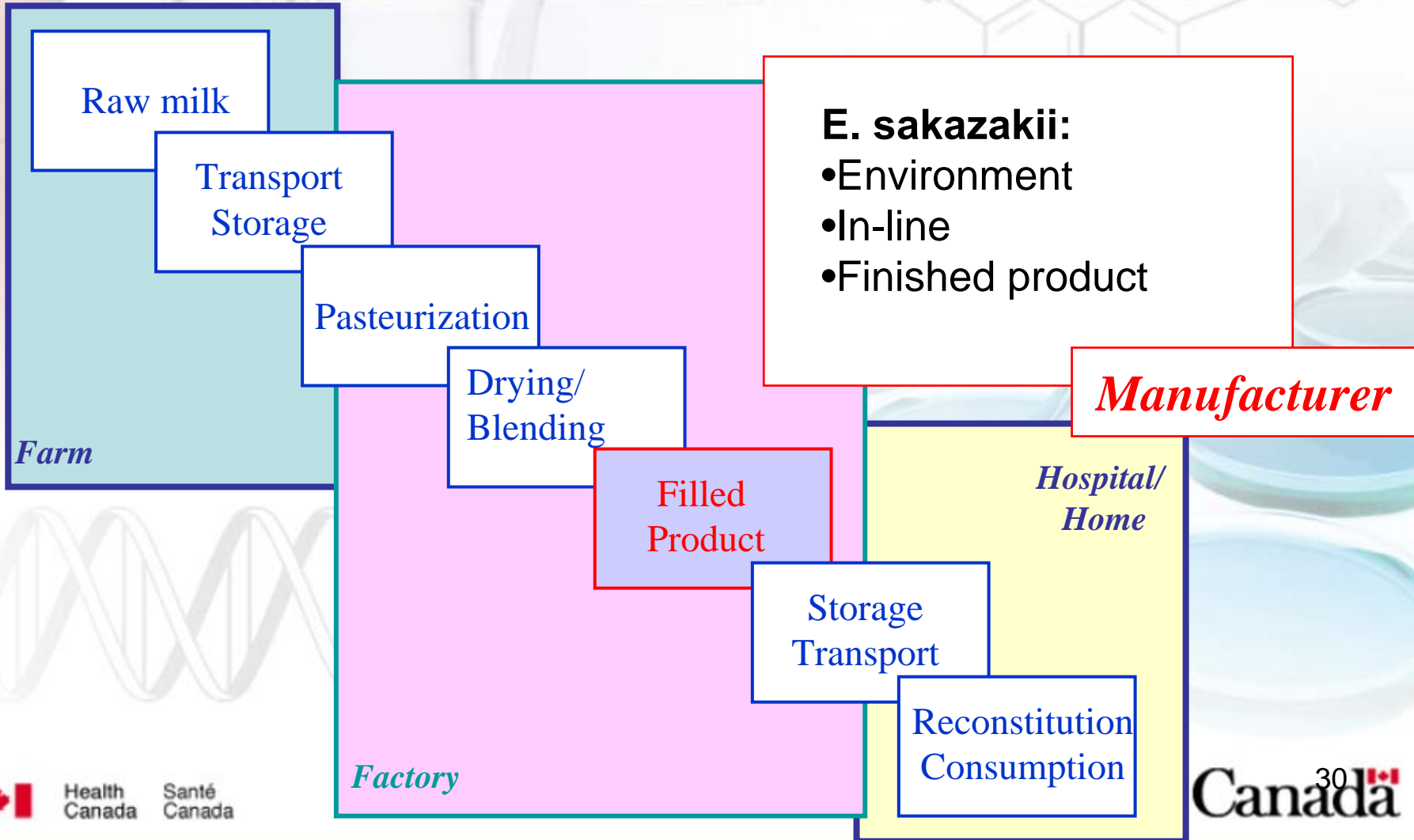


ICMSF/Codex Criteria for *E. sakazakii*

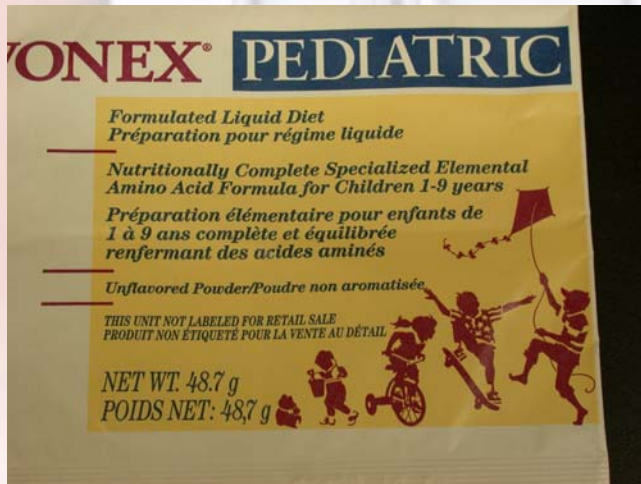


- 2-class sampling plan
- $N=30$; $c=0$; $m=0/10g$
- Assuming
 - Log normal distribution
 - SD 0.8
- Plan would have a 95% probability of rejecting a lot with a mean level of 1 cfu/112g

Microbiological Criteria



Education



- Many individuals unaware that PIF is not a sterile product
- Lack information on how handling, storage and preparation practices can influence the risk
- Effective risk communication practices needed for the public and health professionals
- Some publications are available

Summary



- *E. sakazakii* infection is a rare, but very serious disease
- More data needed on true “susceptible” populations
- More growth data needed
- Dose-response models needed to develop FSOs
- More global data needed on PIF handling, preparation and storing practices



Future Work



- **ICMSF – criteria/specifications**
- **ILSI grant to look at animal models**
- **Risk assessment (FAO/WHO)**
- **Codex – Recommended International Code of Hygienic Practices for Foods for Infants and Children (May, 2006)**



Thank you very much !



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