Thermobacteriology – Lessons Learned

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Introduction

- Focus
  - Thermal resistance of spores
  - Food applications
  - Classic D and z model
  - Taking away the “Art” in microbial inactivation measurements

Models

Model - General

$$\frac{dN}{[N]} = -k(x)dt$$

- Change in concentration [*N*] is first order
- The rate of change in *N* can be expressed by the function '*k(x)*'
- '*k*' is a function of unknown factors – (*x's*)

Model - Dependency

$$dk(T,P,pH) = \left[\frac{\partial k_T}{\partial T}\right]_{P,pH} dT + \left[\frac{\partial k_P}{\partial P}\right]_{T,pH} dP + \left[\frac{\partial k_{pH}}{\partial pH}\right]_{T,P} dpH + etc.$$

- '*k*' can be a function of many factors
- Without prior knowledge, independence must be demonstrated (pressure, temperature, pH, salt, enzymatic activity, competitive microflora, growth history of microorganisms, Aw, formulation, patch hold time)

T=temperature, P=pressure, pH=log of H^+ concentration

Model - Traditional

$$\log \frac{N_o}{N} = \frac{t}{D_T}$$

$$D_T \perp\!\!\!\perp (A_w, pH, Salt, P, etc.)$$

$$\log \frac{D_o}{D_T} = (T - T_o)/z$$

Model - Assumptions

- Validated/accurate methods used to measure D and z
- Destruction of all microorganisms can be described with the same kinetic parameters
- Surviving organisms can be counted
- Factors that affect destruction rate are held constant or are measurable (temperature - z value)
- Rate of destruction is independent of initial concentration

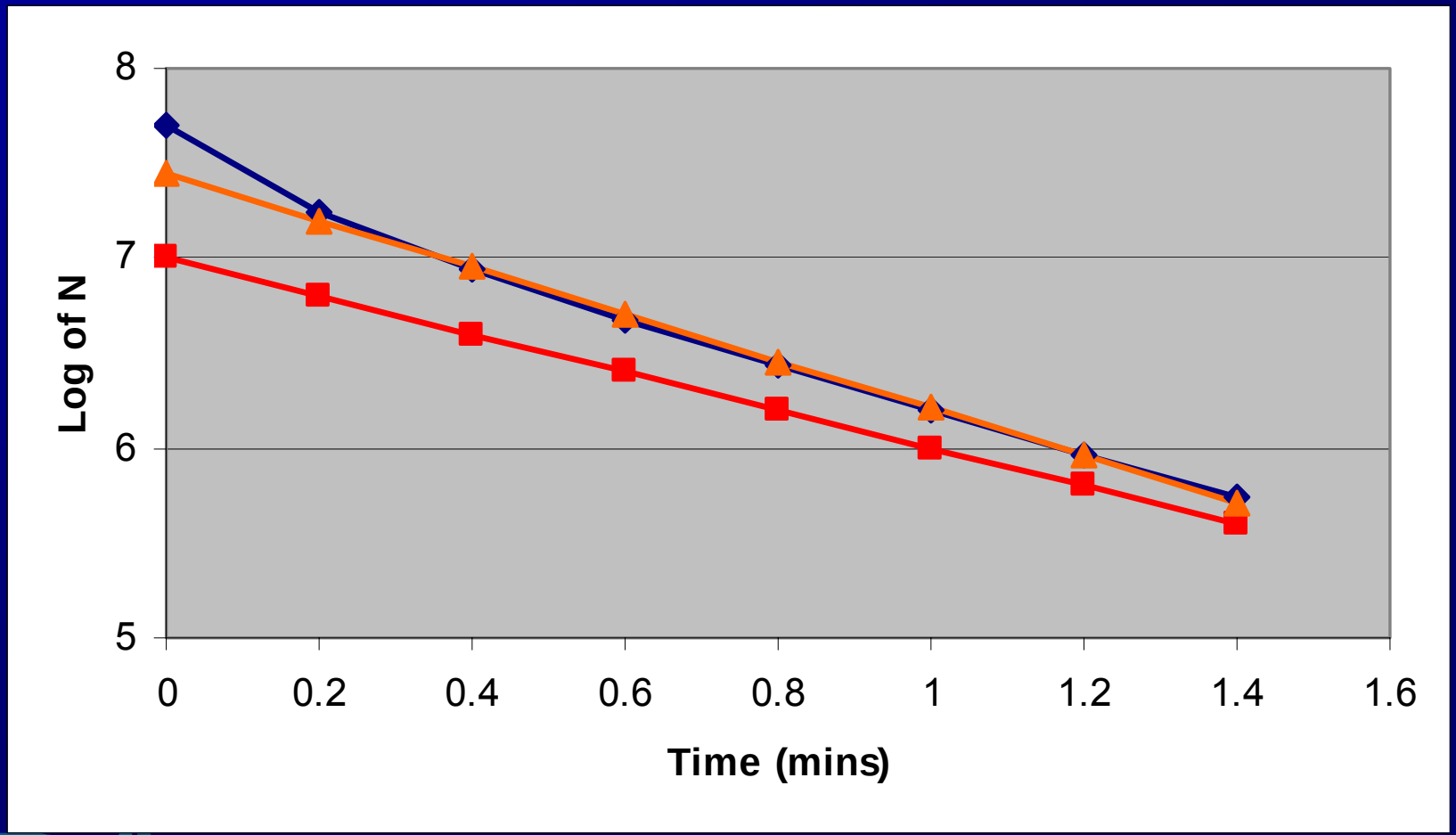
Model – Assumptions (cont'd)

- Population of microorganisms is homogeneous (crops from one spore)
- Destruction rate is first order
- Microbial counts are of individual organisms (no clumping)
- The change in rate of destruction with respect to temperature can be expressed by a constant – z
- The change in rate of destruction is unbounded (extrapolation valid?)

Measuring Process Treatment

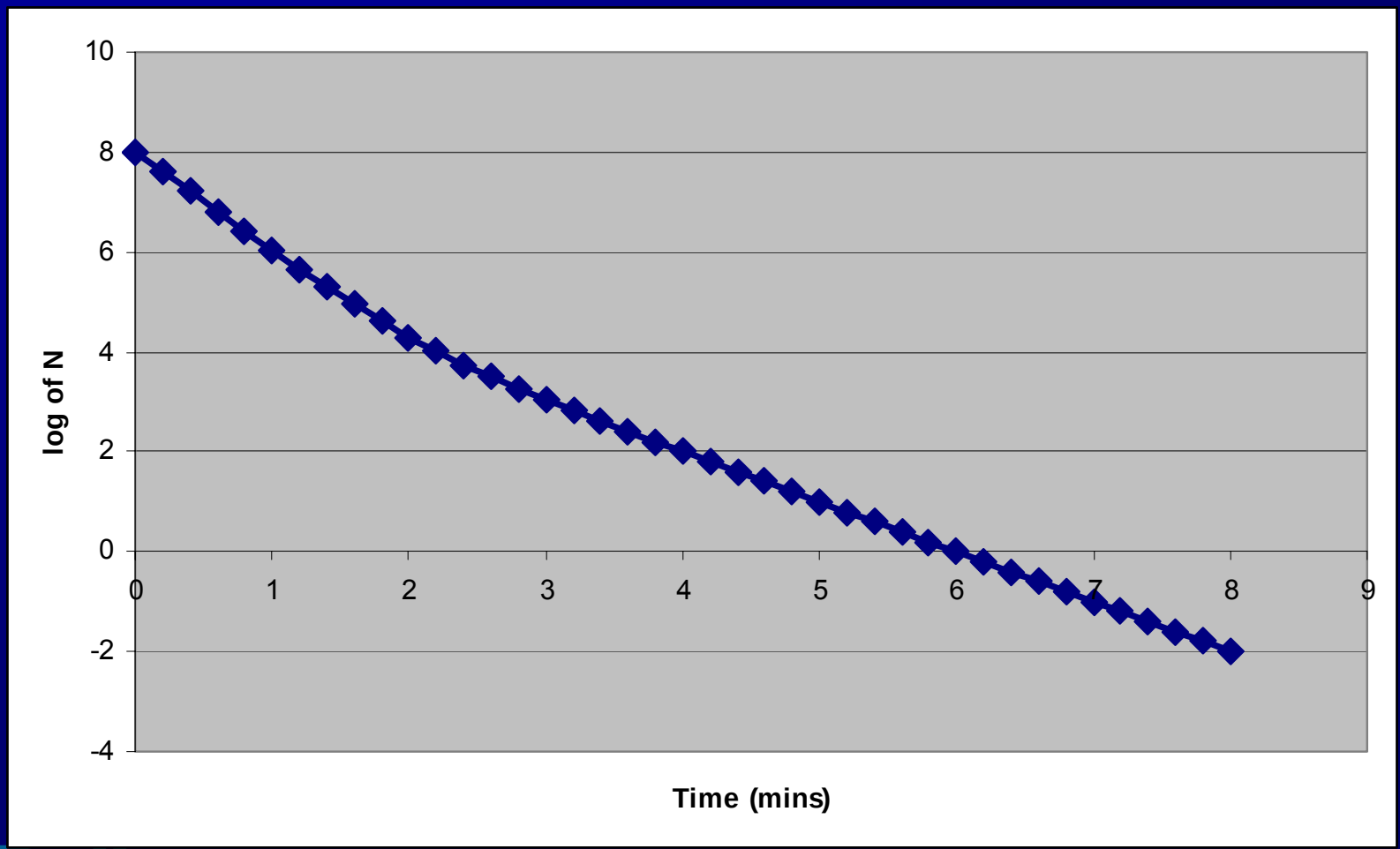
Homogenous Populations

Mixed Population – Equal Mixture(10^7) (D values – 1.0, 0.75, 0.5, 0.25, 0.1)

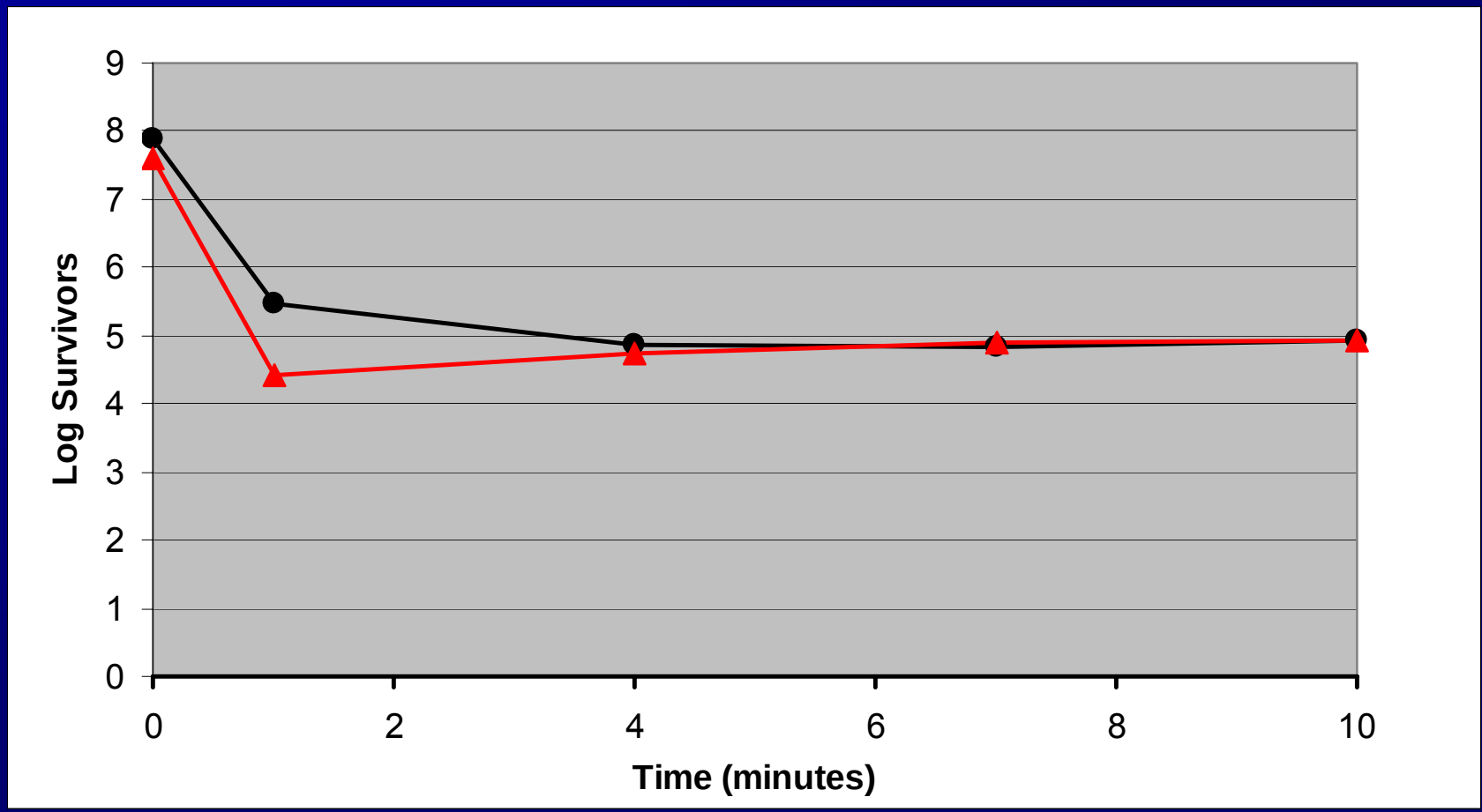


■ – D=1 min, ▲ – Calculated D=0.81, ♦ - measured counts

Mixed Population – Unequal Mixture (D values, 0.5 at 10^8 and 1.0 min at 10^5)



C. botulinum type E Beluga *spores* *Temperature at 75°C (duplicates)*



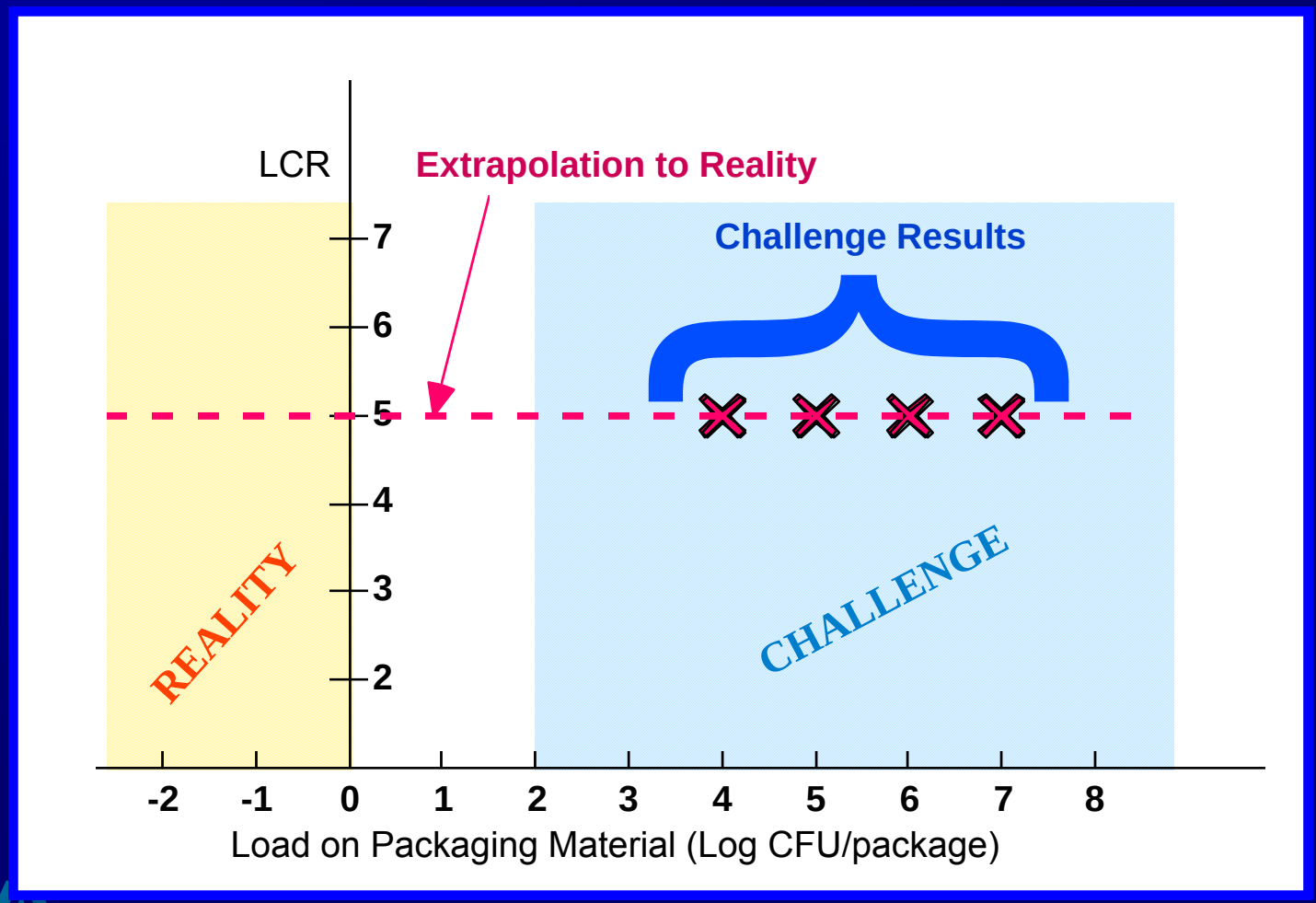
Effect of Population Load

- Independence between dose and treatment
- Halvorson, H. 0. and Ziegler, N. R. equation; Stumbo, Murphy, Cochran method; MPN assumptions

$$D_T = \frac{t}{\log(N_o) - \log(N_t)}; \; N_t = \ln\left(\frac{n}{r}\right)$$

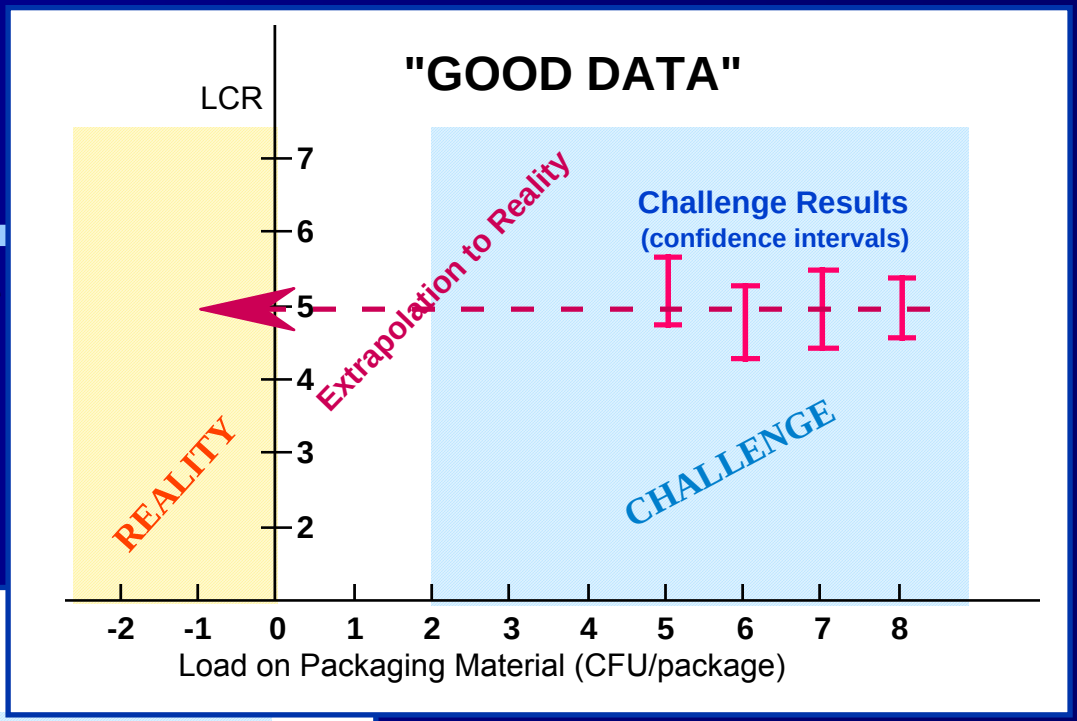
$$n = total \;\; tested, \; r = negatives$$

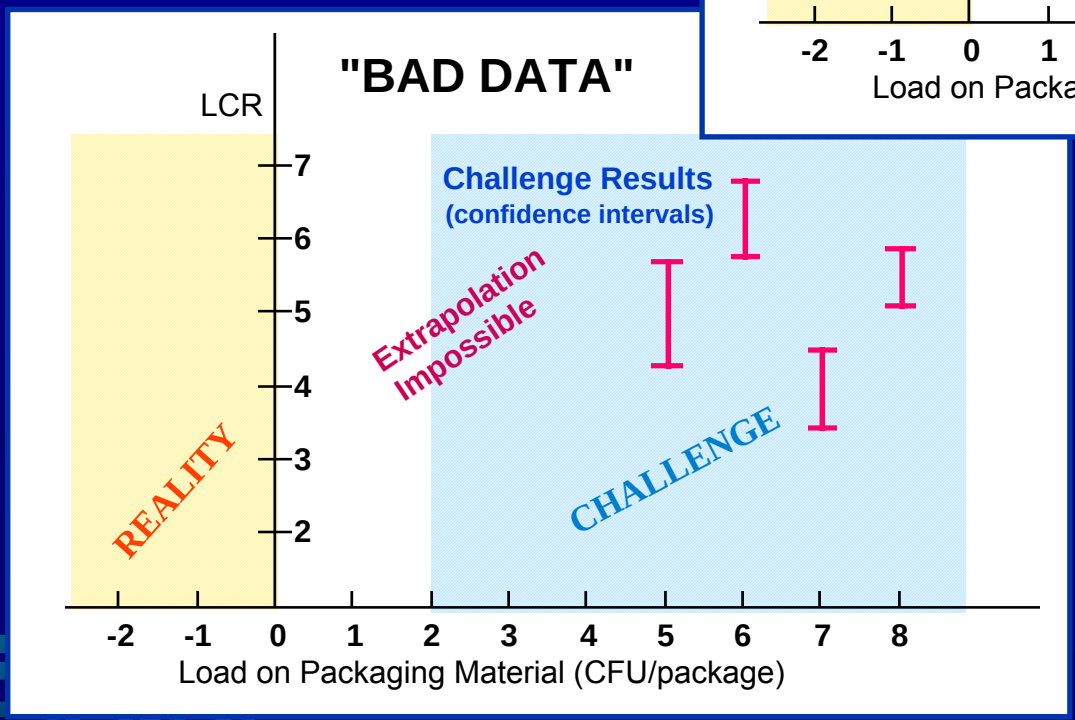
Log Count Reduction (LCR) as a Property of the Challenge Level*



* Moruzzi et al., Food Control, 2000, page 57

LCR at different challenge levels is constant; LCR is a property of the process, and extrapolation to "reality" can be tried





LCR at different challenge levels is not constant; extrapolation to "reality" is impossible

* **Moruzzi et al., Food Control, 2000, page 57**

Underlying MPN Assumptions

W.G. Cochran (1950) - "There are two principle assumptions."

- "The first is that the organisms are distributed randomly throughout the sample…the organism level is equally likely to be the same in each sample.“ – equal treatments
- "The second assumption is that each sample when incubated is certain to exhibit growth whenever the sample contains one or more organisms."

Measuring Process Treatment

- Calibrated/Self-contained BI's (D/z determined – as part of the vial)
- Process extinguishing (temperature, catalase)

Data Handling/ Experimental Procedures

Making Sure Correct Calculations are Used

- (F and C) degrees
- Reasonable results?
- Validated software
- Correct units for complete equation
- Peer review calculations (Ball and Olson book equation error)

Data Handling and Experimental Procedures

- Proper analytical procedures
  - Equipment calibration
  - Recovery – test media
  - Growth promotion
  - Replicates – samples, treatments
- Culture preparation

Data Handling and Experimental Procedures

- Sample enumeration
  - Initial count (assayed/recovered)
  - Positive identification (is the positive the one you put in there?)
  - Positive control – were the organisms in the sample; positive not from effect of treatment
  - Negative control – background contamination, aseptic technique
  - Effect of enumeration procedures and recovery media (kill artifact)

Summary

- Know your destruction rate model
- Confirm your analytical and mathematical techniques (validation)
- Ask fulminous questions about the procedures and results – make sure each step is conducted correctly