



International Association for  
**Food Protection**®

**WEBINAR**

**PRACTICAL APPLICATIONS  
OF MICROBIAL MODELING -  
WEBINAR SERIES**

November 29, 2017

10:00 a.m. EST

# Practical Applications of Microbial Modeling Webinar Series

**Webinar Series:**  
**Part I of III**

- This IAFP webinar is sponsored by the following Professional Development Groups:
  - ▣ Microbial Modelling and Risk Analysis
  - ▣ Meat and Poultry Safety and Quality

# Practical Applications of Microbial Modeling

**Webinar Series: Part I of III**



International Association for  
**Food Protection**<sup>®</sup>  
**WEBINAR**

# Dr. Betsy Booren



Senior Policy Advisor  
Olsson, Frank, Weeda,  
Terman, and Matz PC  
Washington, DC

# WEBINAR HOUSEKEEPING

For best viewing of the presentation material, please click on 'maximize' in the upper right corner of the 'Slide' window, then 'restore' to return to normal view.

Audio is being transmitted over the computer so please have your speakers 'on' and volume turned up in order to hear. A telephone connection is not available.

Questions should be submitted to the presenters during the presentation via the **Q & A section** at the right of the screen

# WEBINAR HOUSEKEEPING

It is important to note that all opinions and statements are those of the individual making the presentation and not necessarily the opinion or view of IAFP

This webinar is being recorded and will be available for access by IAFP members at [www.foodprotection.org](http://www.foodprotection.org) within one week.

# Agenda

- Introduction
  - ▣ Dr. Betsy Booren
- Overview of Predictive Microbial Modeling
  - ▣ Dr. Tom Ross
- Tertiary Models for Estimation of Microbial Behavior in Real Situations – Meat Products
  - ▣ Dr. Peter Taormina
- Questions and Answers

# Dr. Tom Ross

Director  
ARC Industrial  
Transformations  
Training Centre for  
Innovative Horticultural  
Products  
University of Tasmania  
Tasmania, Australia





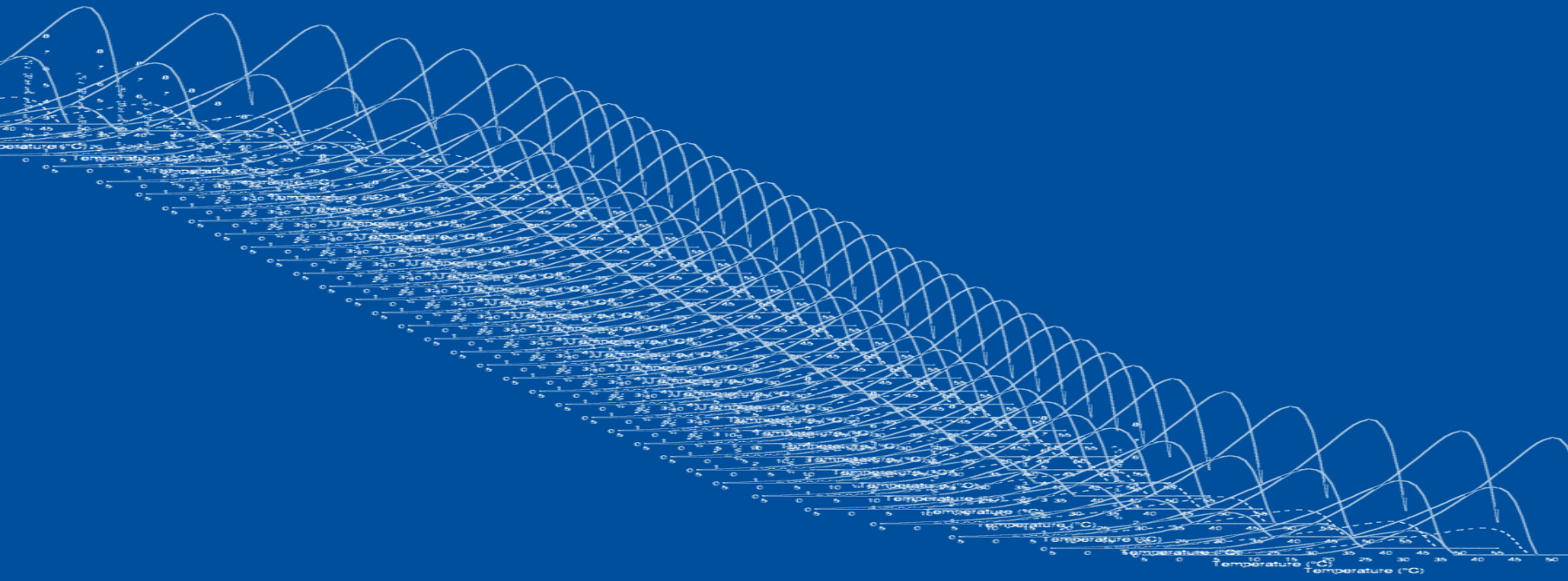
# Dr. Peter Taormina



President  
Etna Consulting Group  
Cincinnati, OH

# Overview of Predictive Microbial Modeling

Assoc Prof Tom Ross



# Predictive Microbiology



- The ***quantitative*** microbial ecology of foods

# Overview



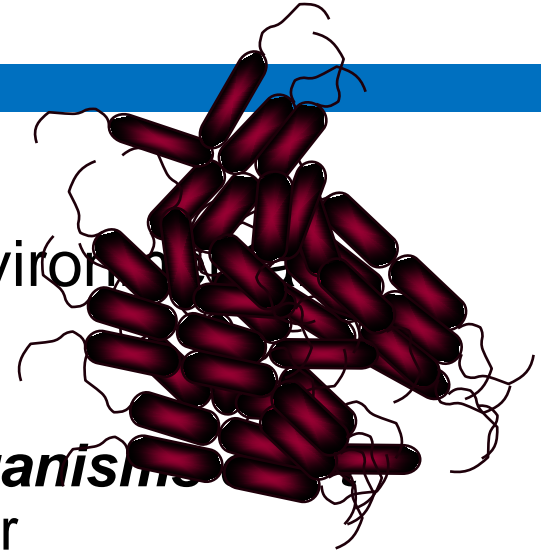
- Basic concept of predictive microbiology
- Basic mathematical ideas
- Kinds of microbial responses that can be predicted
- Current status of predictive microbiology
- Building models
- How can predictive microbiology models help the food industry
- Critically assessing model applicability and reliability

# Predictive Microbiology – basic ideas

- microorganisms react reproducibly to environmental conditions
  - ▣ the fundamental premise is that **microorganisms think**, so that they behave reproducibly (or “predictably”) in ways dictated by their environment.

*thus*

- ▣ if we can measure their environment, we can predict what they will do, and how quickly they will do it.





do *you* all believe this?

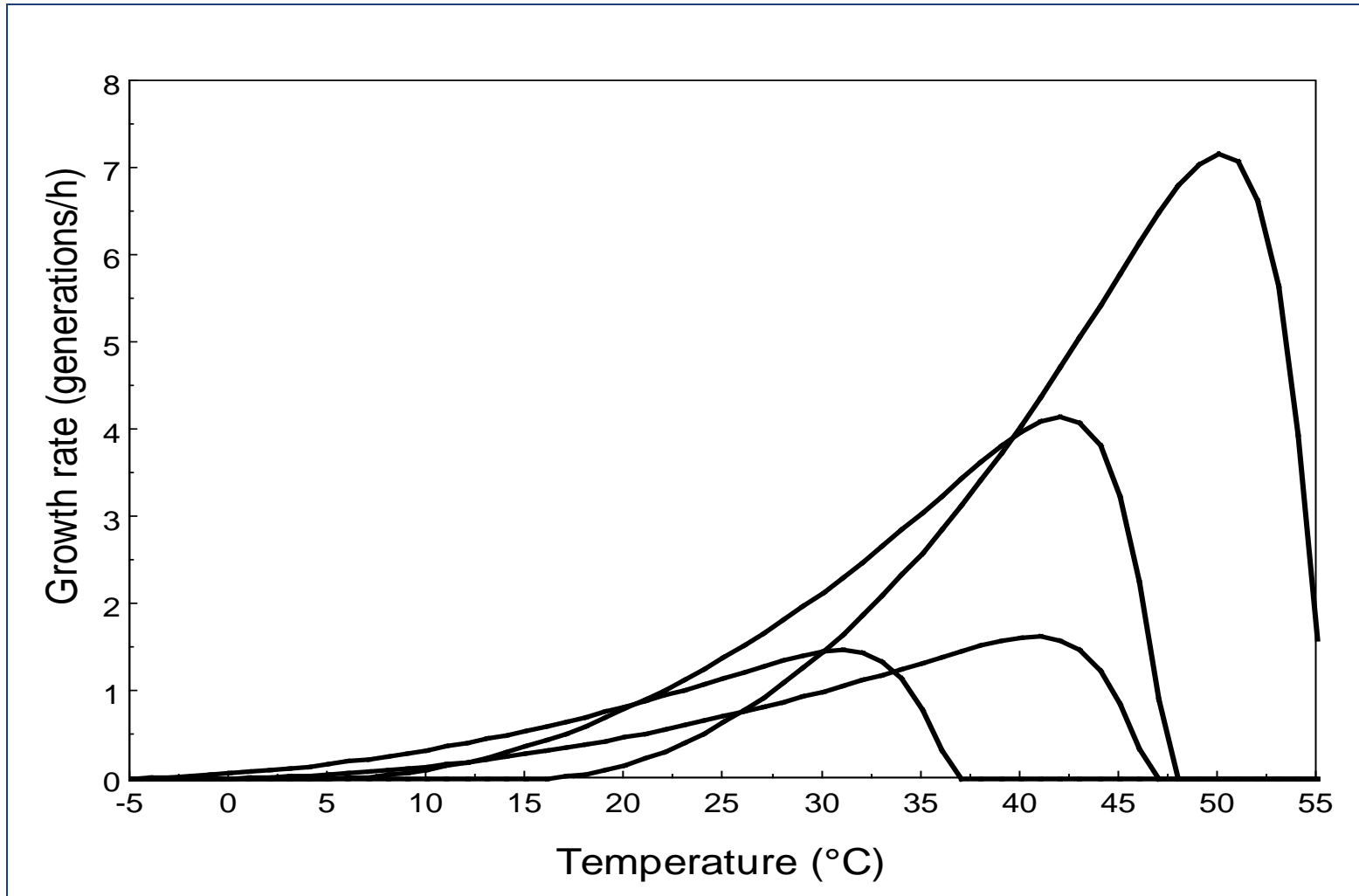
# Modern Predictive Microbiology

*“the growth responses of the microbes of concern would be modelled with respect to the main controlling factors such as temperature, pH and  $a_w$  ...*

*... Models relevant to broad categories of foods would greatly reduce the need for ad hoc microbiological examination and enable predictions of quality and safety to be made speedily with considerable financial benefit.”*

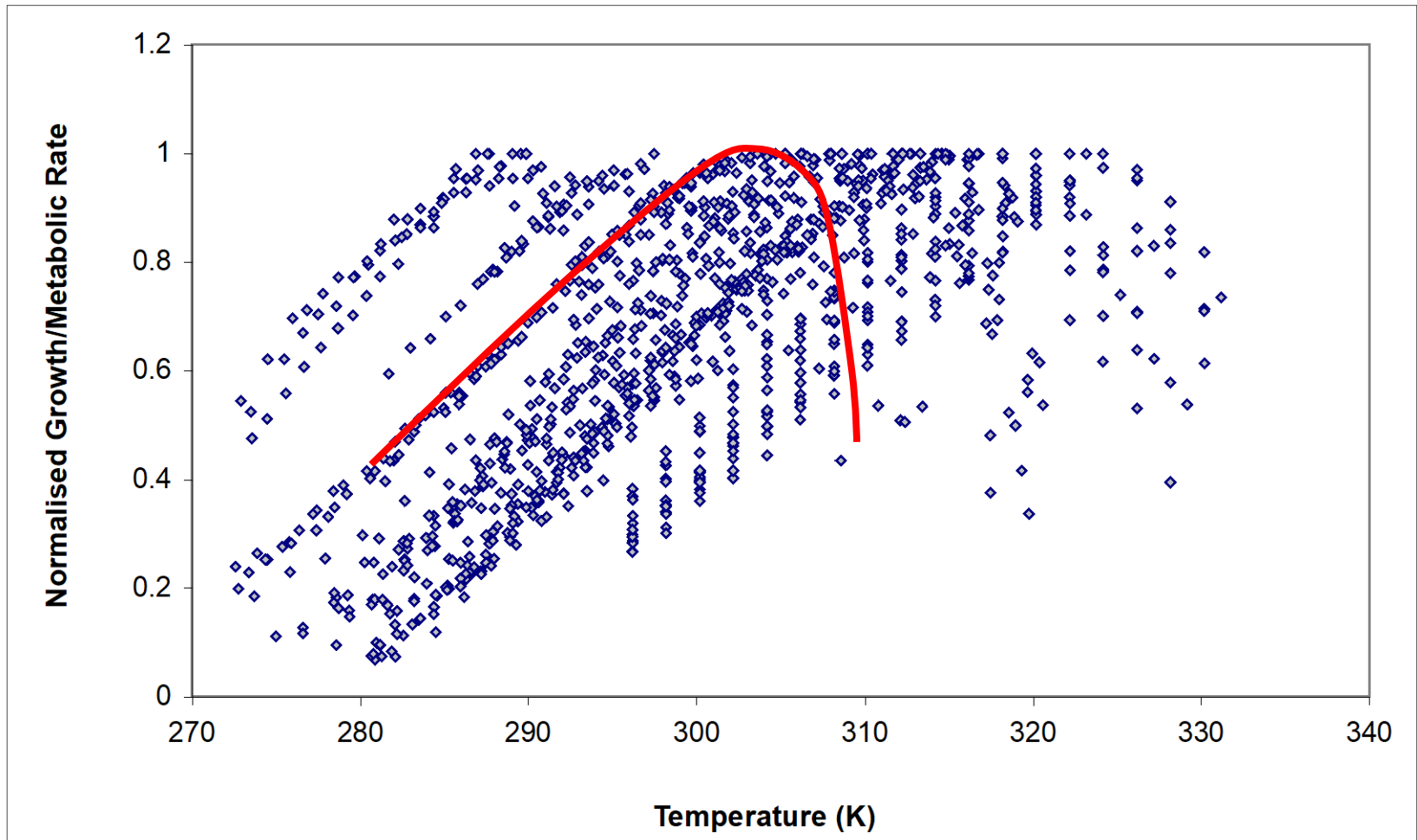
Roberts and Jarvis (1983; *Food Microbiology: Advances and Prospects*, Academic Press, New York, NY)

# Consistent patterns of response: *the temperature-growth rate relationship*





# Universality of the temperature-growth rate relationship



# Predictive Microbiology - concepts

- there is a small number of environmental factors of main importance, namely:
  - ▣ *temperature*
  - ▣ *pH*
  - ▣ *water activity*
- for some foods this works, for processed foods its probably an oversimplification, so
  - ▣ *increasingly, as we adopt 'hurdle technology', other factors need to be considered explicitly: e.g., organic acid type and level, nitrite, gaseous atmosphere, smoke compounds, other microbes in the food, etc.*

# Predictive Microbiology - concepts

- main controlling factors - ***microbial death***

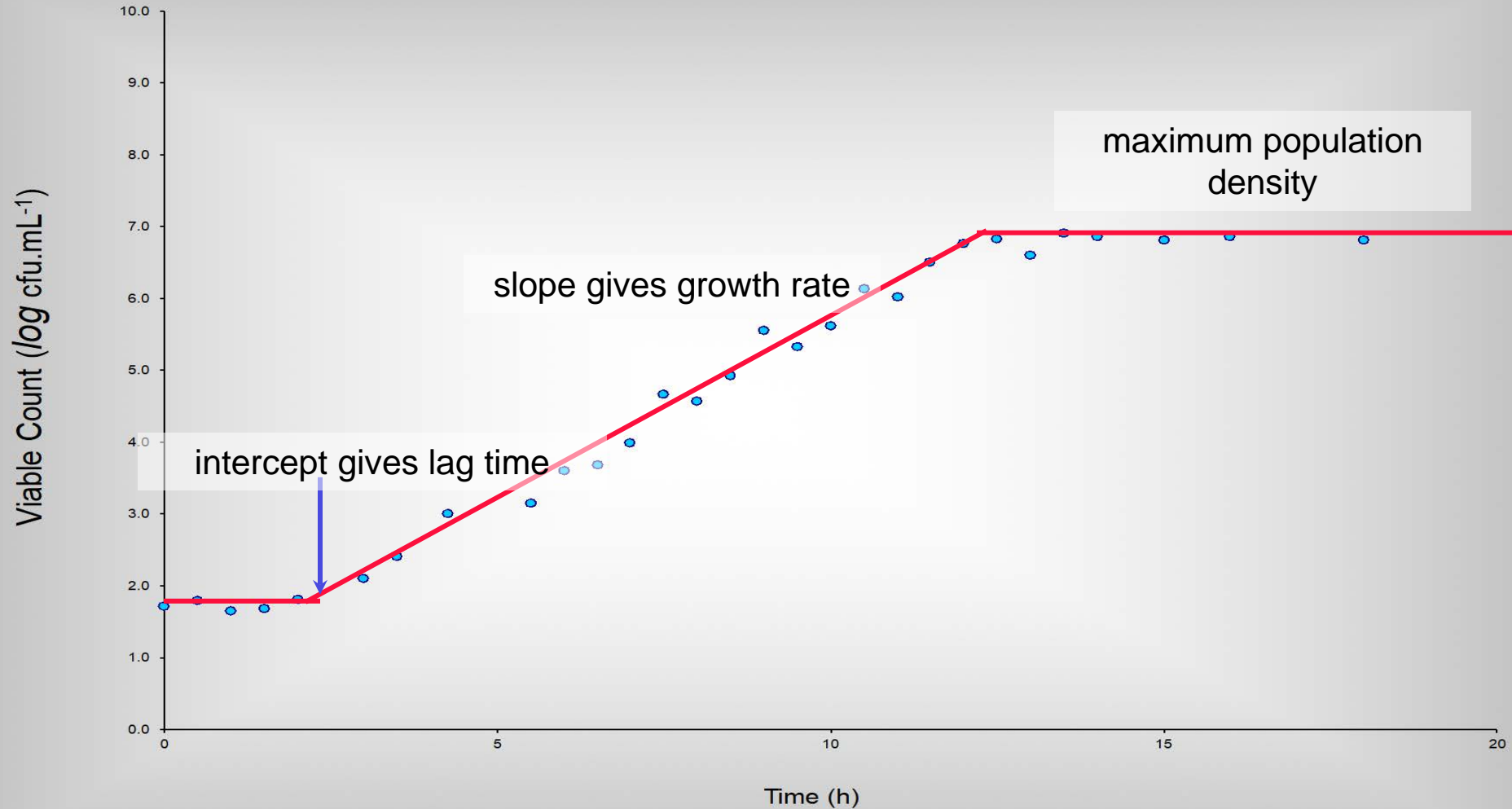
its also assumed that *death rate* is affected by physicochemical conditions in the food, but normally death rate is most strongly governed by the treatment, *e.g.:*

- *temperature*
- *pressure*
- *irradiation (UV, gamma etc.)*
- *electric field strength*

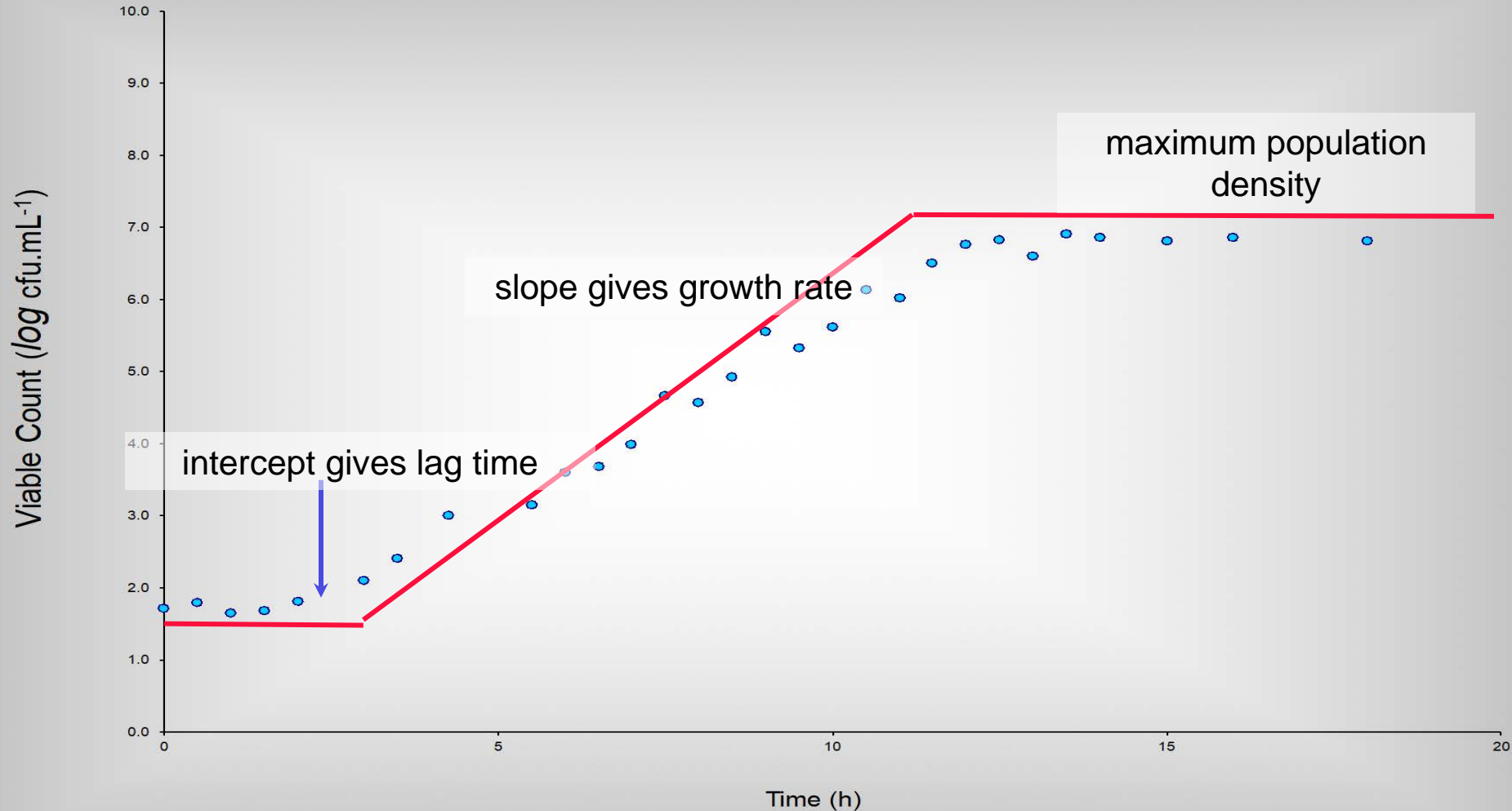
# Basic mathematical ideas



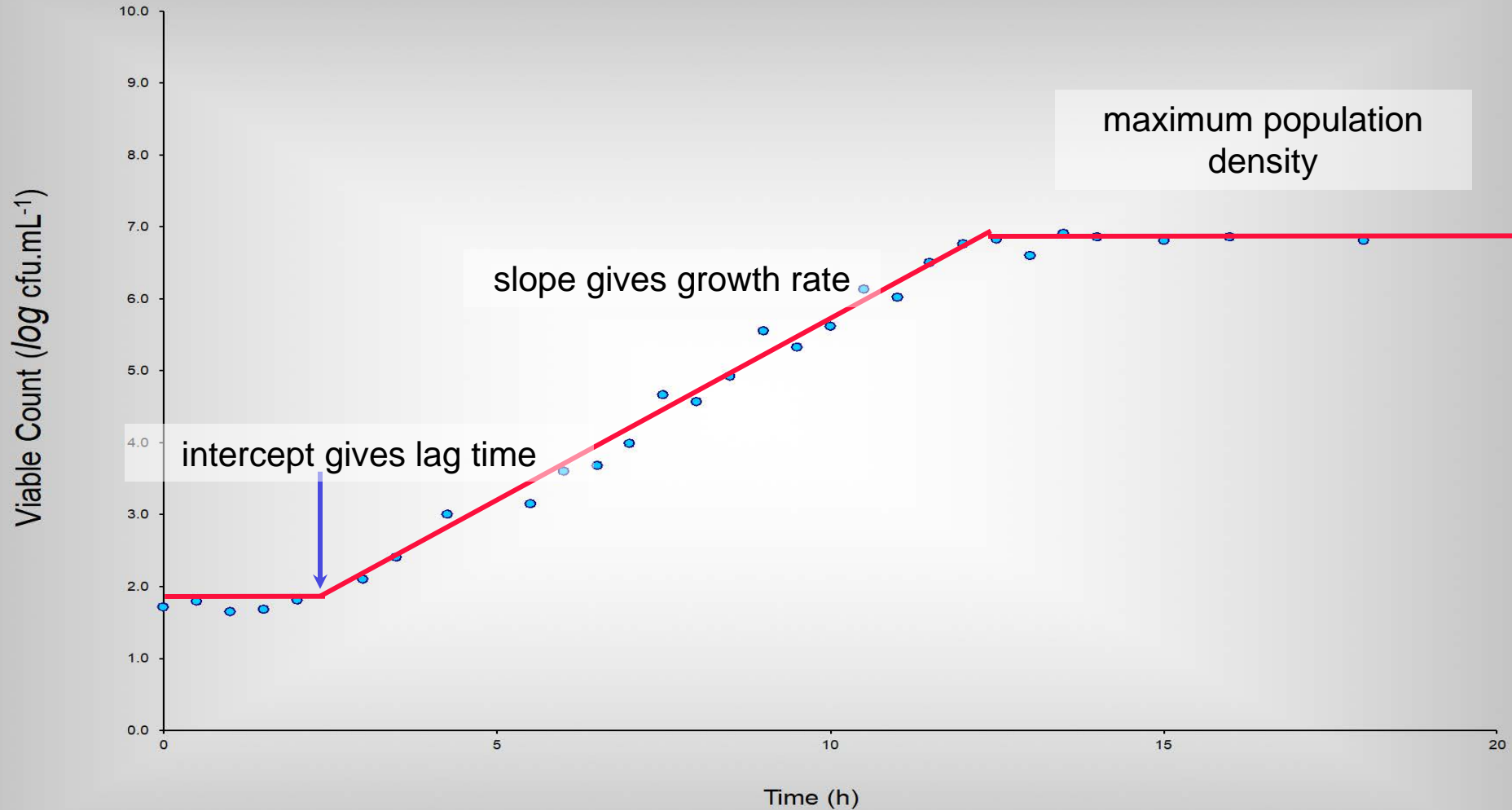
# Summarising the microbial growth curve



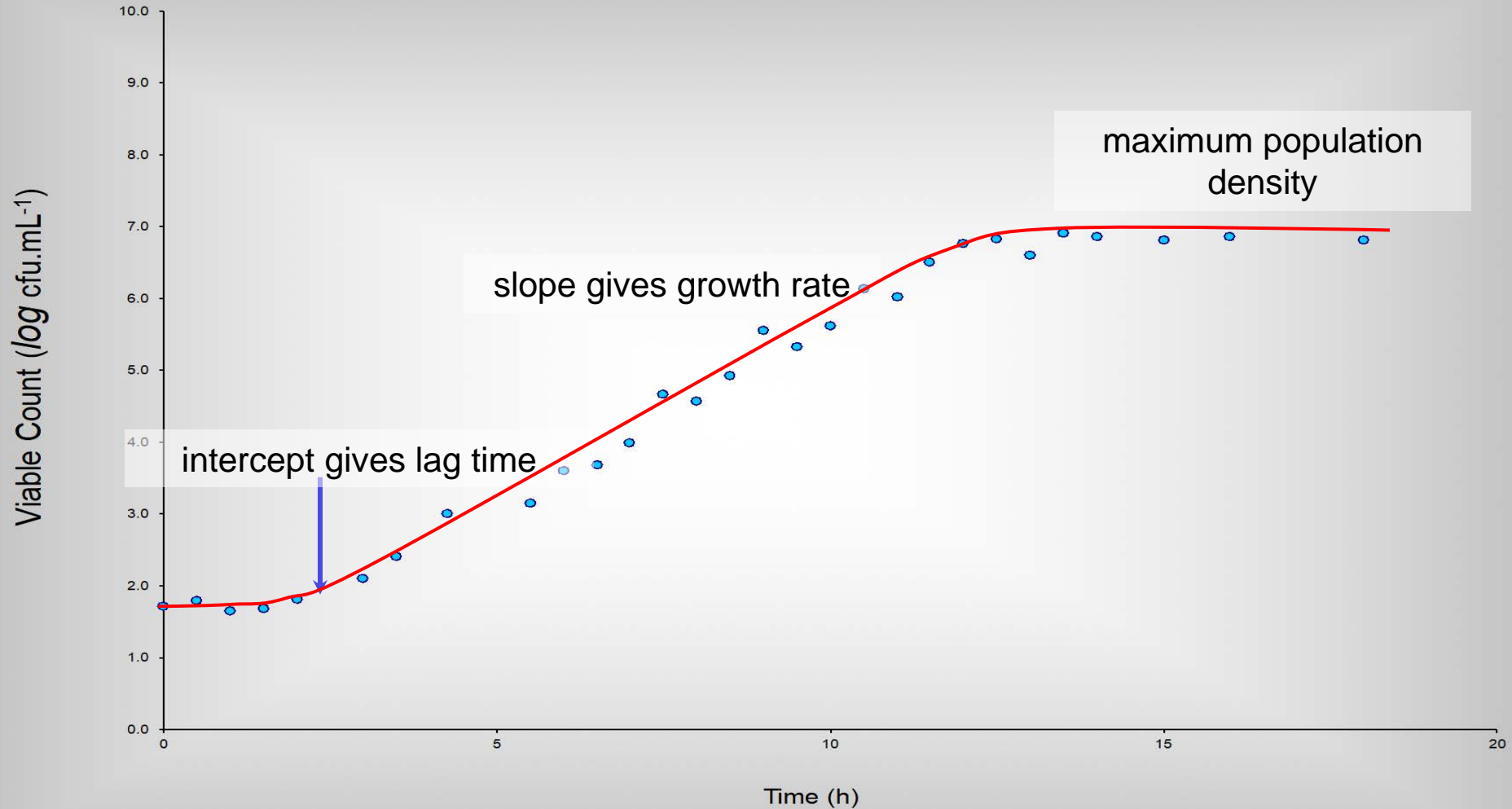
# Summarising the microbial growth curve



# Summarising the microbial growth curve

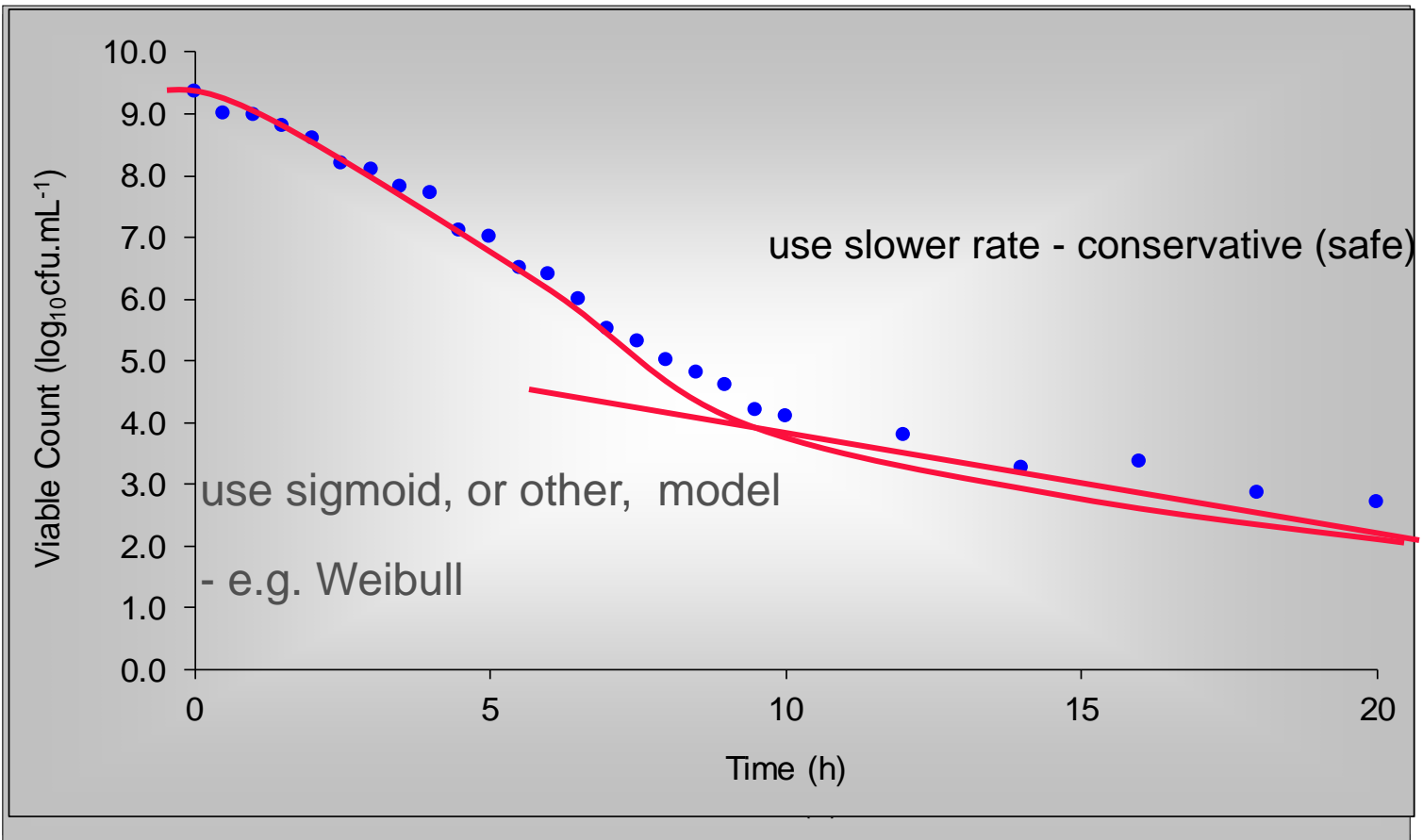


# Summarising the microbial growth curve





# Inactivation modelling



# Thermal inactivation kinetics: summary

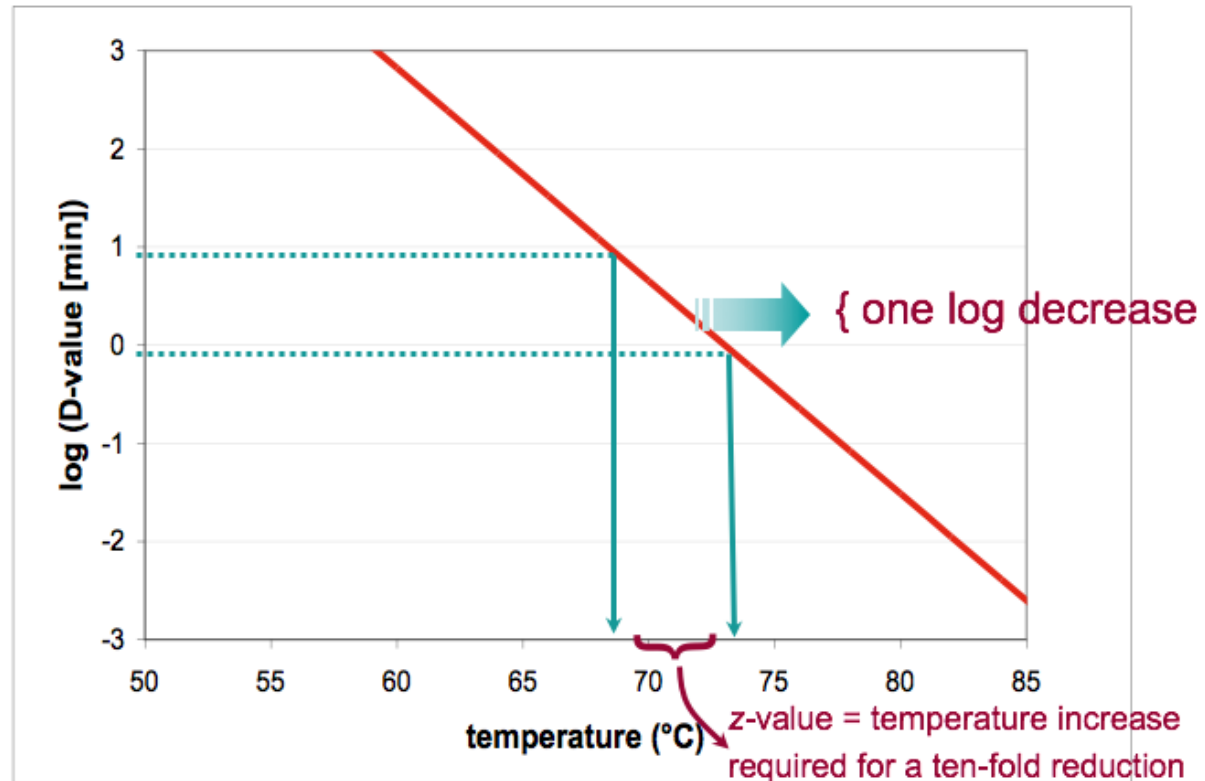
## D-value

time required at given temperature to reduce microbial load by a factor of 10

## z-value

temperature increase required to reduce D-value by a factor of 10

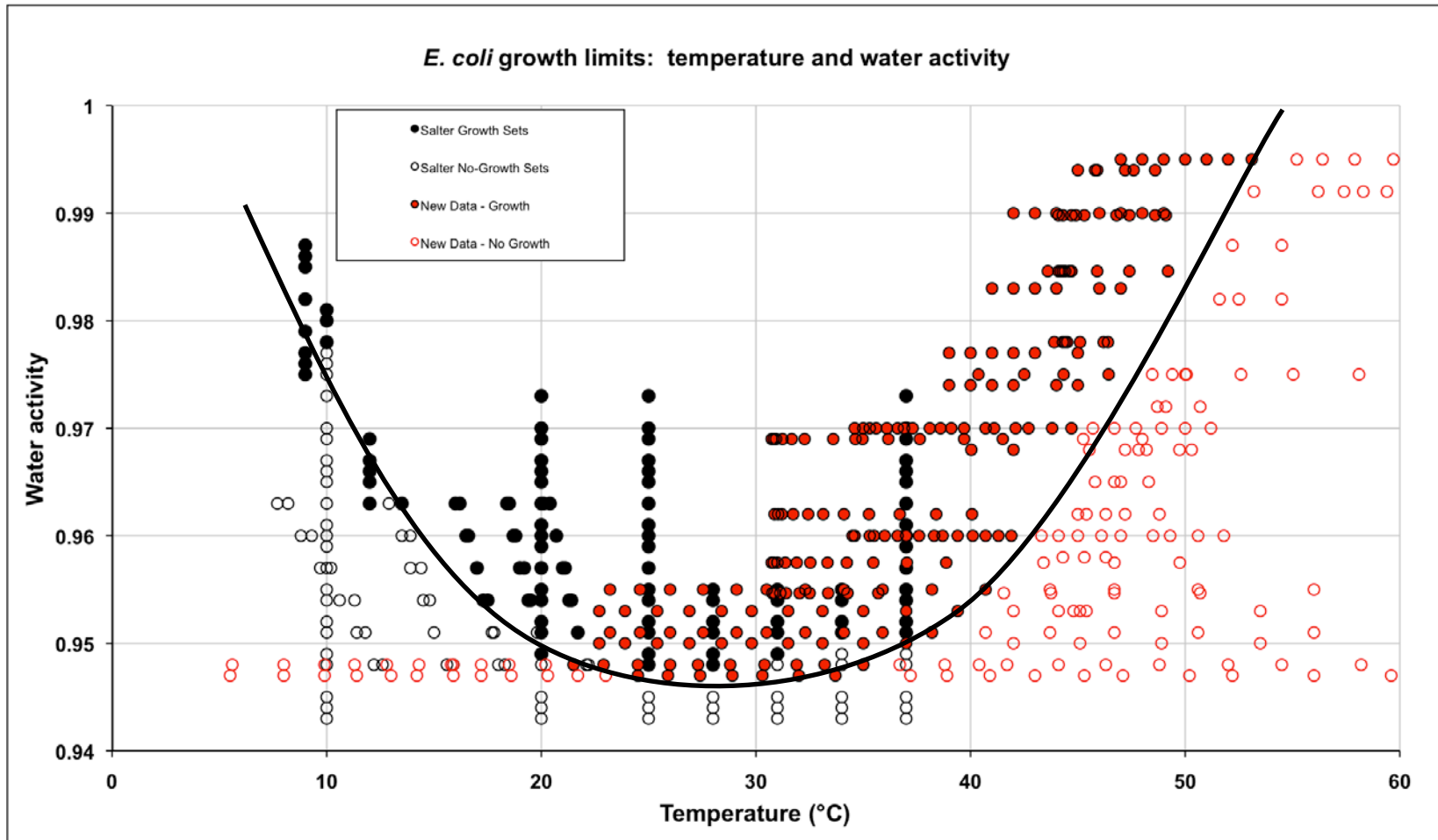
Analogous terms ( $D_p$ ,  $Z_p$ ,  $Z_{pH}$ ) proposed for other lethal factors



# What else can (or would) we 'predict'

- amount of microbial **growth** after time, (from temperature and product formulation; includes lag time, growth rate, germination and outgrowth of spores)
- **reduction** in microbial numbers over time, from knowledge of treatment conditions and product formulations (includes delay, death rate)
- **probability of growth/toxin** production
  - stability of foods (absolute or within defined time)
- **sporulation**
- **recontamination/cross-contamination processes**

# Interaction of $a_w$ and temperature on *growth limits* of *E. coli*



Data of Salter *et al.* (2000) for *E. coli* (augmented 2002)

# What is modelled?

- growth rates
  - ▣ *bacteria*
  - ▣ *yeasts and molds*
- inactivation (death) rates
  - ▣ *bacteria*
  - ▣ *yeasts and molds*
  - ▣ *viruses*
  - ▣ *protozoa*
  - ▣ *microbial toxins?*
- probability of growth/toxin formation
  - ▣ *bacteria*
  - ▣ *yeasts and molds*
  - ▣ *micro-algae\**

# Current status of Predictive Microbiology ...

- PM approach is now firmly established:
  - in the scientific literature
  - in industry for HACCP planning, product/process design
  - supports food safety risk assessment and “Science-based” risk management decisions
  - is used in setting government regulations/laws
    - e.g., Codex (and (EFSA) regulations for *Listeria monocytogenes* in RTE foods
    - e.g., *C. perfringens* model for meat cooling (USDA)
    - e.g., *E. coli* growth in raw meats (NZ and Australia)

# How models are 'built'

- based on measurements of changes in microbial numbers over time and environmental conditions
- can be from
  - ▣ *deliberately designed de novo studies*
  - ▣ *“data mining”*
  - ▣ *studies in broths, or in foods*
    - *n.b.*, assumed that the actual food is less important than the physico-chemical properties of the environment (*i.e.*, the food and its storage conditions), so long as basic nutritional needs are met, *i.e.*, nutrients are non-limiting
    - Sometimes a model is developed *for just one food*

# How models are 'built'

- data are analysed and patterns of response are identified
- these patterns are expressed in the form of mathematical relationships
- the relationships are turned into equations by finding the best values of the parameters to describe individual sets of data, *i.e.*, specific to a particular organism - this is the process of 'model fitting'
- ***performance of the model is then evaluated*** and, if necessary, the model revised or new models constructed



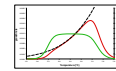
# Predictive Microbiology modelling



- systematically make/collate lots of observations
- summarise the data as mathematical equations

# Mathematical descriptions of temperature-growth rate relationship

- 'mechanistic model'



$$rate = \frac{CT \exp(\Delta H^\ddagger / RT)}{1 + \exp(-n(\Delta H^* - T\Delta S^* + \Delta C_p[(T - T^*_H) - T \ln(T/T^*_S)]) / RT)}$$

- square root model ("empirical")

$$\sqrt{rate} = b \times (T - T_{min}) \times (1 - e^{(c \times (T - T_{max}))})$$

# Predictive Microbiology models

- systematically make/collate lots of observations
- summarise the data as mathematical equations
- convert into computer software, spreadsheets
- users enter data/numbers in, get answers out

# 'Nomenclature' of models

- *Primary model*
  - describes the response of microbial numbers over time (e.g., inactivation curve, growth curve, etc.)
- *Secondary model*
  - describes how outputs of the primary model (death *rate*, growth *rate*, combined limits to growth) are affected by environmental conditions (*i.e.*, quantifies microbial ecology)
- *Tertiary model*
  - makes the knowledge and data contained in the primary and secondary models available for prediction via an accessible software interface (software program, web-site, 'app')

Welcome to the

# Refrigeration Index Calculator

Version 2.0.1896.19881



Paste temperature data here:

	A	
13	23.7	
14	22.3	
15	20.9	
16	19.8	
17	18.8	
18	17.7	
19	16.7	
20	15.6	
21	15.4	
22	13.5	
23	12.8	
24	11.7	
25	10.6	
26	9.9	
27	8.6	
28	8	
29	6.9	
30	6.2	
31	5.4	
32	4.6	
33		

Select the product type:

- Carcase
- Boxed Trim
- Primal where the slowest cooling point is lean
- Primal where the slowest cooling point is fat OR a mixture OR you're not sure
- Offal
- Recovered meat products

The starting temperature is hot (as for initial cooling of a carcass):

- Yes
- No

Specify other parameters and information:

Temperature measurement interval:  min

Date of data collection:

Description of product, processing conditions, etc.:



Welcome to the

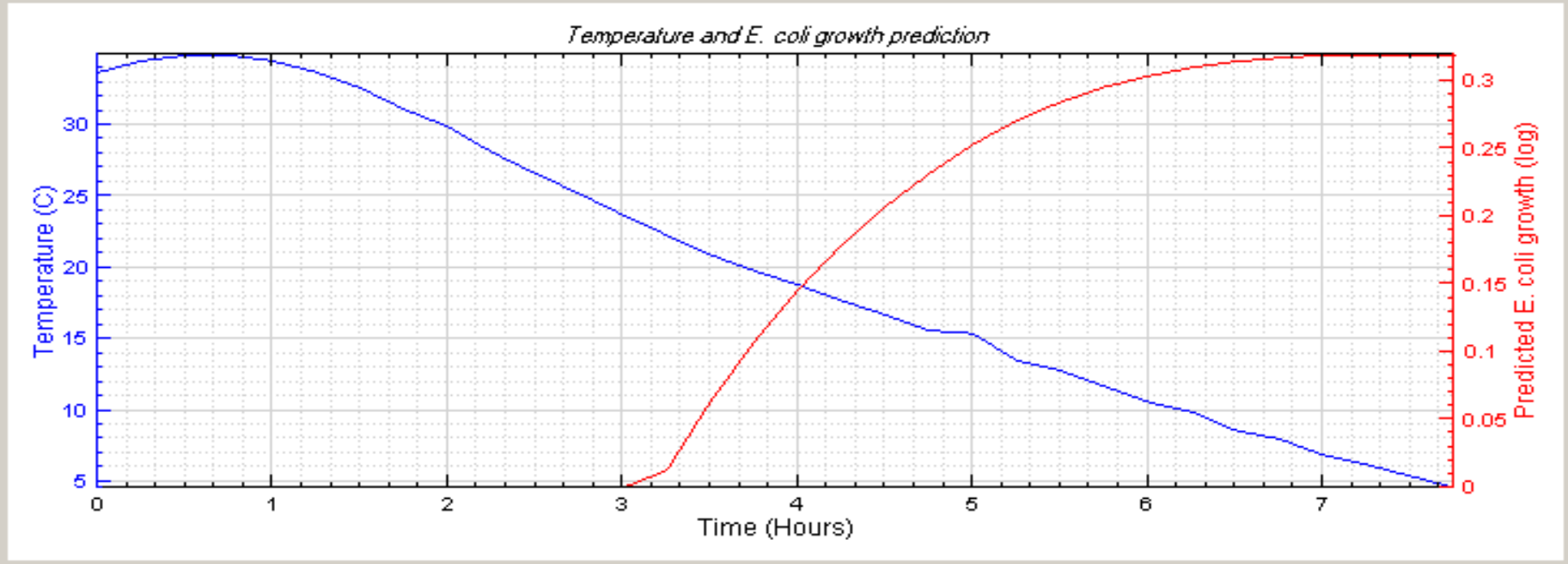
# Refrigeration Index Calculator

Version 2.0.1896.19881



The refrigeration index is: **0.32**  
Date of data collection: 15/03/2005  
Type of product: Primal (lean)  
Lag phase utilized: Yes

Description:



Save

Print

Previous









Finish

Close



Sym'Previus is a complete tool for microbiological data prediction. It helps food manufacturers, from international groups to SMB, in their product development. Recognized by the scientific community and regulations, it provides a guarantee in quality and food safety. Thanks to the expertise provided by its partners and to its ease of use, Sym'Previus supplies its users with personalized results, adapted to their industrial issues.

### Sym'Previus in brief

- 
 Multi-matrix, multi-strain, multi-process tool
- 
 A network composed of industrial and technical centres bringing their expertise
- 
 A user-friendly interface and dynamic graphics allow for quick analysis
- 
 New product formulation and development assistance
- 
 Recommended and recognised by regulation (ISO WG19, EC 2017/2005)
- 
 Its use allows increased result relevancy and so reduces costs
- 
 Over 40 microorganisms available and the possibility of adding customised strains
- 
 The Sym'Previus network of experts can provide made-to-measure solutions

# Applications of Predictive Microbiology

- proactive
  - ▣ product or process design
- reactive
  - ▣ recognising, averting or minimising a problem
  - ▣ managing consequences of loss of control



# Pro-active Predictive Microbiology

using knowledge of microbial ecology in models  
we can

- design products to extend their shelf life
- find minimal conditions (better product quality)  
to achieve required shelf life
- design shelf-stable products
  - ▣ *e.g.*, processed cheese spread

# Re-active Predictive Microbiology

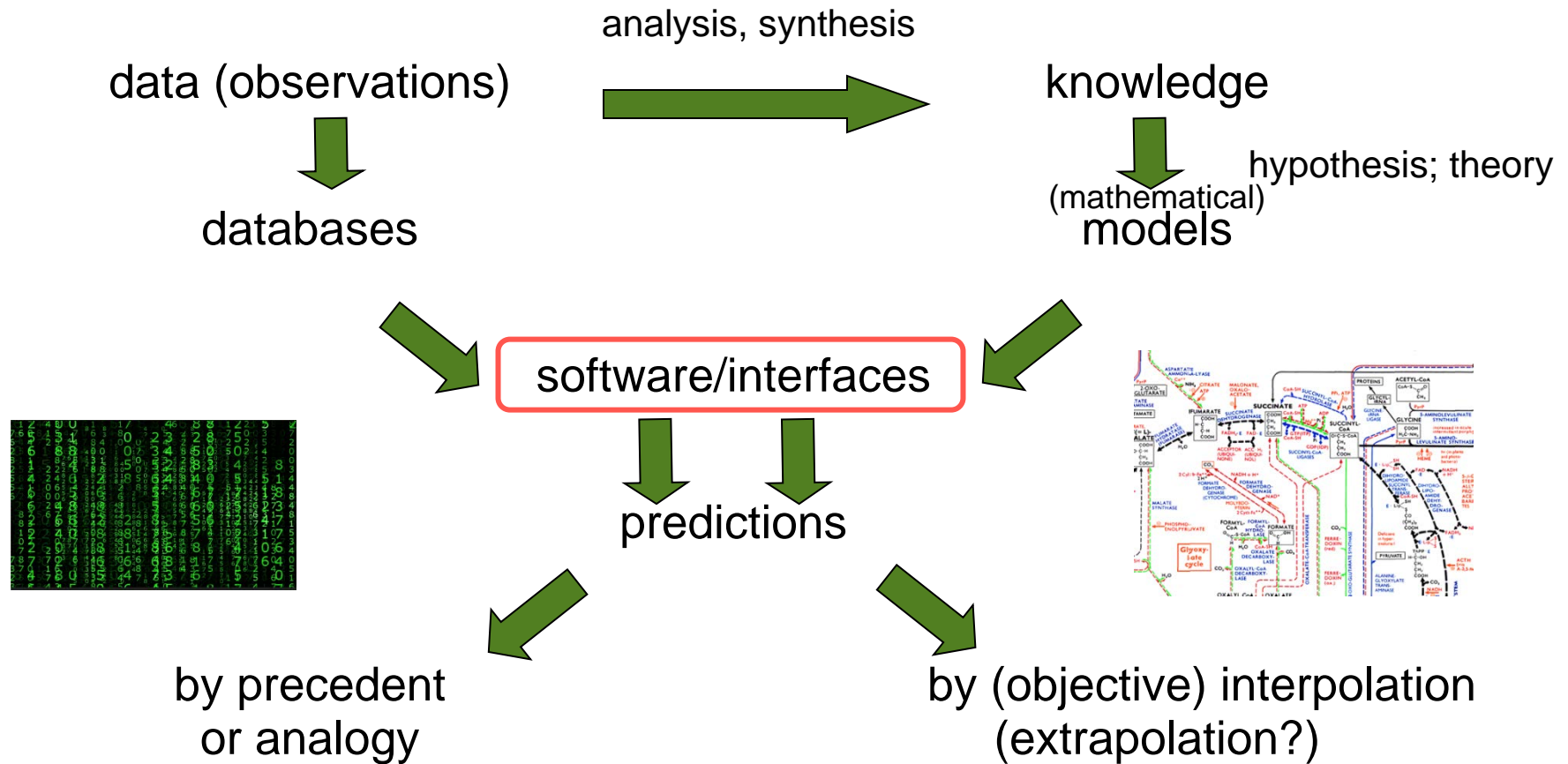
using knowledge of microbial ecology and models

- how much growth has already occurred?
- how much loss of shelf life has occurred?

or

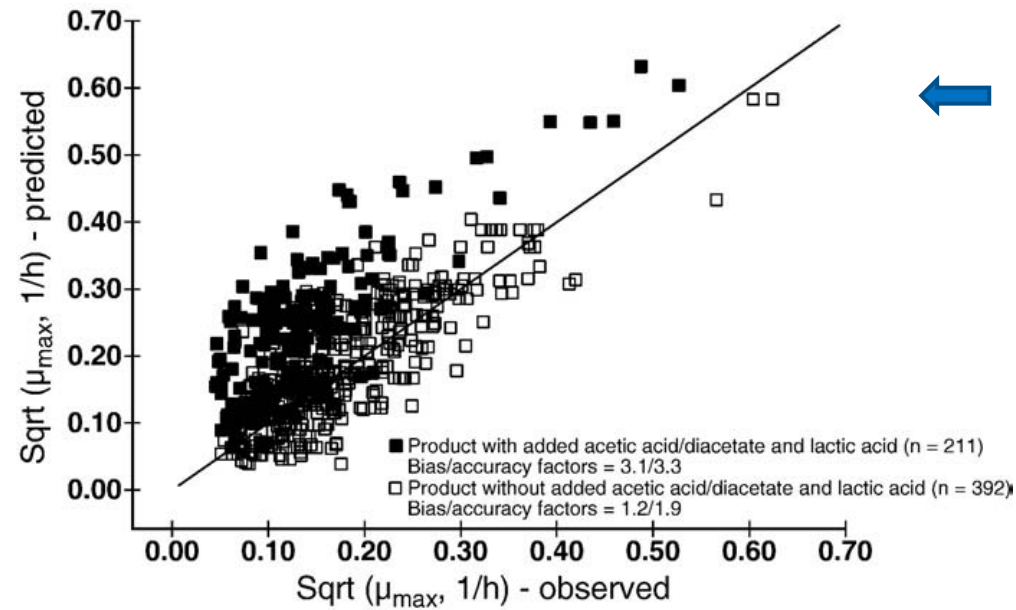
- how long will it take for the initial levels of SSOs to grow to the spoilage level, if we know:
  - ▣ product characteristics (pH,  $a_w$ , etc)
  - ▣ storage conditions (temperature, atmosphere)
  - ▣ characteristics (growth rate, limits) of the SSO

# Data and Models



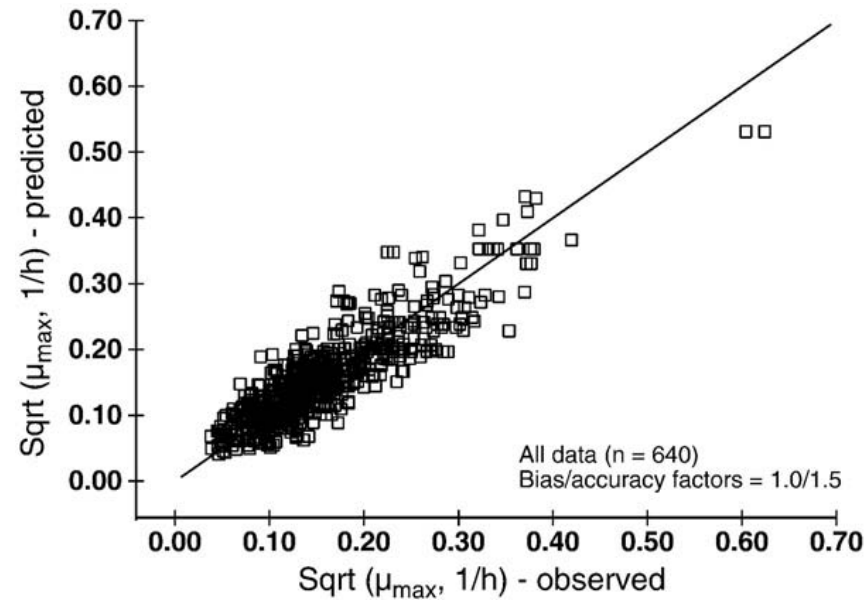
# Choosing a Model/Assessing its Reliability

- Model applicability
  - ▣ Does the model describe the right organism? Are there strain differences?
  - ▣ Is the model relevant to the food – does it include all influential factors?
- Model reliability
  - ▣ Does the model accurately describe the biological phenomena?
- Various measures of reliability
  - ▣ Two aspects – goodness of fit, systematic lack of fit
  - ▣ e.g., Bias and accuracy factors; bias and precision factors; t-test and F-test; etc.



← Model without terms for effect on growth rate of common organic acids

→ Model with terms for effect on growth rate of common organic acids



# Variability

- Variability
  - ▣ between strains
  - ▣ experimental (e.g., variations within food matrix, sampling error)
  - ▣ models should detail ‘confidence intervals’ on predictions, or ‘error term’

# Growth Model

[ Static | Dynamic ]

[ Aw | NaCl ]

Listeria monocytogenes/innocua

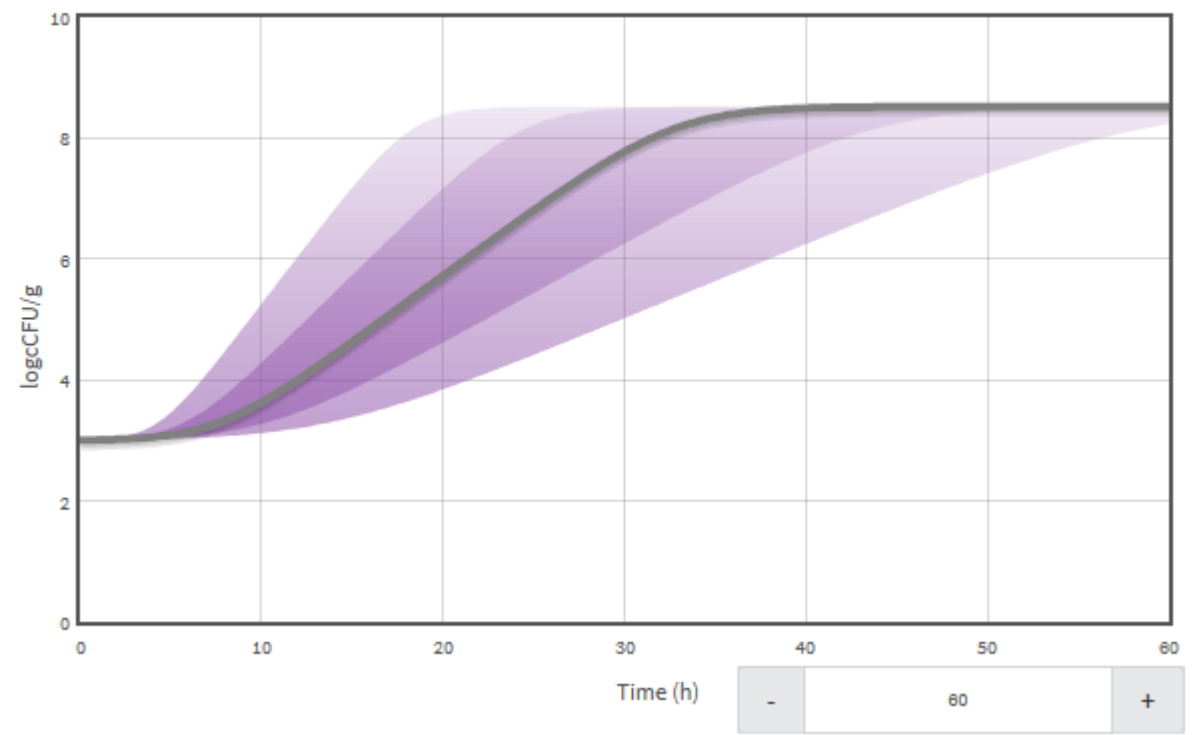
Init. level	3
Phys.state	2.1e-2
Temp (°C)	20
pH	7
NaCl (%)	0.5

0 7  
0 1  
1 40  
4.4 7.5  
0 10.2

Max.rate (log.conc/h) 0.221      Dbl.time(Hours) 1.364

Prediction    Uncertainty

Rate uncertainty    Phys. state uncertainty    Combined uncertainty



# Thank you...

□ and over to Peter 😊



# **Tertiary Microbial Models**

**Utilization of Tertiary Models for  
Estimation of Microbial Behavior in  
Meat-Containing Products**

**Dr. Peter Taormina**

# Reasons for Using Predictive Models

- Hazard Analysis
  - ▣ Assessing microbiological risks
- Process Preventive Control Limits
  - ▣ Times, Temperatures
- Product Development
  - ▣ Formulation (e.g., pH,  $a_w$ , preservatives)
  - ▣ Shelf Life
- Preliminary Assessment of Process Deviation
  - ▣ Cooking, cooling deviations

# Types of Tertiary Models

- Bacterial Transfer
  - ▣ Surface/Product/Human
- Survival
  - ▣ Shelf Life
- Growth
  - ▣ Boundary
  - ▣ Rate
- Inactivation

# Available Predictive Tools

- [Baseline Software Tool](#)
- [Bioinactivation SE](#)
- [ComBase Predictor](#)
- [Dairy products safety predictor](#)
- [DMRI – predictive models for meat](#)
- [E. coli Inactivation in Fermented Meats Model](#)
- [EcSF – E. coli SafeFerment](#)
- [FDA-iRISK®](#)
- [Food Spoilage and Safety Predictor \(FSSP\)](#)
- [FISHMAP](#)
- [GroPIN](#)
- [Listeria Control Model 2012](#)
- [Listeria Meat Model](#)
- [Microbial Responses Viewer \(MRV\)](#)
- [MicroHibro: Predictive Models](#)
- [MLA Refrigeration Index Calculator](#)
- [PMM-Lab](#)
- [Process lethality determination spreadsheet](#)
- [Perfringens Predictor](#)
- [Praedicere](#)
- [Salmonella predictions](#)
- [Shelf Stability Predictor](#)
- [SWEETSHELF](#)
- [Sym'Previus](#)
- [Therm 2.0](#)

# Some Examples of Tertiary Models

-  Pathogen Modeling Program

- <https://pmp.errc.ars.usda.gov>

- 

- <https://www.combase.cc>

-  **DANISH MEAT**  
**RESEARCH INSTITUTE PREDICTIVE MODELS FOR MEAT**

- <http://dmripredict.dk>

# Pathogen Modeling Program 7.0

**PMP70**  
File View Models >> Bacterium Bacteria >> Model References Window Help

Microorganism: *Bacillus cereus (vegetative) (Broth Culture)*

**Input Conditions**  
 Aerobic  Anaerobic

**Temperature:**  
Range: 5 to 42  
19.0 °C 66.2 °F

**pH:**  
Range: 4.7 to 7.5  
6.5

**Sodium Chloride (% [g/dL]):**  
Range: 0.5 to 5  
2.5 (% [g/dL]) Water Activity 0.986

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

**Calculate Growth Data**

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

**Level of Concern**  
6.0 log(CFU/ml) 1,000,000 CFU/ml

**Source and/or Relevant Publications**  
R.C. Benedict, T. Partridge, D. Wells and R.L. Buchanan, *Bacillus cereus*: Aerobic Growth Kinetics: Journal of Food Protection (1993) 56(3):211-214 - <http://www.arsenrc.gov/MFS/HTML/ERRCPubs/5835.pdf> **Related Publications**

**Calculate Model with:**   **Time Scale:**   **Display Format:**

**Modeled Growth Parameters:**

Hours	
<b>Lag Phase Duration:</b>	13.9
Lower Confidence Limit:	10.3
Upper Confidence Limit:	18.7
<b>Generation Time:</b>	1.1
Lower Confidence Limit:	0.8
Upper Confidence Limit:	1.5
<b>Time to Increase 3.0 logs:</b>	25.2
Lower Confidence Limit:	18.5
Upper Confidence Limit:	34.2

**Bacillus cereus (vegetative) in Broth Culture**

— log(CFU/ml) - - LCL ··· UCL

# Corbion® *Listeria* Control Model



## Corbion® *Listeria* Control Model

### Food characteristics

Enter the characteristics of your finished cured meat product as specifically as possible. If you are unsure of a food parameter, please use the default value. You may also select a Corbion ingredient and enter an addition level.

Moisture  %  
 pH   
 NaCl  %  
 Sodium nitrite  ppm (on total formulation)

### Storage conditions

Temperature  °F

### Corbion Solution

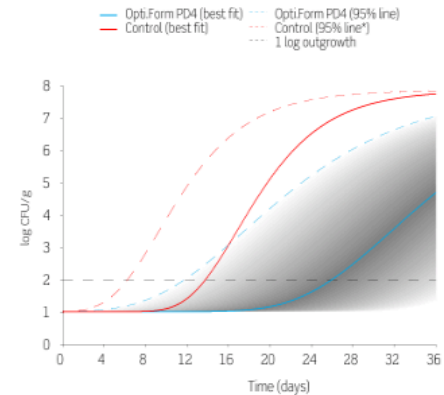
%

### Microorganism data

Initial level  log CFU/g  
 Maximum allowed level  log CFU/g

Reset to defaults

### *Listeria* growth in chicken



### About this graph

Time to 1 log outgrowth in days

	Control	With Opti.Form PD4
Best fit	14	26
95% line*	6	12

\* The lines are based on specifically designed and validated *Listeria* challenge studies. According to these studies, 95% of growth is expected to be slower than the 95% line.

Calculate

# Spoilage Model

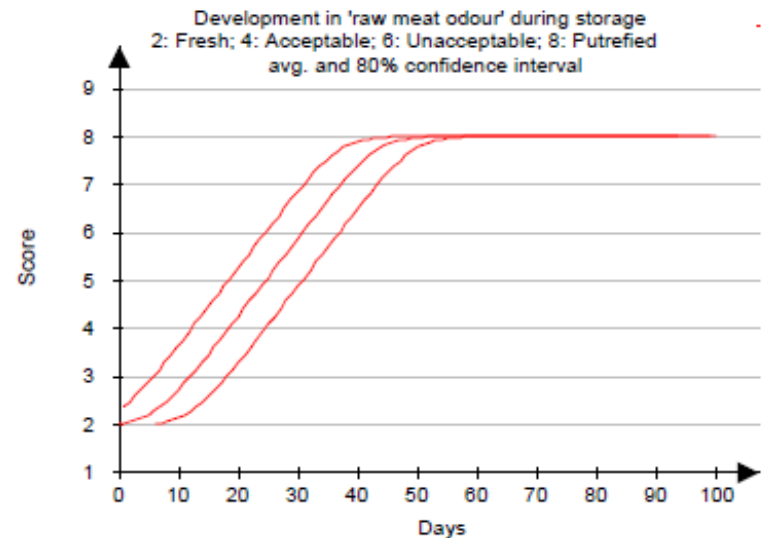
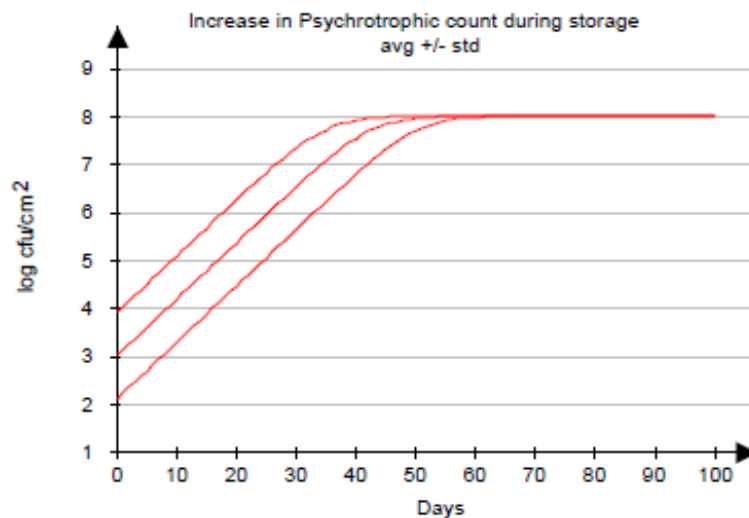
## PREDICTIVE MODELS FOR MEAT



### Shelf life of Bacon (cured pork)

Vacuum packed, 2-5,5 % salt in aqueous (% Sodium Chloride in the water phase, w/w), With/without Ascorbate, 60-120 ppm nitrite/nitrate added, No smoke. Version 1.0

Average	3.00	log cfu/cm <sup>2</sup>		
Standard deviation (std)	0.90	log cfu/cm <sup>2</sup>		
% salt in aqueous	2.00	%		
1. Temperature	7.00	°C in	100	days
2. Temperature	5.00	°C in	0	days
3. Temperature	5.00	°C in	0	days
4. Temperature	5.00	°C in	0	days





# Australia's Food Safety Information Portal

The screenshot shows the homepage of the Food Safety Centre's Australia's Food Safety Information Portal. The page features a green and white color scheme. At the top left is the Food Safety Centre logo, which consists of a stylized green and yellow leaf. To the right of the logo is the text "food safety centre". In the top right corner, there are links for "home", "privacy statement", and "copyright and disclaimer". Below the logo is a navigation menu with links for "About us", "Education", "Research", "Tools", "Subscribing", "Member portal", and "Contact us". The main content area is titled "Australia's food safety information portal" and is divided into two columns. The left column is titled "Predictive Models" and lists three models: "MLA E. coli in Fermented Meats", "MLA Refrigeration Index", and "Oyster Refrigeration Index". Each model has a brief description. The right column contains four buttons: "Subscribers" (with a login form), "Food Safety News Service", "R&D Capability Map", and "R&D Locator Service". At the bottom of the page, there is a footer with two links: "Find out more about the benefits of [subscription](#)." and "Download the [Food Safety Centre Brochure](#)." Below the footer is a photograph of a large agricultural field with rows of crops.

home | [privacy statement](#) | [copyright and disclaimer](#)

**food safety centre**

## Australia's food safety information portal

[About us](#) | [Education](#) | [Research](#) | [Tools](#) | [Subscribing](#) | [Member portal](#) | [Contact us](#)

### Predictive Models

**MLA *E. coli* in Fermented Meats**

A model that describes the inactivation of *E. coli* in fermented sausage.

**MLA Refrigeration Index**

A model that predicts the growth of *E. coli* during the chilling process.

**Oyster Refrigeration Index**

A predictive model that estimates the growth and survival of *V. parahaemolyticus* and total viable count (TVC) bacteria in Pacific oysters (*Crassostrea gigas*).

**Subscribers**

Username

Password

**Food Safety News Service**

**R&D Capability Map**

**R&D Locator Service**

Find out more about the benefits of [subscription](#).

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**UW**  
**Extension**

# HACCP

University of Wisconsin - Madison

*University of Wisconsin - Madison  
Center for Meat Process Validation*

Home

Validation

Pathogen Modeling

Additional Info

Deviations

Model HACCP Plans

Documentation/Support

Prerequisite Programs

Research Results



Navigation : [Home](#) > Pathogen Modeling

## Pathogen Modeling

**THERM 2.0**

**Shelf Stability Predictor**

**USDA Pathogen Modeling 7.0**

**USDA Predictive Microbial  
Information Portal**

### Pathogen Modeling

Pathogen modeling programs can be helpful in setting critical limits, determining hazard severity, and justifying corrective actions.

### Search

### Questions?

**Dr. Barbara Ingham**  
Food Safety Specialist  
Phone: 608-263-7383  
Email: [bingham@wisc.edu](mailto:bingham@wisc.edu)

[Contact](#) | [Term of use](#)

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Cooked Chicken Cooling

Marinated Chicken Breast

# Cooked Chicken Cooling – Marinated, Cooked Chicken Breast

- Chicken breasts are vacuum tumbled in a marinade containing phosphates, sea salt, seasonings, and potassium lactate.
- Marinated chicken breasts are cooked through continuous impingement oven, then cooled on racks.
- pH 6.8,  $a_w$  0.987, 1.7% salt
- Temperature probes:
  - 5 hours from 130 to 60°F (54.4 to 15.6°C)



□ Which Model?

# Pathogen Modeling Program (PMP) Online



## Pathogen Modeling Program (PMP) Online

[PMP Home](#)

**[PMP Online](#)**

[About PMP](#)

[Tutorial](#)

[Frequently Asked Questions](#)

[Reference Material](#)

[Project Scientists](#)

You are here: [PMP Home](#) / PMP Online

SELECT A PATHOGEN MODEL



The models are based on extensive experimental data of microbial behavior in liquid microbiological media and food.

There can be no guarantee that predicted values will match those that would occur in any specific food system. Before the models could be used in such a manner, the user would have to validate the models for each specific food of interest.

OK

# Pathogen Modeling Program (PMP) Online

## Model >> Bacterium

### COOLING ▾

Clostridium botulinum (broth culture)

Clostridium perfringens in cooked cured pork

Clostridium perfringens in cooked beef

Clostridium perfringens in cooked uncured beef

Clostridium perfringens in cooked uncured chicken

Clostridium perfringens in cooked uncured pork

GROWTH ▶

HEAT INACTIVATION ▶

SURVIVAL ▶

TRANSFER ▶

## Bacteria >> Model

AEROMONAS HYDROPHILA ▶

BACILLUS CEREUS ▶

CLOSTRIDIUM BOTULINUM ▶

CLOSTRIDIUM PERFRINGENS ▶

ESCHERICHIA COLI [O157:H7] ▶

LISTERIA MONOCYTOGENES ▶

SALMONELLA DUBLIN ▶

SALMONELLA ENTERITIDIS ▶

SALMONELLA HADAR ▶

SALMONELLA KENTUCKY ▶

SALMONELLA TYPHIMURIUM ▶

SALMONELLA SPP. ▶

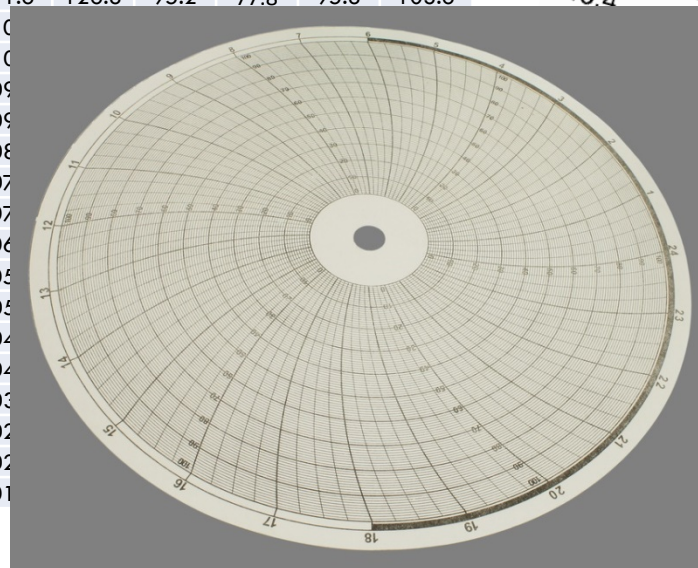
SHIGELLA FLEXNERI ▶

STAPHYLOCOCCUS AUREUS ▶

YERSINIA PSEUDOTUBERCULOSIS ▶

# Time and Temperature Data

18.85	133.0	114.2	114.1	101.8	149.5	122.6	137.0	106.4	89.8	105.9	115.0
18.87	132.2	113.4	113.4	100.9	148.9	121.8	136.3	105.6	89.4	105.2	114.2
18.88	131.5	112.7	112.7	100.1	148.2	121.0	135.6	104.8	89.0	104.5	113.3
18.90	130.7	111.9	112.0	99.3	147.6	120.3	134.8	104.0	88.6	103.8	112.6
18.92	129.9	111.1	111.3	98.5	146.9	119.5	134.1	103.2	88.1	103.1	111.8
18.93	129.2	110.4	110.6	97.8	146.3	118.8	133.4	102.4	87.8	102.5	111.0
18.95	128.4	109.6	109.9	97.0	145.6	118.0	132.7	101.7	87.2	101.8	110.2
18.97	127.7	108.9	109.3	96.2	145.0	117.3	132.0	100.9	85.9	101.1	109.4
18.98	127.0	108.1	108.6	95.5	144.3	116.6	131.4	100.2	84.7	100.4	108.7
19.00	126.2	107.4	108.0	94.7	143.7	115.9	130.7	99.4	83.5	99.7	107.9
19.02	125.5	106.7	107.4	94.0	143.0	115.1	130.0	98.7	82.4	99.1	107.2
19.03	124.8	105.9	106.7	93.3	142.4	114.4	129.4	98.0	81.3	98.4	106.5
19.05	124.1	105.2	106.1	92.6	141.7	113.7	128.7	97.3	80.3	97.8	105.7
19.07	123.3	104.5	105.5	91.9	141.1	113.0	128.0	96.6	79.5	97.1	105.0
19.08	122.6	103.8	104.9	91.2	140.4	112.3	127.4	95.9	78.7	96.5	104.3
19.10	121.9	103.1	104.2	90.5	139.8	111.6	126.8	95.2	77.8	95.8	103.6
19.12	121.2	102.4	103.6	89.8	139.1	111.0	126.1	94.5	77.0	95.1	102.9
19.13	120.5	101.8	103.0	89.2	138.5	110.4	125.5	93.8	76.2	94.4	102.2
19.15	119.9	101.1	102.4	88.5	137.9	109.8	124.9	93.1	75.4	93.7	101.5
19.17	119.2	100.5	101.8	87.9	137.2	109.2	124.3	92.4	74.6	93.0	100.8
19.18	118.5	99.8	101.2	87.3	136.6	108.6	123.7	91.7	73.8	92.3	100.1
19.20	117.8	99.2	100.7	86.6	135.9	108.0	123.1	91.0	73.0	91.6	99.4
19.22	117.2	98.6	100.1	86.0	135.3	107.4	122.5	90.3	72.2	90.9	98.7
19.23	116.5	97.9	99.5	85.4	134.6	106.8	121.9	89.6	71.4	90.2	98.0
19.25	115.8	97.3	98.9	84.8	134.0	106.2	121.3	88.9	70.6	89.5	97.3
19.27	115.2	96.7	98.4	84.2	133.4	105.6	120.7	88.2	69.8	88.8	96.6
19.28	114.5	96.1	97.8	83.7	132.7	105.0	120.1	87.5	69.0	88.1	95.9
19.30	113.9	95.6	97.3	83.1	132.1	104.4	119.5	86.8	68.2	87.4	95.2
19.32	113.2	95.0	96.7	82.5	131.4	103.8	118.9	86.1	67.4	86.7	94.5
19.33	112.6	94.4	96.1	82.0	130.8	103.2	118.3	85.4	66.6	86.0	93.8
19.35	112.0	93.8	95.6	81.5	130.2	102.6	117.7	84.7	65.8	85.3	93.1
19.37	111.3	93.3	95.1	80.9	129.5	102.0	117.1	84.0	65.0	84.6	92.4



114.5		
92.3	3/15/2014 2:30	elapsed time in hrs
63.2	3/15/2014 3:30	0
47.6	3/15/2014 5:30	1:00
42.4	3/15/2014 7:30	3:00
41.8	3/15/2014 9:30	5:00
41.8	3/15/2014 10:30	7:00
42.1	3/15/2014 11:30	8:00
42.6	3/15/2014 12:30	9:00
43.3	3/15/2014 13:30	10:00
44.0	3/16/2014 14:30	11:00
44.8	3/15/2014 15:30	12:00
45.6	3/15/2014 16:30	13:00
46.4	3/15/2014 17:30	14:00
	3/15/2014 18:30	15:00
	3/15/2014 19:02	16:00
	3/15/2014 19:30	16:32
	3/15/2014 20:02	17:00
	3/15/2014 20:30	17:32
	3/15/2014 21:02	18:00
	3/15/2014 21:30	18:32
	3/15/2014 22:02	19:00
	3/15/2014 22:30	19:32
	3/15/2014 23:02	20:00
	3/15/2014 23:30	20:32
	3/16/2014 0:02	21:00
	3/16/2014 0:30	21:32
		22:00
		21.00
		21.53
		22.00



# Pathogen Modeling Program (PMP) Online

## Time-Temperature Data Format

This page loads with sample data in the textbox. This format should be followed when entering or pasting data into the textbox. Specifically, please enter cooling profile data as follows:

- Start with header row (e.g. time(hour), temperature)
- Enter each data point on a separate line, separated by a comma (e.g. 1.0, 44)
- Specify time first, in cumulative hours (e.g. 15 minutes = 0.25 hours)
- Specify temperature second, in the units selected using the radio buttons (°C or °F)
- A minimum of five (5) data time-temperature points must be provided
- At least three of the data points must be above 70°F (21°C)
- The time point in row 1 must always be 0 hours
- Data not following this format will be ignored

If using the **Browse** button to load the data from a text file, the file must:

- Follow the same format as above
- Be in text format (e.g. .txt, .csv)

Clicking on the **Calculate Growth Data** button will use data provided in the file, if specified, or the data in the textbox if no file is specified. The data from the file will be loaded into the textbox. Please confirm the temperature profile is charted correctly.


# Prepare Time/Temp Data

- Download or Manual Entry
- Select data range or points
  - ▣ Convert units
- Save as .CSV file (optional)
- Adjust for temperatures outside of model range

11/14/17 0:34	54.4				
11/14/17 0:44	52.0				
11/14/17 0:54	50.0				
11/14/17 1:04	48.0				
11/14/17 1:14	47.5				
11/14/17 1:24	45.2				
11/14/17 1:34	44.3				
11/14/17 1:44	42.1				
11/14/17 1:54	40.5				
11/14/17 2:04	38.8				
11/14/17 2:14	37.2				
11/14/17 2:24	35.6				
11/14/17 2:34	33.9				
11/14/17 2:44	32.2				
11/14/17 2:54	30.5				
11/14/17 3:04	30.0				
11/14/17 3:14	29.6				
11/14/17 3:24	29.1				
11/14/17 3:34	28.1				
11/14/17 3:44	27.7				
11/14/17 3:54	27.2				
11/14/17 4:04	26.6				
11/14/17 4:14	26.0				
11/14/17 4:24	25.4				
11/14/17 4:34	21.6				
11/14/17 4:44	21.7				
11/14/17 4:54	20.4				
11/14/17 5:04	19.2				
11/14/17 5:14	18.0				
11/14/17 5:24	16.8				
11/14/17 5:34	16.1				
11/14/17 5:44	15.8				
11/14/17 5:54	15.3				
11/14/17 6:04	14.6				
11/14/17 6:14	14.3				
11/14/17 6:24	14.1				
11/14/17 6:34	13.9				

		time (hour)	temperature (°C)
		0,	54.4
		1,	44.3
		2,	35.6
		3,	28.1
		4,	21.6
		5,	16.1
		5.25,	15.3
		6,	12.5
		7,	10.5
		8,	8.2
		9,	7.4
		10,	5.8
		11,	4.4

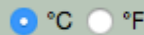


# PMP Online – Marinated, Cooked Chicken Breast Example

## Growth of Clostridium perfringens during cooling of cooked uncured Chicken

### Input Conditions

Temperature in:



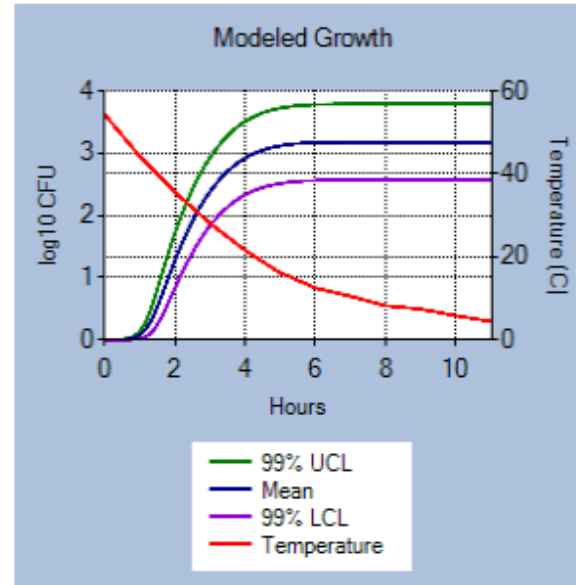
### Cooling Profile (Linear Model)

time(hour),	temperature
0,	54.4
1,	44.3
2,	35.6
3,	28.1
4,	21.6
5,	16.1
5.25,	15.3
6,	12.5
7,	10.5
8,	8.2
9,	7.4
10,	5.8

Or import cooling profile from a file in comma-separated values (.csv) format. (See instructions after the table of results below.)

No file chosen

Note: Model based on parameters in Table 1. However, after further analysis, a value of 0.02 has been substituted for  $q_0$  for pork. Growth is computed assuming a starting concentration of 1 cfu/g (0 log cfu/g). As such, it will not exceed the maximum population density.



### Modeled Growth Parameters

- Total Cooling Time: 11.00 (hours)
- Predicted Mean Growth: 3.18 (log<sub>10</sub> CFU)
- 99% UCL: 3.80 (log<sub>10</sub> CFU)
- 99% LCL: 2.57 (log<sub>10</sub> CFU)
- Maximum Population Density: 8.00 (log<sub>10</sub> CFU/g)

# PMP Online – Marinated, Cooked Chicken Breast Example

- 3.18-Log Increase (2.57 to 3.80 99% CL)
- **Source:** Juneja V.K., Marks H., Huang L., and Thippareddi H. Predictive model for growth of *Clostridium perfringens* during cooling of cooked uncured meat and poultry. Food Microbiology 28 (2011). P. 791-795.
- What about pH,  $a_w$ , % salt?



## Access ComBase

ComBase is the world's largest, freely-accessible database of quantified microbial responses in diverse food environments.

Freely accessible | 60,000 + records | 42,000 + users

[Login/Register](#)



Supported by



## 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
- ✓ ComBase Predictor and Food Models – to predict the growth and inactivation of microorganisms in food

### It can be used for:

- ✓ Informing the design of food safety risk management plans
- ✓ Producing Food Safety Plans and HACCP plans

## News, Events and Jobs

- > [Job: Microbiology Risk Assessor - Unilever](#)
- > [10th International Conference on Predictive Modelling in Food](#)

[↶ See all](#)

## Predictive Microbiology and Risk Assessment News

# Combase – Perfringens Predictor

The image shows a screenshot of the ComBase website interface. The top navigation bar is dark blue with the ComBase logo. A left-hand navigation menu is visible, listing various tools: Browser, ComBase Predictor, Food Models, Perfringens Predictor (highlighted with a white oval), Salmonella in egg, DMFit, Resources, and Help. The main content area is titled "Search" and features a search form with an "Organism" dropdown menu and a text input field containing "Type or click here". Below the search form is a "+Add another field" link. At the bottom of the search area, there are "Environmental conditions" buttons for "Any", "Static", and "Dynamic".

ComBase

Browser

ComBase Predictor

Food Models

**Perfringens Predictor**

Salmonella in egg

DMFit

Resources

Help

## Search

Organism

[+Add another field](#)

Environmental conditions

**Any** Static Dynamic

# 1.3-Log Increase

## Perfringens Predictor

Uncured meat Cured meat

pH [5.2-8.0] 6.8

[Aw | NaCl]

Aw [0.977-1] .987

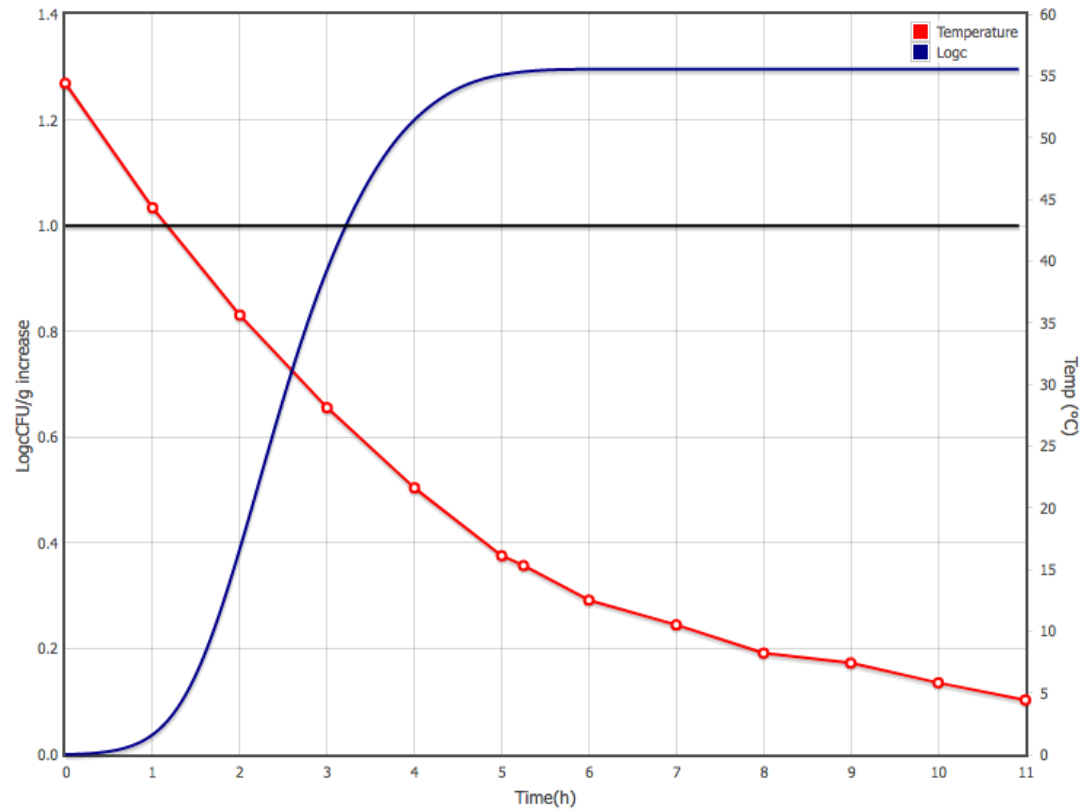
Time(h)	Temp (°C)
0.00	54.40
1.00	44.30
2.00	35.60
3.00	28.10
4.00	21.60
5.00	16.10
5.25	15.30
6.00	12.50
7.00	10.50
8.00	8.20
9.00	7.40
10.00	5.80
11.00	4.40



Predict

Chart

Data points



# 1.92-Log Increase

## Perfringens Predictor

Uncured meat Cured meat

pH [5.2-8.0] 6.8

[ Aw | NaCl ]

NaCl [0-4] 1.7

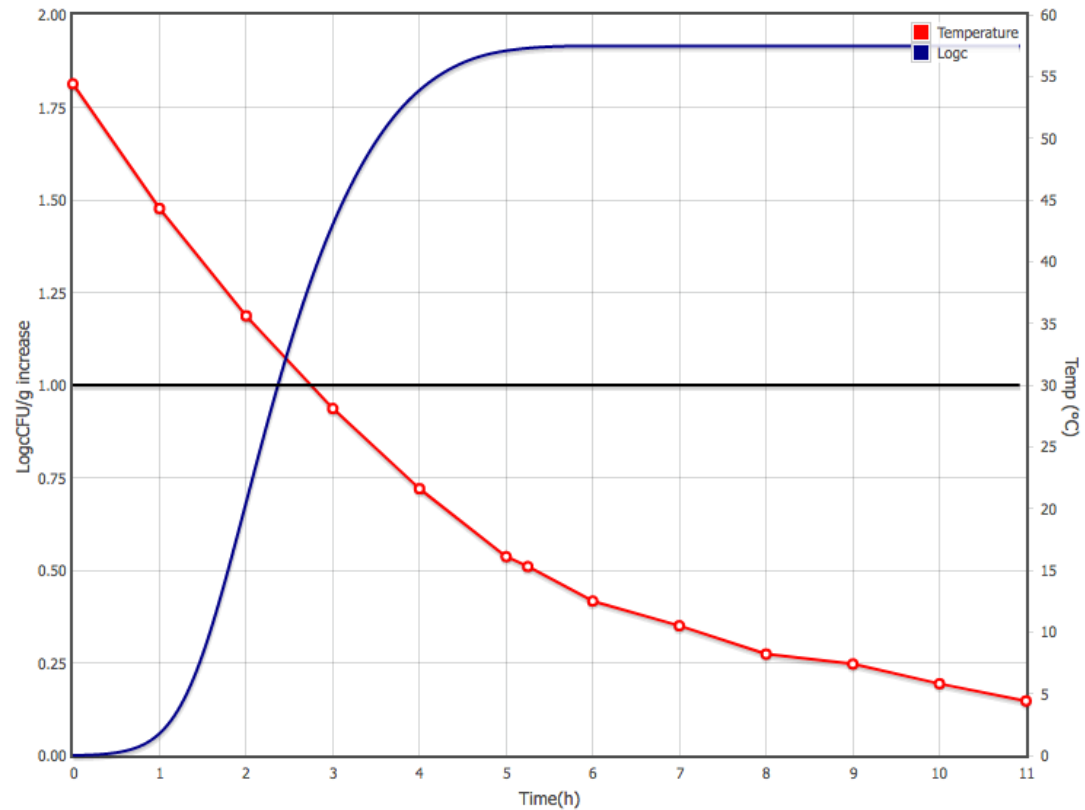
Time(h)	Temp (°C)
0.00	54.40
1.00	44.30
2.00	35.60
3.00	28.10
4.00	21.60
5.00	16.10
5.25	15.30
6.00	12.50
7.00	10.50
8.00	8.20
9.00	7.40
10.00	5.80
11.00	4.40



Predict

Chart

Data points





# Cooked Chicken Cooling – Marinated, Cooked Chicken Breast

- Chicken breasts are vacuum tumbled in a marinade containing phosphates, sea salt, seasonings, and **potassium lactate**.
- Marinated chicken breasts are cooked through continuous impingement oven, then cooled on racks.
- pH 6.8,  $a_w$  0.987, 1.7% salt
- Temperature probes:
  - 5.5 hours from 130 to 60°F (54.4 to 15.6°C)

## Research Note

# Inhibition of *Clostridium perfringens* Growth by Potassium Lactate during an Extended Cooling of Cooked Uncured Ground Turkey Breasts

KATHERINE M. KENNEDY,<sup>1†</sup> ANDREW L. MILKOWSKI,<sup>1,2</sup> AND KATHLEEN A. GLASS<sup>1\*</sup>

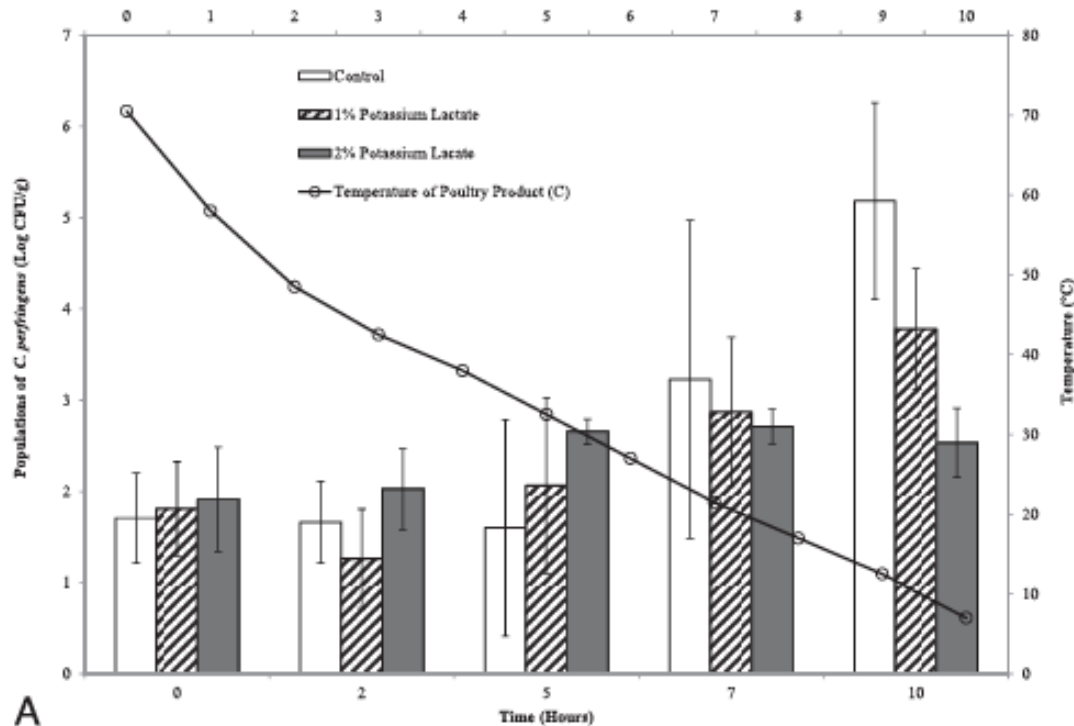
<sup>1</sup>Food Research Institute, University of Wisconsin–Madison, 1550 Linden Drive, Madison, Wisconsin 53706; and <sup>2</sup>Meat Science and Muscle Biology Laboratory, University of Wisconsin–Madison, 1805 Linden Drive, Madison, Wisconsin 53706, USA

MS 13-106: Received 17 March 2013/Accepted 26 June 2013

## ABSTRACT

The U.S. Department of Agriculture's Food Safety and Inspection Service compliance guideline known as Appendix B specifies chilling time and temperature limits for cured and uncured meat products to inhibit growth of spore-forming bacteria, particularly *Clostridium perfringens*. Sodium lactate and potassium lactate inhibit toxigenic growth of *Clostridium botulinum*, and inhibition of *C. perfringens* has been reported. In this study, a cocktail of spores of three *C. perfringens* strains (ATCC 13124, ATCC 12915, and ATCC 12916) were inoculated into 100-g samples of ground skinless, boneless turkey breast formulated to represent deli-style turkey breast. Three treatment groups were supplemented with 0 (control), 1, or 2% potassium lactate (pure basis), cooked to 71°C, and assayed for *C. perfringens* growth during 10 or 12 h of linear cooling to 4°C. In control samples, populations of *C. perfringens* increased 3.8 to 4.7 log CFU/g during the two chilling protocols. The 1% potassium lactate treatment supported only a 2.5- to 2.7-log increase, and the 2% potassium lactate treatment limited growth to a 0.56- to 0.70-log increase. When compared with the control, 2% potassium lactate retarded growth by 2.65 and 4.21 log CFU/g for the 10- and 12-h cooling protocols, respectively. These results confirm that the addition of 2% potassium lactate inhibits growth of *C. perfringens* and that potassium lactate can be used as an alternative to sodium nitrite for safe extended cooling of uncured meats.

- Compare pH,  $a_w$ , percent lactate in published study with your product.



Kennedy et al., 2013



Prepared Meal Cooling Example

Gumbo with Rice

# Prepared Meal Cooling - Gumbo

- Gumbo ingredients: Cooked rice, Andouille sausage, vegetables, shrimp, seasonings
- Rice is cooked in a commercial rice cooker. Meat, shrimp, and vegetables are cooked separately in a kettle.
- Rice is dispensed into plastic trays, and Gumbo mixture is ladled onto rice. Trays are sealed, placed on racks and wheeled into walk-in freezer.
- Reaches 41°F (5°C) in 6 h.

# Prepared Meal Cooling - Gumbo

- Oops!
- Temperature Deviation



# Combase Predictor – Growth Model

ComBase English 中文 peter@etnaconsulting.com

Browser

ComBase Predictor

**Growth**

Thermal Inactivation

Non-thermal Survival

Food Models

DMFit

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]

Chart Data points

Plot custom points

Time (h) - 60 +

# Gumbo Example - Combbase

## Growth Model

Prediction | Uncertainty

[ Static | Dynamic ] [ Aw | NaCl ]

Time(h)	Temp (°C)
0.00	30.00
1.00	28.20
2.00	25.00
3.00	23.10
4.00	25.20
5.00	28.00
6.00	30.00
7.00	29.70
8.00	27.00
9.00	18.00
10.00	15.00
11.00	7.20
12.00	4.40

**Bacillus cereus**

Temperatures range [5,34]

Init. level 1 0 7

Phys. state 2.7e-4 0 1

pH 7.2 4.9 7.4

Aw .979 0.94 1

[Add prediction]

Chart | Data points

Invalid temperature profile: temperature out of allowed range

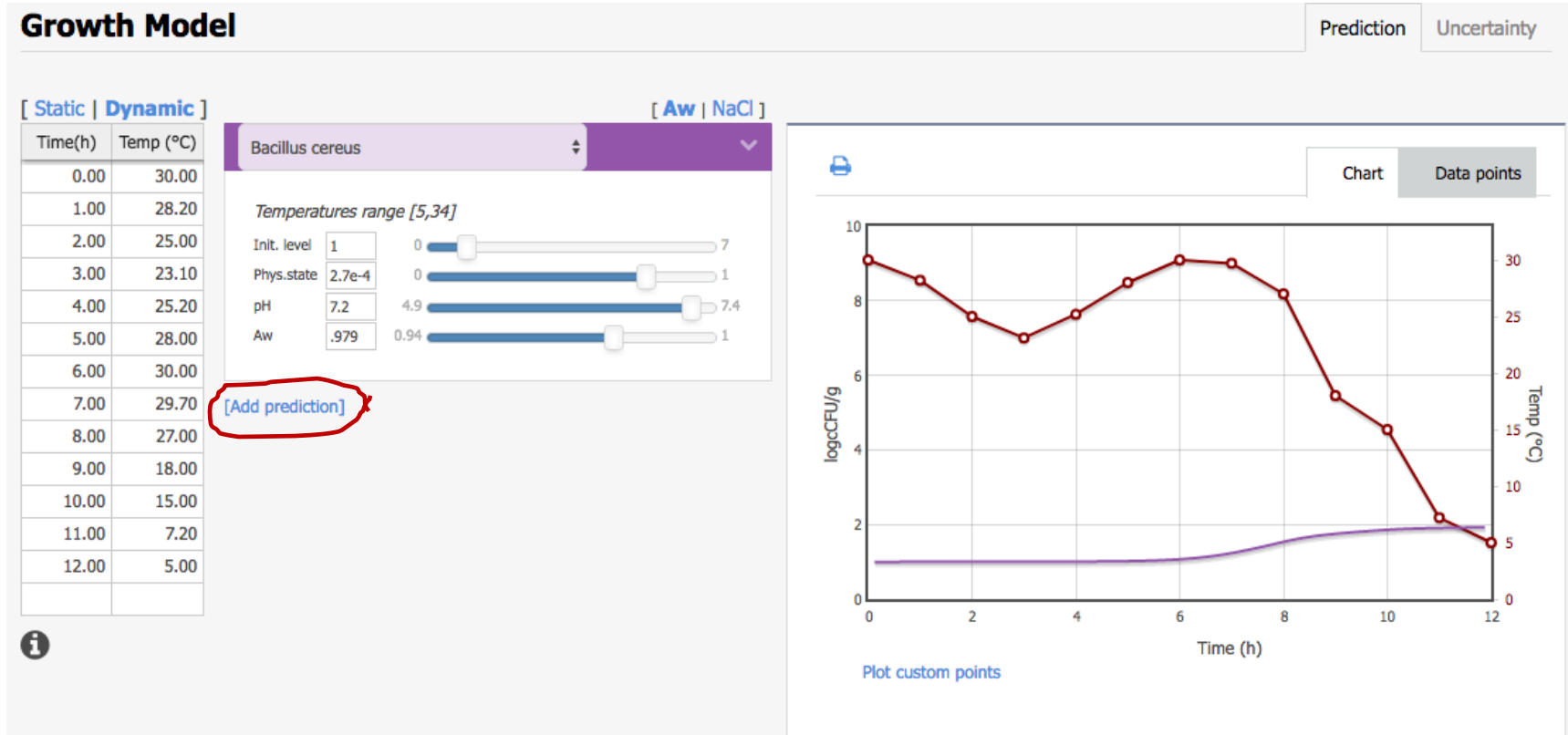
Temp (°C)

Time (h)

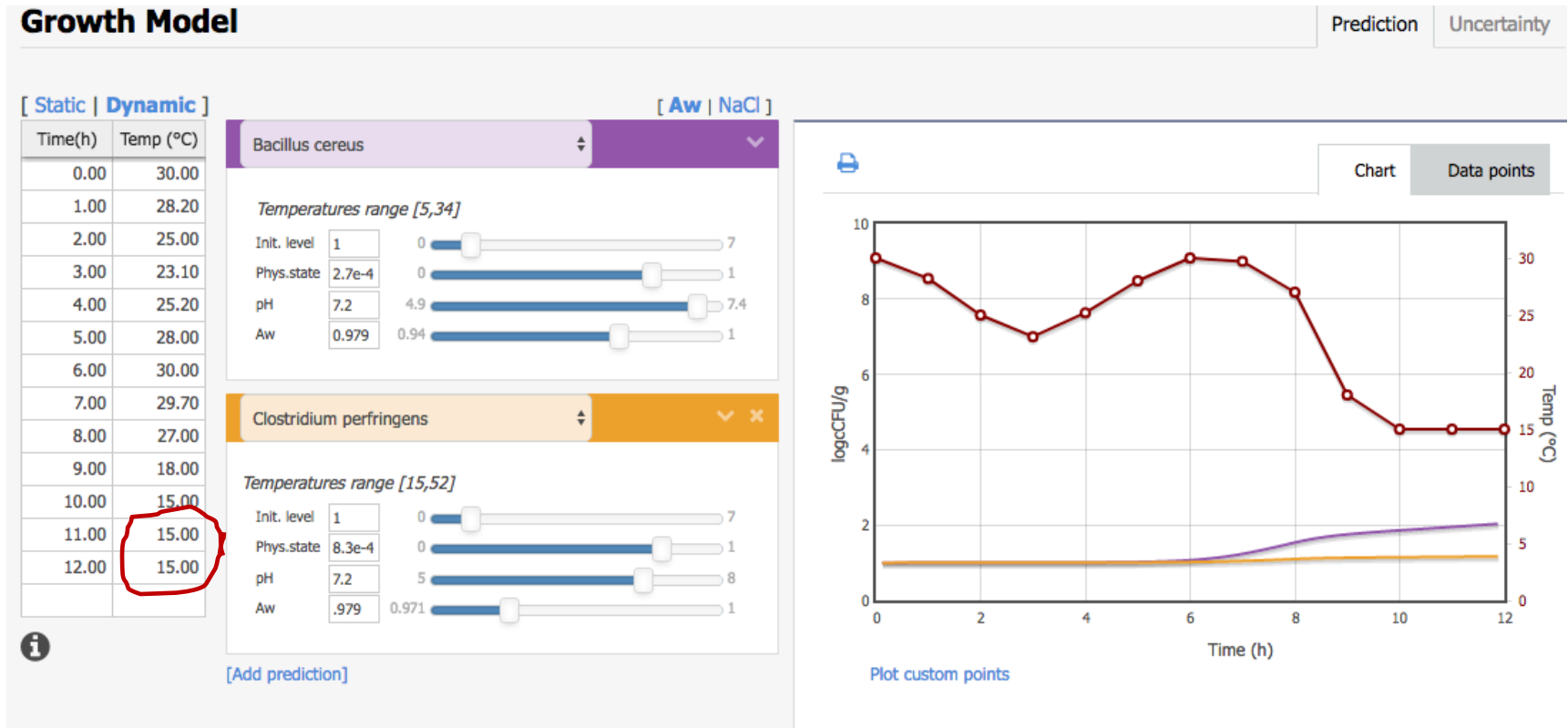
Plot custom points



# Gumbo Example – Combase 0.92-Log Increase *Bacillus cereus*



# Gumbo Example – Combbase



# Growth of non-proteolytic *C. botulinum* at 5°C within about 40 days

## Growth Model

Prediction Uncertainty

[ Static | Dynamic ]

[ Aw | NaCl ]

Clostridium botulinum (non-prot.)

Init. level 1

Phys.state 4.6e-5

Temp (°C) 5

pH 7.2

Aw 0.979

0 7

0 1

4 30

5.1 7.5

0.974 1

Max.rate (log.conc/h) 0.004

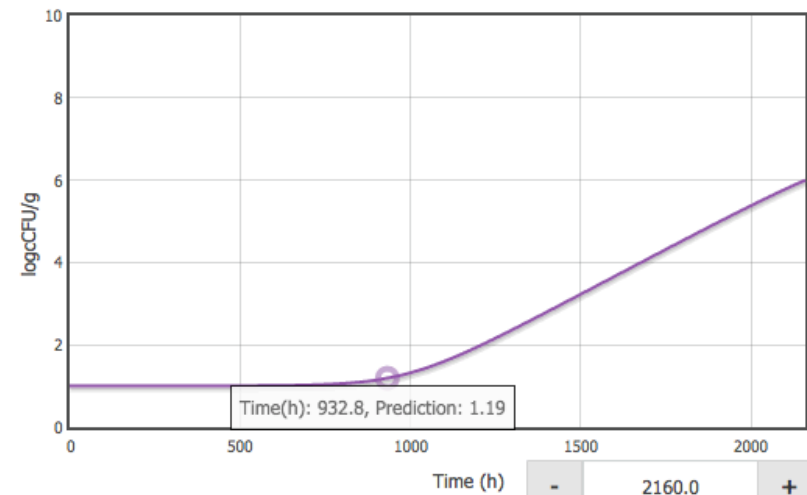
Dbl. time(Hours) 68.775

[Add prediction]



Chart

Data points



Plot custom points

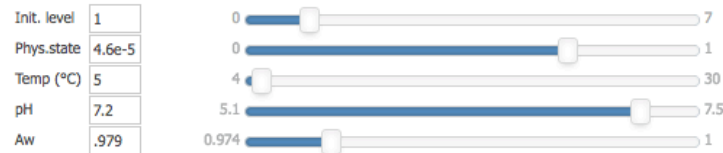
# Gumbo Example – Combbase Shelf Life?

## Growth Model

Prediction **Uncertainty**

[ Aw | NaCl ]

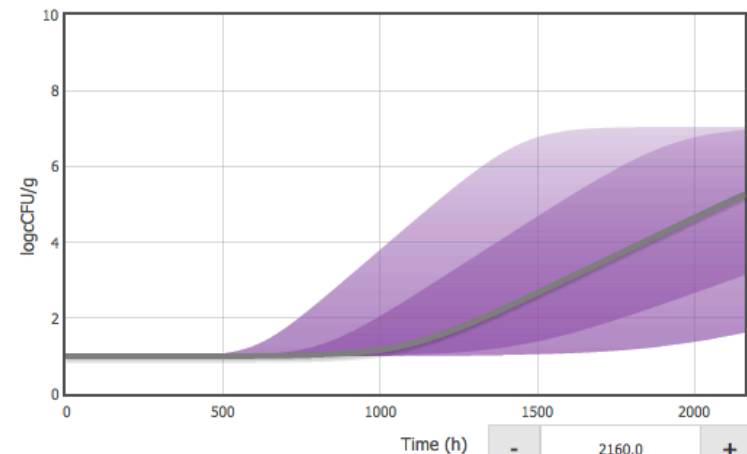
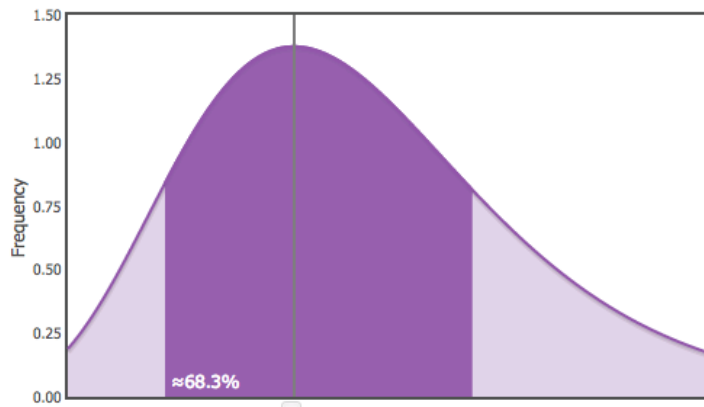
Clostridium botulinum (non-prot.)



Max.rate (log.conc/h) 0.004

Dbl. time(Hours) 68.775

Rate uncertainty Phys. state uncertainty Combined uncertainty



Plot custom points

# Gumbo Example – Combase Shelf Life?

## Growth Model

Prediction Uncertainty

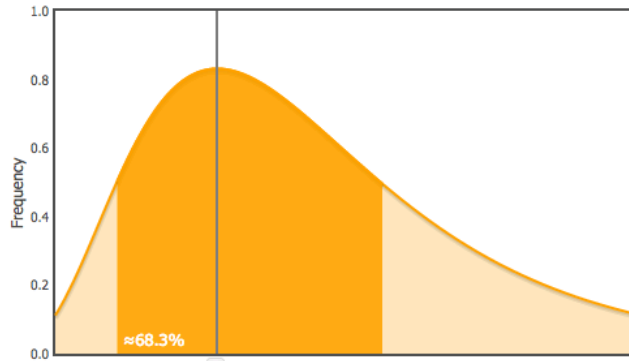
[ Aw | NaCl ]

Clostridium botulinum (non-prot.)

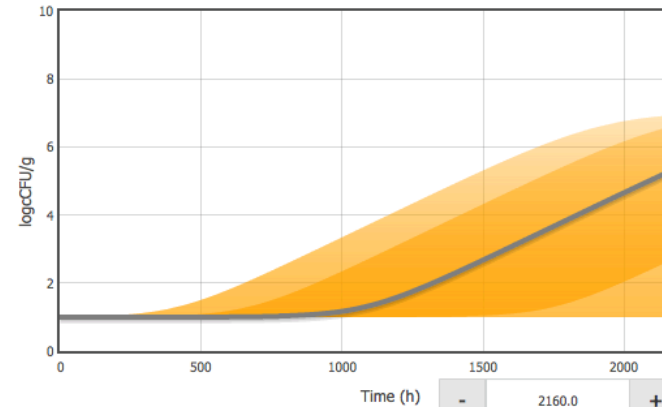
Init. level	1	0	7
Phys.state	4.6e-5	0	1
Temp (°C)	5	4	30
pH	7.2	5.1	7.5
Aw	0.979	0.974	1

Max.rate (log.conc/h) 0.004 Dbl. time(Hours) 68.775

Rate uncertainty Phys. state uncertainty Combined uncertainty

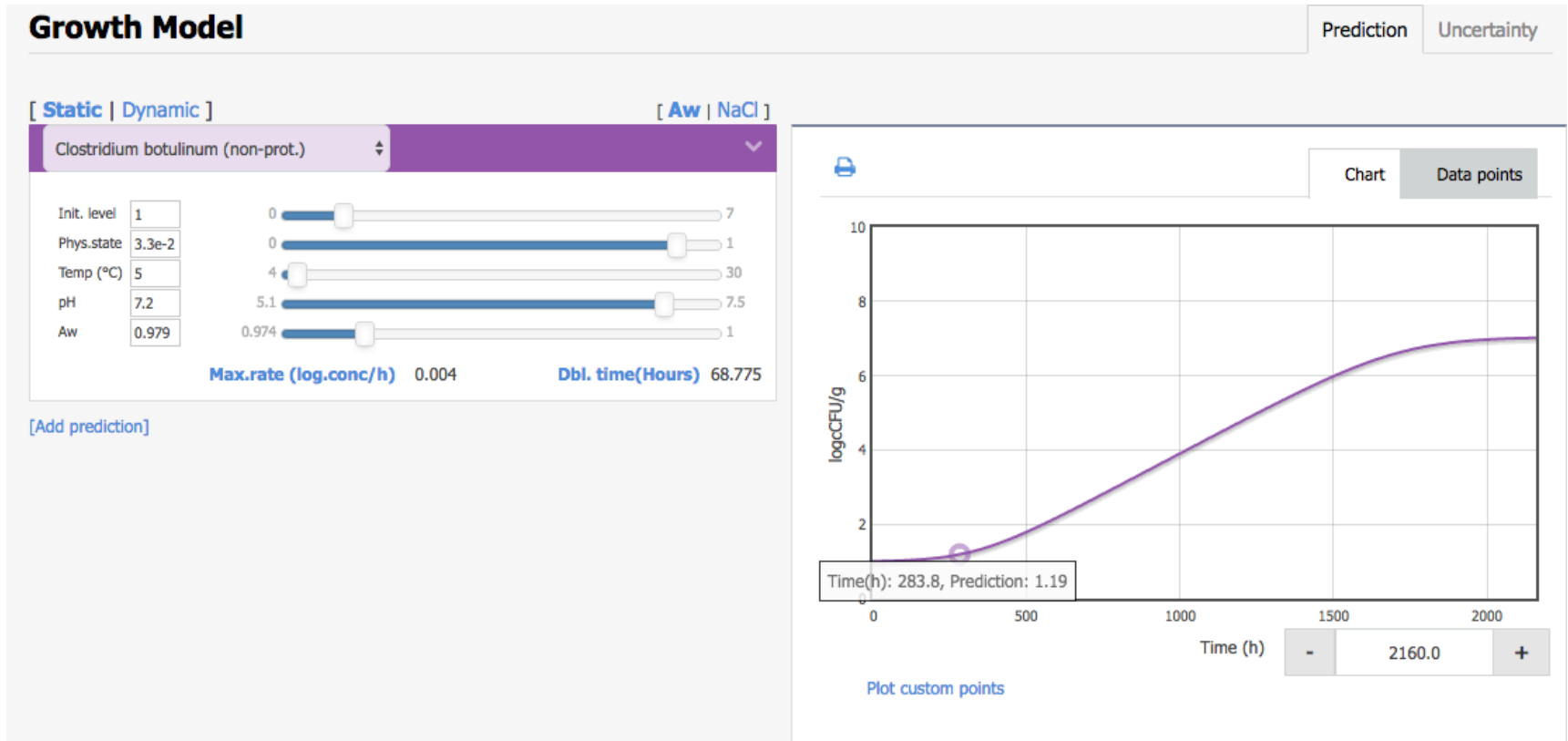


Apha0 = 4.59e-5  
 $\Pr(2.07e-3 \leq X \leq 9.74e-8) = 0.68$



Plot custom points

# Growth of non-proteolytic *C. botulinum* within about 14 days



# Growth Model

Prediction | Uncertainty

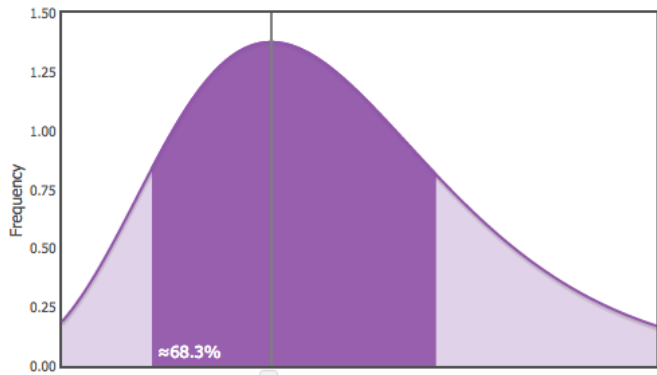
[ Aw | NaCl ]

Clostridium botulinum (non-prot.)

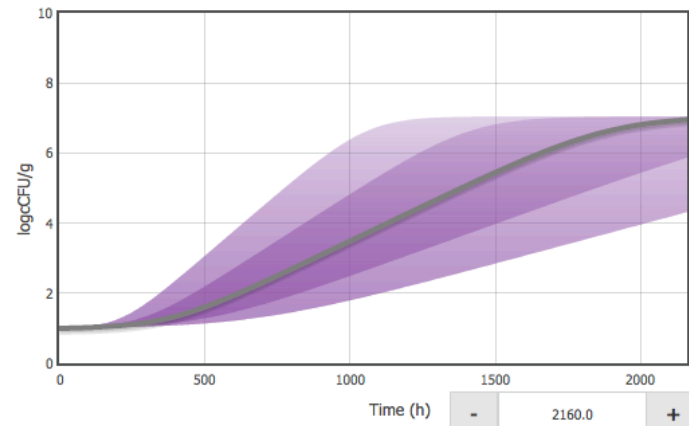
Init. level	1	0	7
Phys.state	3.0e-2	0	1
Temp (°C)	5	4	30
pH	7.2	5.1	7.5
Aw	0.979	0.974	1

Max.rate (log.conc/h) 0.004      Dbl. time(Hours) 68.775

Rate uncertainty | Phys. state uncertainty | Combined uncertainty



Max. rate =  $4e-3$   
 $Pr(3.00e-3 \leq X \leq 5.35e-3) = 0.68$



Plot custom points

# Summary

- Various Tertiary Models Exist
  - ▣ Some of which were demonstrated in this webinar
- Select Model Based Upon Your Unique Situation and Parameters
- Be Careful with Assumptions and Interpretation
  - ▣ Read and follow guidelines and disclaimers
- Validate and Verify







**Dr. Betsy  
Booren**



**Dr. Tom Ross**



**Dr. Peter Taormina**

# QUESTIONS & ANSWERS

# Acknowledgements

## Organizing Committee

- Dr. Yuhuan Chen
- Dr. Marcel Zwietering
- Dr. Bala Kottapalli

## IAFP

- David Tharp
- Sarah Dempsey
- Tamara Ford

# Practical Applications of Microbial Modeling Webinar Series

## Future Sessions

- Part II – Poultry
  - ▣ Winter 2018
- Part III – Risk Modeling
  - ▣ Spring 2018