

1 **Appendix: Predictive Microbiology**

3 **A. Overview**

5 The purpose of this appendix is to present the evidence for predicting growth and decline
6 of *E. coli* O157:H7 in ground beef to encourage deliberation about the merit of the data
7 and approaches for use in risk assessment. Full analytical details for some sections of the
8 discussion herein are presented in a published risk assessment (Marks 1998). In addition,
9 input was also obtained from the Interagency Food Risk Assessment Group (IFRAG
10 participants, particularly Dick Whiting (HHS, Food & Drug Administration) and Tom
11 Oscar (USDA, Agricultural Research Service) and Paul Uhler (USDA, Food Safety &
12 Inspection Service).

14 Modeling growth and decline under changing conditions of temperature during animal
15 production and meat processing, storage, distribution, and preparation is not a simple
16 process. The effect of changing temperature on the lag time has been researched for a few
17 bacteria. Zwietering (1994) reports that shifts in temperature can cause an increase in the
18 lag time, which could vary by organism.

20 The particular conditions for modeling growth may merit consideration of different
21 approaches in this risk assessment. For example, one might model growth of the
22 pathogen in a 2,000-pound combo bin differently than in a quarter-pound serving of
23 hamburger. An issue for the combo bin is that if contamination or temperature abuse
24 occurs, many servings may be contaminated during subsequent mixing and grinding
25 operations. The distribution of the pathogen in ground beef may not be homogeneous, but
26 the effect of clustering might be more crucial to model for contamination and temperature
27 abuse for a serving of hamburger than a combo bin. An opposing view might be that
28 growth in a combo bin could contaminate more servings and result in more cases of
29 illness. In order to provide transparency into the risk assessment process, deliberation of
30 such issues before model development is desirable.

32 **B. Predictive Microbiology**

34 The term Predictive Microbiology describes the scientific discipline of predicting
35 microbiological growth or decline as a function of environmental factors (McMeekin
36 1993). Growth is characterized by properties such as the "lag" time, (the amount of time,
37 from an initial equilibrium state, after an environmental change such as temperature
38 before growth begins), and generation time (an approximation of the amount of time
39 required for a population to double in size). Decline is characterized by a quantity called
40 the "D-value", which is a measure of the amount of time to achieve a 1 common
41 logarithm decline of the population at a given temperature. The common logarithm of the
42 D-values themselves are often linearly related to temperature (Line 1991). The negative
43 of the inverse of the slope of the regression of the log- D-values to temperature is defined
44 as the "z-value."

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1 Experiments to measure the kinetics of microbial growth have traditionally been
2 conducted in rich broth culture media at high initial densities (10^3 - 10^5 CFU per mL;
3 Oscar, personal communication). Such *E. coli* O157:H7 data were the basis for modeling
4 growth in the two published risk assessments; Cassin (1998) utilized a commercial
5 software application, the UK Food MicroModel (FMM), and Marks (1998) used portions
6 of the raw data most pertinent to ground beef from extensive experimental work at the
7 USDA Agricultural Research Service (Whiting, personal communication). The full
8 polynomial model, ARS Pathogen Modeling Program (PMP), includes a large number of
9 observations of growth kinetics at a variety of temperature, pH, salt and nitrite conditions
10 in pure culture broths from as many as 300 growth curves. The intent of such
11 experimental work is to broadly represent growth in many conditions that might be
12 typical of a host of food matrices.

13
14 Some validation of *E. coli* O157:H7 growth kinetics was conducted in many food
15 matrices according to the UK FMM manual. At the writing of this draft, little of the data
16 supporting this model appears to be publicly available. In addition, Walls (1996a) also
17 performed validation studies, one comparing bacterial kinetics in food with predictions of
18 both the ARS PMP and UK FMM. Both programs predicted longer lag phases (0.5-130
19 hours and <47 hours) for *Staphylococcus aureus* than actually observed in the food
20 matrix. Both models also underestimated growth rate. Similar results were also reported
21 in non-sterile food matrices (Smittle 1994). In addition, Walls (1996b) inoculated ground
22 beef with *E. coli* O157:H7 ($\sim 10^4$ /g) and compared results to the predictions of the ARS
23 PMP. Differences between the models were noted. Lag periods were shortened for
24 inoculated ground beef at pH 5.7 and 6.3 and at temperatures of 12 and 20 °C (reductions
25 from 31 hours in broth to 16 hours in beef, 27 to 3 hours, 8 to 2 hours, and 8 to 1 hour).
26 These differences might be significant to consider in risk assessment.

27
28 Further concerns about the available data include the very high level of inoculum for both
29 broth and validation studies. At present, most predictive microbiology models predict
30 growth independent of the initial density of pathogens per gram or per unit surface area.
31 Many other models, including those of Monod and Baranyi, have a specific term for
32 initial inoculum (Buchanan 1997; Alavi 1996; Marks, in preparation. Bacterial growth
33 kinetics may be independent of initial density in pure culture systems with rich media and
34 relatively high initial densities ($>10^2$), but may not hold true for low densities of *E. coli*
35 O157:H7 detected in ground beef (Oscar, personal communication).

36 37 **C. Microbial Ecology of Ground Beef**

38
39 Some data are available on the microbial ecology of ground beef. Raw meats are a good
40 substrate for growth of some bacteria. The specific microbial associations that dominate
41 meats include lactic acid bacteria and *Brochothrix thermosphacta* under anaerobic
42 conditions and *Pseudomonas*, *Acinetobacter*, and *Moraxella* under aerobic conditions
43 from classical work in food microbiology (ICMSF 1980; Jackson 1997). Ecologically
44 speaking, these bacteria appear to have evolved as the most effective microbial
45 competitors in raw meat and poultry products.

46

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1 Aerobic plate counts (APCs) are generally considered an indicator of the level of the
 2 spoilage flora (NRC 1985). The similarity in composition of the microflora of meat has
 3 long been recognized to mirror that of animal hides. However, additional sources of the
 4 microflora of meat may include fecal material, soil, hooves, hair, ingesta or
 5 gastrointestinal contents, and contacts in the slaughter environment from equipment
 6 surfaces, aerosols, and handling (Jackson 1997). The microflora of meat can be
 7 considered an inherent defect of animal production and processing perhaps best
 8 controlled by HACCP systems (NRC 1985). However, even under the best of production
 9 and slaughter conditions, raw meats cannot be expected to be sterile without further
 10 processing, such as thorough cooking. The National Research Council (1985) considered
 11 APCs an indicator of meat quality or shelf life, not safety. APC counts in foods are
 12 correlated with shelf life or time to spoilage, not to foodborne disease (Miskimin 1976;
 13 USDA 1993).

14
 15 Data from USDA (1996) and other studies (ICMSF 1980, 1996; Goepfert 1975) depict an
 16 extensive microflora associated with raw ground beef. A summary of the FSIS data for
 17 raw ground beef is tabulated below (USDA 1996). Note that every sample of 563 tested
 18 was positive for APC at a 95% upper limit of <13,300. None of the 563 samples analyzed
 19 in this study were positive for *E. coli* O157:H7. Data from MPN enumeration is available
 20 from the 1993 outbreak in the western states (USDA 1993; Johnson 1995). Of six
 21 samples analyzed, most probable numbers per gram for 25-gram samples of raw ground
 22 beef, unadjusted for recovery from frozen product, were 0.3 – 15 MPN/g. Competition
 23 from other microorganisms in foods is not considered in most predictive microbiology
 24 models. The growth of a pathogen is dependent not only on its initial population density
 25 in ground beef, but also the density of competing flora (Goepfert 1975; Coleman 1996).
 26 Smittle (1994) reported that the indigenous flora on foods is important to consider in
 27 describing the kinetics of growth of pathogens. Data or comments on the roles for
 28 predictive microbiology and microbial ecology in risk assessment are invited.

Table A.1: Results of baseline study in ground beef (USDA 1996)

Microorganism	Number of samples positive by quantitative methods of 563 samples tested	95% Confidence Interval
Direct enumeration (CFU/g)		
Aerobic Plate Count @ 35 C	563	4,700; 13,300
Total coliforms	563	60; 154
<i>Escherichia coli</i> Biotype I	563	31; 95
<i>Clostridium perfringens</i>	563	42; 107
<i>Staphylococcus aureus</i>	563	23; 41
Most Probable Number enumeration (MPN/g)		
<i>Listeria monocytogenes</i>	99	0.42; 19.7
<i>Salmonella</i> spp.	29	0.001; 2.84
<i>Campylobacter jejuni/coli</i>	0	NA
<i>Escherichia coli</i> O157:H7	0	NA

1 **D. Modeling Growth Directly**

2
3 Many empirical model forms have been compared for predicting growth as a function of
4 time for a given temperature. The available models are simplifications of the complexity
5 of modeling growth of bacterial populations. Hauschild (1993) and Zwietering (1990)
6 report that the Gompertz equation provides a better fit than other sigmoidal model forms,
7 such as the logistic. In addition, Buchanan (1997) describes another alternative, simple
8 linear three-phase models. However, the Gompertz equation has been very widely used
9 for fitting and predicting the expected growth for many organisms (Buchanan 1990). The
10 Gompertz equation appears to provide a reasonable, but imperfect model for predicting
11 growth of *E. coli* O157:H7 as discussed below.

12
13 Despite its properties of good empirical fit to bacterial growth data and common usage,
14 the Gompertz equation is not derived from mechanistic microbiological considerations.
15 The lack of a biological basis for parameters makes interpretation of the equation
16 parameters difficult (Marks 1998). For example, the lag time, l , is interpreted as the time
17 component of the point of intersection of the two lines: $Y = L(0)$ and the tangent line
18 containing the point $(M, L(M))$. Biologically, lag time calculated by the Gompertz
19 equation is a misnomer, because growth can occur before the predicted lag time (Marks
20 1998).

21
22 Further, the solution for the Gompertz equation used to calculate the maximum
23 population density (MPD) is based on the assumption that the sum of A (asymptotic
24 minimum growth parameter) and C (asymptotic difference in cell numbers) can be treated
25 as a constant (Buchanan 1990). This assumption is not innocuous. It implies that at the
26 initial time point, the organism is early in the lag phase of growth where the Gompertz
27 curve is flat. In practice, this assumption may cause bias in predictions of longer lags than
28 may occur with varying initial densities of inocula.

29
30 Several weaknesses of the available models are of concern to risk assessors. Some
31 evidence indicates that predicted lag times from response surface models may vary from
32 predictions in broth when the pathogen is inoculated directly into food matrices (Walls
33 1996a,b; Smittle 1994). In addition, for practical reasons, the experimental systems for
34 generating data to both construct the predictive microbiological models and to validate
35 the derived models use very high initial inoculum densities. A great concern for risk
36 assessors is predicting growth under changing temperature conditions. At least two
37 published studies (Zwietering 1994; Rajkowski 1995) appear to offer conflicting
38 interpretations of the effect of temperature changes on lag time for bacterial pathogens.
39 Some adjustment to point estimates reported for lag time predictions will be needed to
40 account for variability and uncertainty in risk assessment modeling.

41
42 Therefore, the ARS includes a disclaimer for the PMP that states that their application of
43 the Gompertz equation was designed as a research and instructional tool for estimating
44 the effects of multiple variables on the growth or survival of foodborne pathogens in
45 culture media. The lag times predicted from general polynomial models based on
46 response surfaces for a broad array of experimental conditions possible for all foods may

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1 thus not be appropriate for use in a risk assessment to predict growth in a particular food
2 commodity. Full discussion of the Gompertz model and its parameters is available in
3 various publications (McMeekin 1993; Marks 1998). The equations used in the Marks
4 analysis (1998) are listed in Table A.2.

5
6 Uncertainty is associated with the effects of suddenly changing temperature on microbial
7 growth. The lag times for each period could be assumed 100% of the lag time that would
8 be calculated from the above formula. The assumptions used in predictive microbiology
9 applications may have great impact on growth predictions for risk assessment since
10 growth kinetics are not routinely characterized for bacterial populations at the low
11 densities observed for *E. coli* O157:H7 in outbreak investigations (USDA 1993; Johnson
12 1993).

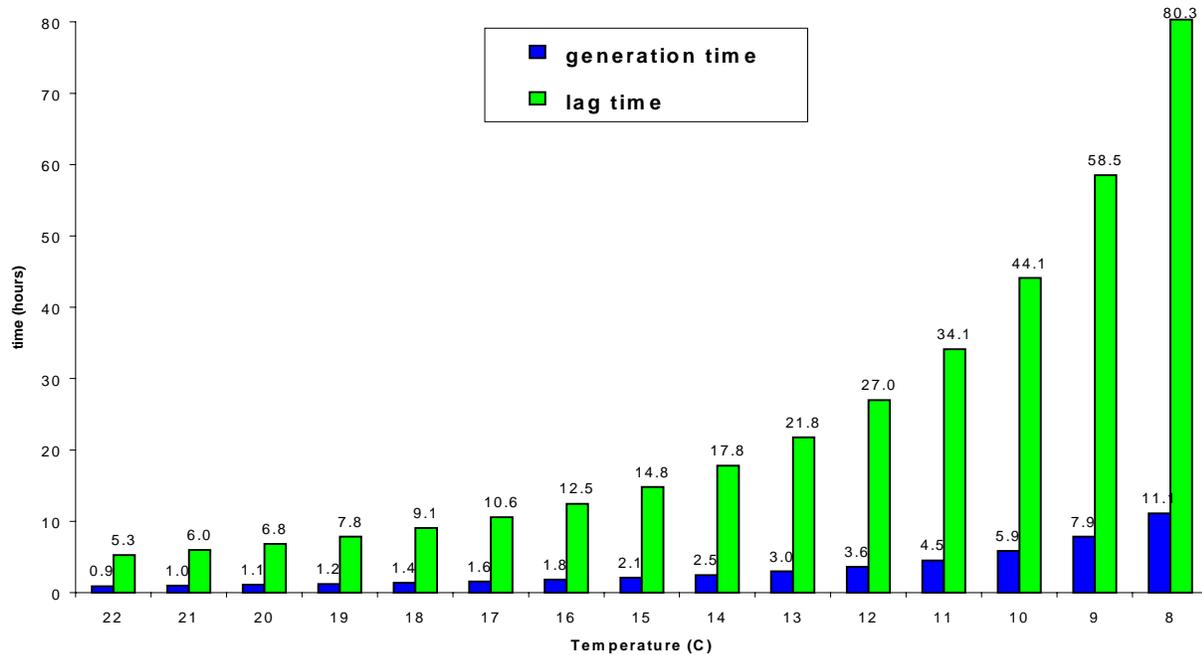
13
14 Agricultural Research Service data (Whiting, personal communication) were generated
15 for *E. coli* O157:H7 growth in broth culture relating temperature, pH, and salt and nitrite
16 concentration to the generation and lag times, which were derived using Equation 2 and
17 assuming that $A = \log_{10}(N_0)$. A subset of these data that reflect attributes of hamburger
18 was analyzed by Marks (1998) for conditions of no added nitrite, 0.5% added salt (the
19 lowest value tested), and pH 5.5-6.5. The experimental data included temperatures from
20 50 to 99° F (10 to 37° C). Some of the details of the analysis (Marks 1998) are further
21 described below.

22
23 Since the Gompertz equations model microbial growth kinetics at constant temperature,
24 the square root transformations of the growth characteristics, generation time, g , and lag
25 period, l is commonly used to model temperature dependencies. Equations using the
26 square root transformation are called Ratkowsky equations (McMeekin 1993). With the
27 data subset provided (Whiting, personal communication), however, other transformations
28 of the data provided more linear fits with temperature than that using the square root
29 transformations (Marks 1998). Specifically, data analysis was used to find parsimonious
30 (linear) models that describe the generation time and lag time. Stepwise regressions of the
31 natural logarithms of the parameters g and l were performed to identify linear
32 relationships, using as independent variables various positive and negative powers of
33 temperature, T , $\ln(T)$ and $\ln(\ln(T))$. For l , $\ln(T)$ was the first and only variable selected.
34 For g , $\ln(\ln(T))$ raised to the 2.5 power was the first and only variable selected. For the
35 latter dependent variable, a stepwise regression was repeated using only the $\ln(T)$ and
36 $\ln(\ln(T))$ variables. Using the log-log of temperature as the independent variable implies
37 that at 1° C (34° F) the generation time, g , is not defined. The residuals from regression
38 equations using the log transformed values of g and l as dependent variables appeared to
39 be homogeneous over the range of temperatures considered, thus simplifying models for
40 use in subsequent Monte Carlo simulations (Marks 1998).

41
42 In addition to determining lag and generation times as simple functions of temperature,
43 MPD values (theoretical maximum population density, assumed approximately equal to
44 $A+C$) were also calculated and were found to be linearly related with temperature
45 (Equation 3 in Table A.2; Marks 1998). Estimates of the parameters and the covariance
46 error matrix of the estimates were determined from linear regression of seemingly

1 unrelated regressions (SUR) using the SAS[®] routine PROC MOD. The estimates are: $a =$
 2 7.03 , $b = -6.31$, $c = 9.98$, $d = -2.69$, $e = 10.08$ and $f = -0.014$ (Marks 1998). Figure 1
 3 depicts, for example, the expected lag time at 22° C (72° F) is approximately 5.3 hours
 4 and the expected value of the generation time is approximately 55 minutes. At 37° C (99°
 5 F), the expected values predicted by Equation 3 are approximately 1.3 hours for the lag
 6 time and 21 minutes for the generation time.

7
 8 Fig. 1: Some Predictions for Gompertz Parameters for *E. coli* O157:H7 Growth in Broth



9
 10 To account for the uncertainty of the estimated parameters for each simulation trial (Marks
 11 1998), values of a , b , c , d , e and f were randomly determined by generating 6 independent
 12 normal random variables with variances equal to the eigenvalues of the covariance matrix,
 13 and transforming these by the transpose of the matrix of eigenvectors (Anderson 1958).
 14 Computations of the vector of eigenvalues, M , and the matrix of eigenvectors, E , were
 15 made on SAS[®] routine PROC IML. Independent normal variables, U , were generated with
 16 variance matrix with diagonal entries equal to the values of M . The simulated parameter
 17 values were then computed from the transformation EU . These computations can be used
 18 to transform the deterministic predictions illustrated above to stochastic equations that
 19 account for uncertainty of the parameter estimates in Monte Carlo analysis.

20
 21 There is additional uncertainty in the estimated growth because the available Agricultural
 22 Research Service data (Whiting, personal communication) were based on experiments
 23 conducted in broth, but not validated in hamburger. Also, the variability in growth is
 24 understated because the data were generated from a cocktail of strains, which does not
 25 permit explicit estimation of strain variability (Whiting, personal communication). The
 26 standard deviations of the residuals of the regression models defined in Equation 3 were:
 27 0.16 for the natural log of the generation time; 0.27 for the log of the lag time; and 0.15

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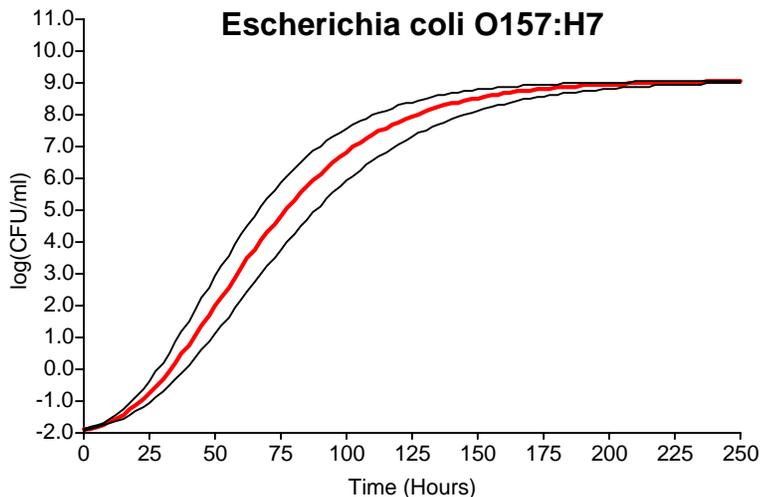
1 for the maximum population density. The source of variability in these error values is
2 unknown. In an attempt to account for unknown differences in variability, the growth
3 characteristic estimates could be adjusted by randomly generated normal variables with
4 mean equal zero and standard deviation equal to the standard deviation of the residuals
5 (Marks 1998). The correlation of these residuals could be taken into account in a similar
6 manner as described above.

8 E. Modeling Growth using Two Predictive Microbiology Modeling Programs

9
10 The UK Ministry of Agriculture, Fisheries, and Food (MAFF) and the USDA
11 Agricultural Research Service have prepared user-friendly deterministic models to
12 predict growth of certain pathogens under specific conditions. Both models include
13 growth of *E. coli* O157:H7. The models permit input of the following variables:
14 temperature, pH, salt content, nitrite content, aerobic or anaerobic growth, and initial
15 population density. The outputs of the model are the expected value and the minimum
16 and maximum observations for lag phase duration and generation time. (In addition, a
17 level of concern may be input, and time to level of concern may be output for the ARS
18 Pathogen Modeling Program.) The model fits the four Gompertz parameters described
19 above to describe growth at constant temperature. It appears that both the ARS PMP and
20 MAFF models simply back-calculate from the estimated Gompertz parameters for a
21 given temperature rather than explicitly fitting the data to the Ratkowsky equations as
22 performed by Marks (1998). Neither the PMP nor the MAFF models appear to
23 incorporate growth under changing conditions of temperature which is of interest to
24 microbial risk assessors.

25
26 Figure 2 below was generated using the ARS Pathogen Modeling Program for *E. coli*
27 O157:H7 specifically to generate dialogue on some difficult technical issues. The specific
28 conditions of the experimental system expected to be most relevant to ground

29
30 Fig. 2: Specific Predictions for *E. coli* O157:H7 Growth in Broth (derived from PMP)



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1 beef were used as inputs: 22 C, 6.5 pH, 0.5% salt, and 0 nitrite. The initial population
2 density selected was 0.01 CFU/mL or 1 CFU/100 mL growing under anaerobic
3 conditions.

4
5 Table 3 below was another output of the PMP for the conditions input for the figure
6 above. Note that the population could increase by less than one log before the lag phase
7 duration was reached. Even though the PMP permitted generation of these results, the
8 shape of the curve is open to interpretation for a number of reasons. The expected value
9 for lag phase duration in the tabulated output was 3.6 hours (2.9, 4.5) and the expected
10 generation time was 0.8 hours (0.7, 0.9). This lag period is not very apparent on the
11 figure. The value of the parameter C, the difference between the densities at time plus
12 and minus infinity, may be inappropriately large (Dick Whiting, personal
13 communication). If this parameter value is unrealistic or implausible, the remaining
14 Gompertz parameters (the constant B and M, the time for which the relative growth is
15 maximal, the asymptotic difference between the densities at maximal density) are
16 inappropriately defined. The parameters estimated from a subset of these ARS data
17 (Marks 1998) were similar to PMP results tabulated below, but variability and
18 uncertainty can be explicitly calculated from the analysis of Marks (1998). Thus, risk
19 assessors using predictive microbiological data and models must be aware of the potential
20 limitations of the experimental data.

21

Inputs				Outputs (expected value and range)	
Aerobic	Anaerobic	pH 5.5	pH 6.5	Lag phase duration	Generation time
	X		X	3.6 (2.9, 4.5)	0.8 (0.7, 0.9)
X			X	4.8 (4.1, 5.6)	0.8 (0.7, 0.9)
	X	X		5.7 (4.7, 7.1)	1.0 (0.9, 1.2)
X		X		6.0 (5, 7.2)	1.1 (1, 1.2)

22 F. Modeling Decline With Cooking

23
24 Rates of decline of *E. coli* O157:H7 depend, in a complex way, upon previous storage
25 and holding temperatures and fat content of foods (Jackson 1996). In addition, Line
26 (1991) reports that rate of decline decreases with the percentage of fat, that is, fat content
27 of food vehicles appears to be protective of pathogens subjected to cooking temperatures.
28 The interaction of changing conditions creates a complex system wherein growth and
29 decline may not be not linear functions of time, and may depend upon the rate of
30 temperature increase or decrease. In addition, models that can incorporate thickness of
31 hamburgers are needed to model preparation of hamburgers at home. Thicker hamburgers
32 are expected to pose greater probability of survival of pathogens if not cooked
33 thoroughly. To develop a model that accounts for these complexities, the thermal heat
34 transfer equations would be needed to determine the temperature changes during
35 preparation, storage, and cooking of hamburgers (Alavi 1996; Singh 1996).

36
37
38 A number of thermal death studies were conducted in which decline of the pathogen in
39 small portions of inoculated ground beef ground beef was measured over time in thermal

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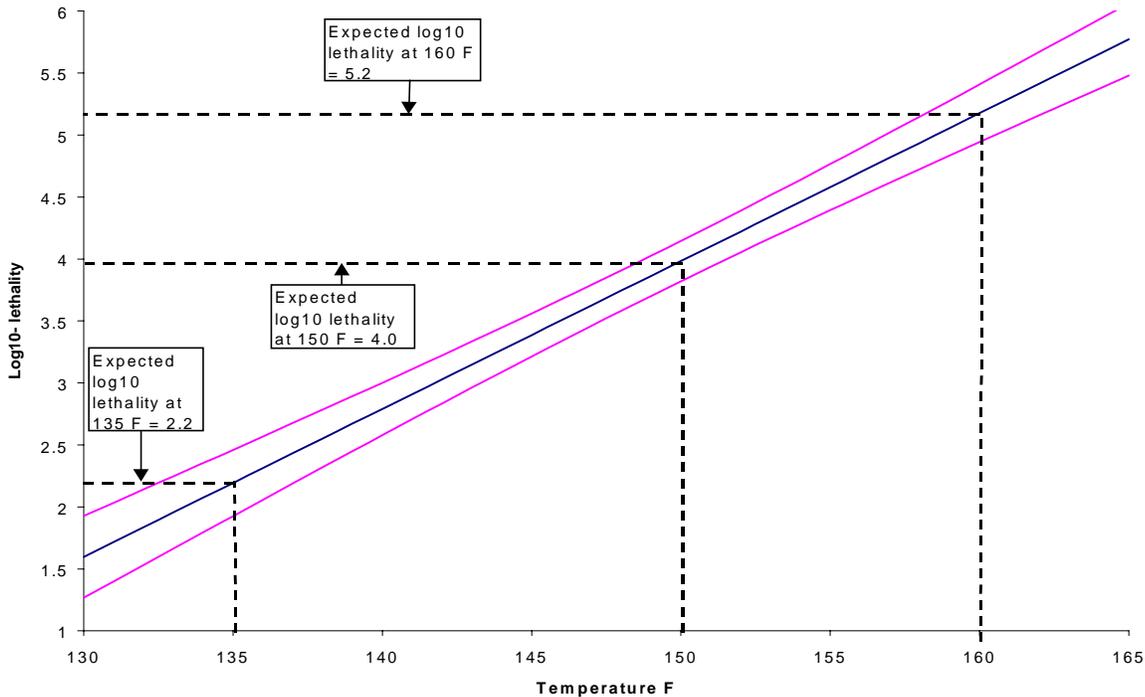
1 death tubes incubated in water baths at different temperatures (Ahmed 1995; Line 1991;
2 Doyle 1984). Results of such studies are subject to regression analyses to estimate
3 parameters D, the time for a 1- \log_{10} decrease in the pathogen level at a defined
4 temperature, and z, the change in the \log_{10} D with temperature. In addition, non-linear
5 models may be considered for estimating parameters from these data since the
6 assumption of linearity may be violated. Thermal death tube studies may be most relevant
7 to modeling decline of the pathogen in ground beef dishes other than hamburger.

8
9 Direct measurements of decline in the number of organisms in hamburger were made by
10 two groups of researchers (Juneja 1997; Jackson 1996). The Juneja (1997) study results
11 illustrated in the figure below (Marks, unpublished results) were selected for modeling
12 decline in hamburgers (Marks 1998) because various sources of experimental variability
13 were better controlled. However, the data source and modeling approach may not be
14 amenable to modeling decline for other ground beef commodities or for hamburgers that
15 vary from these experimental conditions in weight, thickness, and preparation.

16
17 Juneja (1997) inoculated 100-gram (3.5 ounce) hamburgers (27% fat content) with
18 approximately 6.6 \log_{10} *E. coli* O157:H7 and cooked in an electric skillet at 275° F (135°
19 C) until specified internal temperatures ranging from 130 to 160° F (54 to 71° C) were
20 reached. The expected number of surviving organisms thus is a function of only the
21 temperature. The standard deviation, which includes random deviations from the model
22 and hamburger to hamburger variation and was derived through an analysis of variance
23 (AOV), was 0.395 (Marks 1998). In the simulations a normal distribution with standard
24 deviation equal to this value could be used to reflect population variation. The covariance
25 matrix of the coefficients were determined and could be used in the simulations to reflect
26 uncertainty in the estimates (Marks 1998). Figure 3 depicts the expected value and 95%
27 confidence intervals for \log_{10} lethality as a function of internal cooking temperature based
28 on data of Juneja (1997). Three reference points are highlighted in the figure: 160° F (71°
29 C), which corresponds to the current recommendation by USDA for thorough cooking of
30 hamburger, and 150° F and 135° F (68 and 60° C) which represent undercooking. The
31 \log_{10} lethality declines linearly with temperature from an expected value of 5.2 \log_{10}
32 lethality at 160° F to an expected value of only 2.2 \log_{10} lethality at 135° F. The
33 significance of \log_{10} lethality can be illustrated for a hamburger containing 100 *E. coli*
34 cells. Cooking to 160° F suggests that the probability that one pathogen cell survives is
35 much less than <1% (2×10^{-4} or $100/10^{5.2}$), whereas cooking to 135° F might permit 45%
36 survival ($100/10^{2.2}$). This relationship will be useful in modeling decline specifically for
37 hamburgers prepared and cooked under the conditions of Juneja (1997).

38

1 Fig. 3: Decline of *E. coli* O157:H7 in Quarter-Pound Hamburgers with Cooking.



2 **G. Ingested Number of Organisms**

3

4 The actual number of ingested organisms would differ from the expected number of
 5 organisms for a given initial number of organisms, where the previous discussion
 6 concerned predicting the expected number of organisms as a function of time (Marks
 7 1998). To account for the variability in the number of organisms, the number of
 8 organisms at time t was assumed to follow a simple stochastic birth or death process
 9 (Bharucha-Reid 1960). The simple or linear birth process assumes that the probability of
 10 increase at a given time is proportional to the number of organisms, where the
 11 proportionality constant, μ , is independent of the time and the number of organisms. The
 12 value of μ can be thought of as the probability that a single organism will become two
 13 organisms. Independent growth between organisms was assumed. Thus, in a small
 14 interval of time, the change in the probability of n organisms can be described by
 15 Equation 4. From these equations and subsequent work (Marks, in preparation), the
 16 distribution of the random variable $N(t)$ can be determined to be the negative binomial
 17 (Marks 1998). Thus the probability that $N(t) = n$ is expressed in Equation 5 and the
 18 variance in Equation 6. Note that the variance of $N(t)$ depends on time only through the
 19 expected value. For simplicity, the expected relative growth computed from the
 20 Gompertz equation, (Equation 1) can be set equal to $\exp(\mu)$, and a value of $N_1(t_1)$ was
 21 generated using the negative binomial distribution (Marks 1998).

22

23 For decline of organisms when cooking to an internal temperature T , using a similar
 24 derivation, the resulting distribution of the number of surviving organisms is binomial
 25 with two parameters: 1) the number of organisms immediately before cooking and 2) the
 26 probability of a single organism surviving. The expected number of surviving organisms

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1 may be very small. Generating exposures of zero organisms can be problematic. A
2 conditional binomial or Poisson distribution (given that the number of organisms after
3 cooking is positive) can be used to address this difficulty and increase the efficiency of
4 the Monte Carlo simulations (Marks 1998).

5
6 Finally, after cooking, the number of organisms consumed can be generated using a
7 negative binomial distribution (Marks 1998). The approach presented here can be
8 extended to the most general situation describing birth and death together, and dropping
9 the assumption of the lack of time dependence of the probabilities of birth or death within
10 an increment of time (Bharucha-Reid 1960).

Table A.4: Predictive Microbiology Equations (Marks, 1998)

$$L(t) = A + Ce^{-B(t-M)} \quad (1)$$

{L(t) = the common log of the expected number of organisms at time t; A = an asymptotic minimum value for t=-∞; C= asymptotic difference between times t=∞ and t=-∞; B = constant; M = time for which the relative growth rate, dL(t)/d(t), is maximal }

$$l = M - \frac{1}{B} + \frac{\log_{10}(N_0) - A}{CB/e} \quad (2)$$

$$g = \log_{10}(2)e/BC$$

{ g = generation time; l = lag time; log₁₀(N₀) = common logarithm of the initial number of organisms (t=0) }

$$\begin{aligned} \ln(g) &= a + b\ln(\ln(T)) \\ \ln(l) &= c + d\ln(T) \\ MPD &= e + fT \end{aligned} \quad (3)$$

{ g = generation time; l = lag time; MPD = maximum population density, theoretically equal to constant A+C; T = temperature; parameter estimates from SUR: a = , b = -6.31, c = 9.98, d = -2.69, e = 10.08 and f = -0.014 }

$$dP_n(t)/dt = \mu(n-1)P_{n-1}(t) - \mu nP_n(t) \quad (4)$$

{ change in the probability of n organisms, dP_n(t); n = 2,3, ; initial boundary condition P₁(0) = N₀ }

$$P_n(t) = C(N_0, n) e^{-N_0 \mu t} (1 - e^{-\mu t})^{n-N_0} \quad (5)$$

{ probability that N(t) = n; C(N₀, n) is a function of N₀ and n; variance var defined below }

$$\text{var}(N(t)) = N_0 \gamma(t) (\gamma(t) - 1) \quad (6)$$

References

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