Questions/Answers
Modeling Webinar

March 5, 2018

Production of biofilms are characteristics of some microbes and often a problem in food processing surfaces. Where is this parameter treated in the model?

Biofilm is one of the factors determining the physiological state of organisms, relating to the "history" of the initial level. Indeed, in the biofilm environment cells are often more stressed and become stronger than planktonic cells. This stress-hardening "history" of a population can affect the value of the decimal reduction time during inactivation. For example, it can be modelled by a different physiological state parameter, different D-value/z-value (if linear inactivation), and different δ-value and β-value (if non-linear inactivation).

What is the 'right' strain for validation? Is it the most resistant, or a strain with an average resistance?

A single strain with average resistance is not suitable, because there is huge variation in D-value, often by a factor of 10! Consequently, inactivation of the average organism by 6 logs, means that the most resistant strain (the extreme) would be inactivated by only 0.6 logs. Even if this strain is initially 1 percent of the overall population, this would result in less than a 3D overall reduction. Granted, the very distinctive extremes are almost never encountered, so validating with such strains might be a too "fail safe." Generally using the most resistant is safest, except if you clearly have indication that this is really a very extreme strain that is really almost never found in practice. A common approach to account for variations in survival among strains in challenge studies is to use three to five strains either individually or in combination (*i.e.*, a cocktail); for more information on this approach, we refer the audience to section 4.3 "Type and number of strains" in the NACMCF (2010) guidance. Multiple strains may also be necessary for model validation, so long as researchers are cognizant of the potential errors mentioned related to using those with average resistance and adjust accordingly. For model validation, use of too many strains at once in the form of a "cocktail" can confound interpretation, and so using multiple strains individually in parallel is most preferred if possible.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF). 2010. Parameters for determining inoculated pack/challenge study protocols. *J. Food Prot*. 73:140-202.

Do you recommend avoiding the use of non-serotype strains of *Salmonella* (e.g., wild-type strains) for growth studies or inactivation studies?

Yes, it is preferable to use strains that were isolated from the actual matrices being studied, or that are in some other way associated with the food system or model purpose. Such isolates often exhibit the more extreme tolerance to the stresses being modelled and are the true "culprits" in practical situations. It is appropriate and wise to characterize such wild-type isolates using phenotypic analysis as well as DNA sequencing techniques. It is also worth noting that many laboratory stock strains (e.g., outbreak strains) were once considered new, and may still offer valid options for use, particularly when culture conditions can be employed to render populations resistant or hardy.

The food safety team could have chosen a z-value, *i.e.* 9⁰C, from the literature for *Lm* for the product type and determine the D-value for a temperature higher than 65⁰C in the PMP model, instead of doing confirmatory *Lm* testing.

There are many ways a food company can use predictive modelling as well as other methods to demonstrate a food product is safe, wholesome and meets regulatory requirements. Indeed, also using a generic z-value would have been a possibility, especially if the food company had the appropriate data to use these models. A food company has flexibility to use a variety of means to provide the scientific evidence that process deviation has not impacted food safety. Now, there may be other regulatory requirements or actions that may be needed based on the situation, which could include evaluation of available data for the product and the situation, predictive modelling, product testing, and other options. Food companies would need to work with their regulators to ensure those requirements or actions are met or conducted.

Is the D value calculation start from the time when start heating the product until it reaches the expected temperature and the time it takes to reduce one log reduction? If the product is initially cooled for long time in a vacuum (*e.g*. freeze-dried) and then heat to higher temperatures within the same process to achieve 5-log reduction, what should be the starting time for the calculation of D value?

With dynamic approaches you can determine integrated lethal effects (*e.g*. with the shown AMI Foundation Process Lethality Determination Spreadsheet (http://meatpoultryfoundation.org/content/process-lethality-spreadsheet). Since temperature works exponentially on the death rate, however, mainly the "holding part" and the time just before and after the holding determine, generally, almost all of the lethality (except for slow cooking). Drying can increase the resistance of the organisms, and should be considered in selecting appropriate D- and z-values to use.

To cover the strain diversity, do you recommend using a cocktail of strains? Please provide recommendations.

Generally, I do not like that much cocktails since results are difficult to interpret. For validation "sec" maybe it could be considered, but I prefer to do the validation with for example two or three strains in parallel, so not in cocktail. It is of course more work, but gives much better insightful info. We note that some of the published heat resistance studies were conducted using a cocktail of strains, which may reflect in part practical considerations. An important distinction is that combined-strain cocktails are appropriate for other forms of validation, if not for model development.

The ICMSF Sampling Plan Tool requires a defect rate to be determined or assumed. What did the company do in determining that parameter?

The intent of the practical examples was to demonstrate how predictive modelling could be used by a food company to address food safety event. The intent was not to discuss the International Commission on Microbiological Specifications for Foods (ICMSF) Sampling Plans *per se*. The example of ICMSF case use was used to provide information that would "complete" the practical example. The ICMSF Case 11 was selected for a non-growth product (frozen) with a serious hazard (conditions cause no change in concern). We note that assuming a standard deviation of 0.8, case 11 testing would lead to lots having a geometric mean concentration ≥1

CFU/83g (or an arithmetic mean of ≥1 CFU/33g)[1] will be rejected with at least 95% confidence. The decisions Company XYZ made, as discussed in the webinar, was based on FSIS regulatory requirements. As such FSIS guidance documents were utilized as resources as Company XYZ worked through their food safety event.

The FSIS Guidance document utilized was *FSIS Compliance Guideline: Controlling Listeria monocytogenes in Post-lethality Exposed Ready-to-Eat Meat and Poultry Products* (January 2014). The primary discussion regarding ICMSF Sampling Plans for *Lm* begin on page 119 of the Compliance Guideline.

If you would like to learn more about ICMSF and their Sampling Plans, please see http://www.icmsf.org/.

ICMSF. 2018. Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. Second Edition. ISBN 978-3-319-68458-1. Springer International Publishing AG.

The Weibull model has been around for many years. Has it been used in dynamically changing (rising) temperature conditions? Are the food safety authorities ready to use it for process evaluation?

Indeed, the Weibull model is used often to *fit* data, and fits of course curved data better than the log linear model. But it is much more cumbersome for *prediction* in practical condition. Is the curvature at different (practical) initial levels equal? And indeed, in dynamic condition, is it also performing well in dynamic conditions? There is some literature on dynamic use, but I do not think it has very often be applied and taken up by food safety authorities for process evaluation. Often selecting the right strain is much more important than to include the curvature.

How we can predict the growth of a pathogen in a food product using a model with a buffer solution?

Growth is mentioned? Probably inactivation is meant. Or was this related to staph growth? Growth will be investigated in foods or in a broth. Broth is a "model" environment for a food. On micro-scale environment an organism will see something like a "broth". Inactivation can be done in a food, in a broth or in a buffer solution. General practice is to simulate as much as possible conditions like T, pH, a_w, etc. in a buffer, or broth, to investigate the kinetics of growth and inactivation. Thereafter it can be validated in foods. In many cases this works fine, but sometimes things are unexpectedly different (like shown for *Salmonella* in chocolate in the webinar. Or if you would determine the growth of *Listeria* in gin). It will not always work, but often it will. The approach to make only product specific models is not very efficient (but to blindly believe in broth models is dangerous).

How can we use PMP when we don't have data for the initial contamination level?

Yes you can, but be prepared to estimate an initial level or if you determine several scenarios of initial level. Some history or literature data can back up these scenarios. Many tertiary models for growth set the default at 3-log, but prior knowledge about the product and historical data

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[1] The geometric mean of the distribution is better suitable to characterise the distribution, the arithmetic mean is more relevant to describe the risk. A distribution with equal geometric mean (or mean log), but wider distribution can have a much larger risk. This is better accounted for in the arithmetic mean (or log mean)
(ICMSF second edition of Book 7)

might justify altering this initial level. Typically, growth rate is only marginally affected by this initial level, but the lag time could be affected when the initial level is lower than 1-2 log.

If we have some minor ingredients in the food (like spices, salt, or sugar), and the model was developed for a food or conditions in which those in which those minor ingredients were not considered, are the predictions reliable in those cases?

Dr. Zwietering's final slide says it best: "Many models are correct…but they are not perfect." With every change, you do not know….. But the best guess is it does. Minor ingredients like spices do often not change that much the kinetics of inactivation but do influence very much the initial load of organisms, and large levels of spices will even influence (inhibit) growth. In such cases, models can only take you so far, and you should consider the need for data collection, including the use of challenge studies.

What is your main source of information for Meta-Analysis?

The main source is ICMSF Book 5, review articles, and further scientific literature. It needs to be well traceable results, decently carried out experiments.

How big should the death curve be to calculate a D-value? Is 1-log enough or should you follow the death at a certain temperature for a 5 -7 log kill?

Even for linear inactivation, 1 log reduction is very small to determine a rate, so more logs give much better accuracy of the "average" D-value. Using only a 1-log span of the inactivation plot will surely be inadequate when curvature is involved. 7-log kill means you have to start at 8 logs initial contamination. Here maybe the level is so high that it influences inactivation. So 1 is too small, 7 maybe too high, and practically speaking may include the tail of a bi-phasic curve, but 4-6 would be a reasonable range. Again, this is a general opinion not a "law," and it also depends on the target. Examples of target reductions are 5D for apple juice, 6D for milk, 12D for retorted cans. For *Clostridium botulinum* in cans, we recommend using a larger range than for *E. coli* O157:H7 in juice. Some examples of inoculum level:

- *E. coli* O157:H7 in apple juice and other juices, at ~ 5 log CFU/g (Mazzotta 2001)
- *Salmonella* in peanut butter, at 7-8 log CFU/g (Ma et al. 2009)
- *L. monocytogenes* in broth, at 7-8 log CFU/ml (Aryani et al. 2015)

Aryani, D. C., H. Den Besten, W. Hazeleger, and M. Zwietering. 2015. Quantifying variability on thermal resistance of *Listeria monocytogenes. Int. J. Food Microbiol*. 193:130-138.

Ma, L., G. Zhang, P. Gerner-Smidt, V. Mantripragada, I. Ezeoke, and M. P. Doyle. 2009. Thermal inactivation of *Salmonella* in peanut butter. *J. Food Prot*. 72:1596-1601.

Mazzotta, A. S. 2001. Thermal inactivation of stationary-phase and acid-adapted *Escherichia coli* O157: H7, *Salmonella*, and *Listeria monocytogenes* in fruit juices. *J. Food Prot.* 64:315-320.