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ABSTRACT

For the validation of control measures in a food chain, the FSO concept can be used, to structurally combine the initial level, reduction and increase of contaminants. The impact of taking into consideration both the level and the variability of these factors on the proportion of product meeting the FSO has been investigated. In this manner it can be examined where in the process the main factors are found to control the proportion of product meeting the FSO. Furthermore equivalence in performance, either by reducing the level or the variability in a level, is investigated. Both experimental and statistical aspects are described that can together be combined to support the confidence that a process can conform to a set FSO.

Key Words: food safety objective, HACCP, validation, verification

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1. Introduction

Validation of food processes is defined as establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a food product meeting its pre-determined specifications and quality attributes (Keener, 2006), or as determining if an intervention, when properly applied, will effectively control the microbial hazard(s) (Swanson & Anderson, 2000). So validation is the collection and evaluation of scientific and technical information to determine if the process (treatment), when properly applied, will effectively control the microbiological hazard, or in other words, if the process criteria can reliably deliver a specified performance objective. The overall effectiveness of the control measures should be validated according to the prevalence of microbial hazards in the food of concern, taking into consideration the characteristics of the individual hazards(s) of concern, established food safety objectives/performance objectives and level of risk to the consumer (CAC 2007). Validation focuses on the collection and evaluation of scientific, technical and observational information. In order to take full advantage of the flexibility that an outcome based risk management system offers, it is important to be able to demonstrate that the selected control measures actually are capable, on a consistent basis, of achieving the intended level of control. Guidelines for the validation of food hygiene control measures have been proposed by Codex (CAC, 2008). Validation is different from verification and monitoring; verification is used to determine that the control measures have been appropriately implemented, showing that the system is operating as designed, while monitoring is the on-going collection of information on a control measure at the time the control measure is applied to ensure the HACCP system is operating as intended.

Food producers design their processes to meet performance objectives (PO), which can be set at specific points throughout the food chain to assure food safety. Regulatory authorities are concerned with whether a group of products or the consequences of a series of processing steps at the time of consumption meets the food safety objective (FSO) in order to be certain that those foods achieve levels that are consistent with the appropriate level of protection (ALOP).

Various control measures include the appropriate selection of food materials and ingredients at the initial stage of food processing or food chain, and intensive protocols to reduce or eliminate the contamination by washing, heating, disinfecting, and many other measures. Control measures are also designed to prevent possible or predicted increases of microbiological hazards during transportation and storage, by cross-contamination during processing of the foods, or even by re-contamination after processing and during packaging, distribution, retail and consumer storage.

Control measures need to be validated to determine whether the products will meet the objectives, however, depending upon the standpoints, different elements of the food industry may take the role of validating the (critical) control points (CCP's). Food producers may wish to validate the control measures taken in the processes under their responsibility, and validation should be focused on the ability of the control measures to meet the designated PO. For appropriate validation of a process, both within-lot and between-lot variability must be considered.

On the other hand, control measures to be validated under the responsibility of regulatory authorities cover all control actions in the system for multiple companies, products and process controls, including consideration of between-lot variability. In this case the validation is targeted at assessing the established POs and FSOs.

In this paper, the ICMSF equation (ICMSF, 2002) for the prevalence and levels of microorganisms from the initial contamination (H_0), reduction (ΣR), growth and re-contamination (ΣI), and factors influencing these are considered throughout food production until consumption, and in their role in meeting the FSO by the equation $H_0 - \Sigma R + \Sigma I \leq$ FSO. Stochastic aspects of the parameters are taken into account as well as deterministic values. This is illustrated in the following sections with various examples of the use of data to validate one or a series of processes of food production for practical application, including statistical insights.

2. Considerations for validation

Processes can be validated through the use of predictive modeling, microbiological challenge studies, studies to show that certain limiting parameters (e.g. pH<4.5) are achieved and/or use of default criteria (safe harbors, like 72°C, 15s for pasteurization of milk, or 121°C 20 min. for sterilization). Not all these need to be used, however, often several sources of information can be used together to supply sufficient evidence. When a safe harbor approach is used, it is not normally necessary to conduct validation studies for that process. For example, a safe harbor for milk pasteurization is to deliver a

minimum process of 72°C for 15s; this process criterion has already been validated and therefore can be implemented by processors without re-validation of the process. The process would still need to be verified and monitored by the processors.

3. Validation of control measures

When determining the processing criteria (PC) required to achieve a desired PO, generally microbiological studies begin on a laboratory scale, move to a pilot plant scale and then are finally validated on a commercial scale, when possible or necessary. Inactivation kinetic studies can be conducted over a small range of treatments (a unique combination of factors and their levels; for example pH 6.5 and 70ºC) or over a broad range of treatments that would allow for the development of microbiological predictive models. Several good microbiological predictive models are available, including the USDA Pathogen Modeling Programs, which can be found at http://ars.usda.gov/Services/docs.htm?docid=6786 and COMBASE, which can be found at http://wyndmoor.arserrc.gov/combase/. Challenge studies can also be used to determine processing criteria; although they are more limited in scope than models, they are often used as a way of validating the model predictions. Finally, on a commercial scale, challenge studies can be conducted utilizing nonpathogenic surrogate microorganisms; shelf life studies with uninoculated product can also provide useful information for validating a process.

While microbiological challenge testing can also be used for determining the stability of a product with regards to spoilage over the intended shelf life, the remainder of this discussion will focus on product safety with regards to pathogens relevant to foods.

In the following sections, each of the terms in the ICMSF equation, the initial contamination (H_0), reduction (ΣR), growth and re-contamination (ΣI), and factors influencing these are discussed sequentially, including data needs, some experimental considerations, and especially effects of their variability.

3.1 Determining the initial level (H_0), standard deviation and distribution

The design of the food process will determine the importance of incoming material for product safety. The main source of the pathogen of concern may be from a major or minor ingredient, one incorporated in the initial processing steps, or one added later by recontamination. It is important to understand which of the ingredient(s) may harbor the pathogen as well as to understand if there is seasonal effect on the level of the pathogen present [for example the number of lots of ground beef positive for *E. coli* O157:H7 increase over the June-October period in the USA (USDA-FSIS, 2009)]. The geographical source of the ingredient may also play a role in the likelihood of whether a certain foodborne pathogen is present in the raw ingredients. If contamination is not avoidable, the goal is to develop specifications and criteria for the incoming material that will limit frequencies and/or levels of contamination and lead to achievement of the final PO and FSO, in conjunction with the PC for the other steps in the food process. The

176 microbiological specifications for accepting the incoming materials may include the
177 acceptable proportion above a limit or the mean level and standard deviation.
178
179 Information for validating that incoming materials meet required specifications can come
180 from baseline data from government agencies; documentation from suppliers that
181 specifications are met (supplier provides validation and end product testing); baseline
182 data from the processor's experience; or test results of incoming lots.
183
184 *3.2 Inactivation Studies and Modeling of Kinetic Inactivation (ΣR)*
185
186 *3.2.1 Modeling and Laboratory Studies*
187
188 A microbiological predictive model can be defined as an equation that describes or
189 predicts the growth, survival or death of microorganisms in foods. In food microbiology,
190 these models are often empirical and not based on biological mechanisms; in other words
191 they simply relate the observed microbial growth, survival or death responses to the
192 levels of the controlling factors. Empirical models should not be used outside the range of
193 the factors used to create them because there is no underlying principle on which to base
194 extrapolation. Hence, we must carefully consider the range over which they will be used
195 before beginning experimentation (Legan, Stewart, Vandeven, & Cole, 2002). Models
196 that can predict the rate of death of pathogens can be used to design safe and effective
197 processes. A practical guide to modeling, supported by references to primary sources of
198 modeling information is discussed by Van Gerwen & Zwietering (1998), Legan et al.

(2002), Ross & McMeekin (2003), McKellar & Lu (2004), and Whiting & Buchanan (2007).

When designing microbial inactivation experiments, kinetic studies measuring changes with time are preferred as they provide more information than end-point measurements. Additionally, kinetic studies offer flexibility and a depth of understanding that is not obtainable via end point measurements alone (Legan et al., 2002). Therefore, experimental points should be selected to allow the true nature of the microbial response to the lethal agent to be determined. The inoculation level should be sufficiently high to demonstrate the performance criteria without the need for extrapolation, if practically possible. Points should be spaced over the time interval to allow any curvature in the response to be described; ideally this typically involves 10-12 points over a 6-7 $\log_{10}$ (or greater) reduction in population size. This implies an inoculation level of at least 10^8-10^9 CFU/ ml or g. A zero-time point is critical and equidistant time intervals are often selected, except for very slow inactivation rates where intervals that increase geometrically between samplings are often useful.

3.2.2 Growth (ΣI)

The population of a pathogen will increase during storage periods if the food, storage temperature and packaging conditions support growth. Storage periods may occur for raw ingredients or at intermediate points during the manufacturing. After manufacture, there

222 will be a series of storage periods through distribution, including at the retail level, in the
223 home and/or in food service operations. Generally, public health cannot be assured unless
224 the potential for growth of pathogens is minimized. Nevertheless, if the pathogen is not
225 completely inactivated and growth is possible, then an accurate estimation and validation
226 of the amount of growth during storage and distribution that would be expected in normal
227 and occasional abuse becomes an important component in validating that the FSO is
228 achieved.
229
230 As previously described for validating microbial inactivation processes, estimates for
231 growth may be obtained from a variety of sources including the literature, models and
232 challenge tests (Scott et al., 2005). Increasing reliance is given to different studies as the
233 experimental conditions more closely reflect the actual conditions of the food, e.g.,
234 laboratory vs. pilot plant or pure culture vs. food with spoilage flora. For satisfactory
235 validation of a pathogen's growth in a food, challenge tests with the normal background
236 flora will be the authoritative source of information. Models and broth studies can
237 provide support for evaluating minor changes in formulation and strain differences and
238 for interpolating to conditions not explicitly tested in the challenge tests. Applications of
239 predictive models in food microbiology include models that predict the growth rate of
240 bacterial pathogens in response to product or environmental factors such as water activity
241 (a_w), temperature or pH. Growth models can be used to design safe product formulations,
242 to set appropriate storage conditions and to explore the maximum interval between
243 cleaning and sanitizing for process equipment.
244

Factors that should be considered when evaluating growth data include the strain(s) used, surrogates, physiological state of the inoculum, method of inoculation, degree of simulation of the experimental or pilot plant conditions to the commercial process, inclusion of all environmental factors in the food (pH, a_w, acid anions) and external factors (temperature, packaging), and inclusion of the spoilage flora. Detailed information on the design and implementation of microbiological challenge studies (also referred to as inoculated pack studies) has been reported by IFT (2001) and Scott et al. (2005).

3.2.3 Recontamination (ΣI)

If a food process includes pasteurization or another lethal step that eliminates the pathogen, then all of the pathogens present at consumption are the consequence of recontamination. Foods processed to deliver 6 to 8 $\log_{10}$ reduction of the pathogen will result in a very low frequency of contaminated packages after such a process. For example a product containing initially a homogeneous contamination level of 100 cfu/g, in a 100 g package will contain 0.001 cfu/package after a 7 $\log_{10}$ reduction, meaning 1 in 1000 packages contaminated with one (or a few) cells. When determining whether such a food meets a PO at a further step or FSO, calculation of the food process begins after the lethal step. The appropriate parameters to consider are the frequency and level of contamination; essentially, they form a new H_0. Little literature data exists for guidance concerning frequencies and levels of recontamination and few applicable models have been developed to estimate the results of recontamination. Sufficient sampling of the

specific process at this step or at a subsequent step with a back calculation is the only way to obtain valid data on recontamination. A food process without a lethal step and with several potential points of additional recontamination is difficult to predict. Sufficient sampling of the food after the last point of recontamination is a possible way to validate whether a PO or FSO is being achieved. Another approach to controlling contamination is environmental monitoring and monitoring of food contact surfaces and integrating this information into the sanitation program. Other factors to consider are packaging integrity and proper training on handling practices by employees.

4. Validation of FSO compliance, probabilistic aspects: The effect of variability in processing on non-conformance to an FSO/PO

4.1 Introduction

One way to show compliance to an FSO is by using the ICMSF equation:

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO} \qquad (1)$$

By combining information from different sources concerning the initial level (H_0), reductions (ΣR) and increases (ΣI) of the microbiological hazard through the food production and distribution chain, it can be determined if the FSO or PO will be reliably met. It can also be determined how variability in the steps in the process/food chain influences the ability to meet the FSO.

In the following examples, the impact of including the effect of statistical distributions for H_0, ΣR and ΣI on the hazard level and the percentage non-conformance (percentage of product above the PO or FSO) is calculated. First, the problem will be solved by a point-estimate approach. Then the impact on variability in the initial levels, processing (using as an example of washing produce to achieve a reduction in the pathogen of concern) and growth during distribution (increase) in meeting the PO and FSO will be determined. The process and product example is fresh cut, washed and packaged lettuce where *Listeria monocytogenes* is the target pathogenic microorganism of concern. For illustrative purposes, it is assumed that to reach an ALOP, a maximum exposure of *L. monocytogenes* of 100 cfu/g (FSO = 2 $\log_{10}$ cfu/g) for ready-to-eat foods is set.

4.2 Point-estimate approach

In the paper of Szabo, Simons, Coventry & Cole (2003), estimates are made of the initial contamination level of *L. monocytogenes* on pre-cut lettuce, reduction using sanitizing rinses and the increase in levels of the pathogen after packaging and during storage and distribution. For a given initial level of *L. monocytogenes* on lettuce and an expected level of growth (increase) during storage and distribution, the necessary reduction level, in order to achieve a given FSO, can be determined. For example, in Szabo et al. (2003), it is given that for an H_0 of 0.1 $\log_{10}$ cfu/g of *L. monocytogenes* and for a potential increase of ΣI = 2.7 $\log_{10}$ cfu/g during storage for 14 days at 8°C, a $\Sigma R \geq 0.8$ $\log_{10}$ cfu/g is necessary to achieve the set FSO of 2 $\log_{10}$ cfu/g:

$$H_0 - \Sigma R + \Sigma I = 2.0 \quad (2)$$

$$0.1 - 0.8 + 2.7 = 2.0$$

The average process can therefore be considered to exactly achieve the FSO.

4.3 Including variability in the process

Now let the standard deviation, *s*, for ΣI be 0.59 (Szabo et al. 2003; with ΣI, the $\log_{10}$ increase of the levels of *L. monocytogenes* being normally distributed), but still consider the H_0 and ΣR levels as exact. Due to the variability of the increase in levels of *L. monocytogenes* (the distribution), the producer must target a lower average initial level in order to reduce the proportion of defective units (units with *L. monocytogenes* levels higher than the FSO). If the same limit (i.e. FSO = 2 $\log_{10}$ cfu/g) is considered, 50% of the products would not conform to the FSO. The level of reduction needed to achieve a certain level of conformity is given for various other examples in Table 1 which shows the fraction of servings that does not meet the FSO given different reductions (ΣR). The greater the reduction, the lower the frequency of non-conforming servings. This frequency of non-conformity is a risk managers decision.

4.4 Including variability in the process for all process stages

In nearly every process all three variables, H_0, ΣI, and ΣR, will have a distribution with values as for example given in Table 2. The resulting final distribution (which describes

the distribution of levels of *L. monocytogenes* in packages of fresh cut lettuce at the point of consumption) can be described by a mean value that is equal to the sum of the means of H_0, ΣI, and ΣR. The mean, however, is not a correct indicator of the risk, without representing also the variance. The variance of the total distribution is equal to the sum of the variances (the final standard deviation is the square root of the sum of the squares of the variable standard deviations (Snedecor and Cochran, 1989)). The distributions are represented graphically in Figure 1.

Given this distribution of outcomes, the proportion of packages of lettuce not meeting the FSO can be determined, which, in this example, is 0.2% (This proportion can be determined from the area under a normal curve that exceeds the FSO using the Excel or similar function, following the procedure as given in the footnote in Table 1).

4.5 Ineffective washing step

Assuming that the lettuce washing step is not effective ($\Sigma R = 0$) in reducing the level of *L. monocytogenes* (Table 3, Figure 2), the effect on the overall effectiveness of the process can be determined. We can see that the mean level of *L. monocytogenes* in packages of fresh cut lettuce is higher (from –1.2 to 0.2) and the overall standard deviation of the level decreases (from 1.112 to 0.994) compared to the previous calculation (Table 2). The proportion of packages of lettuce having levels of *L. monocytogenes* at the point of consumption that are above the FSO (2 $\log_{10}$ cfu/g) increases to 3.5 %. Note that the standard deviation does not differ much since the overall

356 standard deviation is mainly determined by the largest contributors, which, in this case, is
357 H_0.
358
359 In this example, due to the ineffectiveness of the washing procedure, there is a higher
360 proportion of packages (3.5%) of lettuce with levels of *L. monocytogenes* which do not
361 meet the FSO (2 $\log_{10}$ cfu/g), therefore this may be a condition under which a producer
362 would not want/be able to operate.
363
364 *4.6 Effect of shortening the shelf life of the packaged lettuce*
365 If the product supports growth of the pathogen, the length of the shelf life can influence
366 its impact on public health. In this example, the effect of a shorter product shelf life on
367 the proportion of lettuce packages that do not meet the FSO is evaluated by reducing the
368 predicted value for ΣI (Table 4, Figure 3). If the product is stored for 7 days at 8°C, rather
369 than 14 days, the increase in *L. monocytogenes* is estimated to be 1.9 with a standard
370 deviation of 0.56 compared to the previous growth of 2.7 (Szabo et al., 2003).
371
372 By decreasing the shelf life, which decreases the extent of growth of *L. monocytogenes* in
373 the packages of fresh cut lettuce (and very slightly decreases the standard deviation), the
374 proportion of packages of lettuce that do not meet the FSO is decreased to 0.013%.
375
376 *4.7 Impact of more effective process control*
377 The impact of better process control on the proportion of packages of fresh cut lettuce
378 that meet the FSO can be evaluated. If, for instance, raw materials with less variability

(standard deviation) in the levels of *L. monocytogenes* present on the lettuce can be obtained by supplier selection, changing supplier specifications, or better input control, the standard deviation of H_0 can be reduced (Table 5, Figure 4; compare with Table 2). By this better process control, the average level of *L. monocytogenes* on the raw materials remains the same, but the final standard deviation goes down, resulting in a lower percentage of packages of fresh cut lettuce that do not meet the FSO (going from 0.2% to 0.012%) or, conversely, a larger percentage of product now meets the FSO, comparable to a reduction in shelf life to 7 days (Table 4).

4.8 Ability to meet the FSO at the same level of performance by different means

It can also be determined how an equivalent outcome can be achieved (same proportion of the products meeting the FSO), in this instance only 0.2% of packages of fresh cut lettuce not meeting the FSO (see Table 2), by reducing the variability of one of the inputs. For example, if the variability (standard deviation) of the initial levels of *L. monocytogenes* on the raw materials is reduced from 0.8 to 0.4, the required level of reduction of *L. monocytogenes* during the lettuce washing step (ΣR) could be decreased from 1.4 to 0.7 while still achieving the same proportion of product that meets the FSO (Table 6).

4.9 Relation between log mean value, standard deviation and proportion of products that do not meet the FSO (levels of L. monocytogenes *at the point of consumption are greater than the FSO)*

The proportion of products in which the level of *L. monocytogenes* is above the FSO is determined by both the mean log levels and the standard deviation of the combined distributions for H_0, ΣR and ΣI. Different combinations of the mean and standard deviation resulting in the same overall proportion of products not meeting the FSO can be calculated, and the results are shown in Figure 5.

The values in Figure 5 can also be determined by calculation, since the probability that a value is higher than a certain level can be determined with the *z*-score (Snedecor and Cochran, 1989). For an FSO of 2, the calculation becomes $x+z \cdot s=2$, so for a given mean value x, the s value that gives a certain probability to surpass the FSO equals $s=(2-x)/z$, with z the value determined by the probability level (Table 7). For example, at the line in figure 5 for 0.05 (5%) the probability is described by

$$s=(2-x)/z=(2-x)/1.645 \quad (3)$$

In Table 1 the levels of 1.03, 0.63, and 0.18 and with a standard deviation of 0.59 correspond to a probability level of 0.05, 0.01, and 0.001 respectively: (2-1.03)/1.645=0.59 (*z*-value for 0.05 probability level); (2-0.63)/2.326=0.59; (2-0.18)/3.09=0.59

The effect of reducing the standard deviation in raw materials, or elsewhere, can be converted in a log gain by this approach. Having two different processes that have equal probability to surpass the FSO it can be derived from $x_1+z \cdot s_1=x_2+z \cdot s_2$ that:

$$\Delta x = z\Delta s \quad (4)$$

resulting in a formula that can provide an equivalent change in level following a reduction of the standard deviation.

For example, for an FSO set with a confidence level of 99% (meaning that 99% of the product units do confirm to this level), z equals 2.33 resulting in:

$$\Delta x = 2.33\Delta s \quad (5)$$

Therefore, a 0.1 $\log_{10}$ decrease in the standard deviation is equivalent to a 0.233 $\log_{10}$ decrease in average level.

To calculate the difference in equivalent reduction necessary to achieve a 0.2% defective rate, for an H_0 with a 0.8 standard deviation (Table 2) to a H_0 with a 0.4 standard deviation (Table 6) we can perform the following calculation:

By reducing the s in H_0 from 0.8 to 0.4, the standard deviation of the overall level will reduce from 1.112 (sqrt(0.8^2+0.5^2+0.59^2), see Table 2) to 0.8707 (sqrt(0.4^2+0.5^2+0.59^2) see Table 6), so this translates to a "gain" in log mean of 2.878*(1.112-0.8707)= 0.697 logs. Instead of a 1.4 $\log_{10}$ reduction (Table 2), a 0.7 $\log_{10}$ reduction is sufficient (Table 6).

So how much one could change the mean concentration while retaining the same proportion of defective products, depends both on the change in overall standard

443 deviation, but also on the conformity level (e.g. 1% proportion of product that does not
444 meet the FSO) set (Figure 5).
445
446 **5. Conclusions**
447 From the various examples presented in this paper, the impact of taking into
448 consideration both the level and the variability of H_0, ΣR, and ΣI on the proportion of
449 product meeting the FSO has been demonstrated. With this consideration, a deeper level
450 of understanding is obtained of the influence of both the levels and variability of the
451 initial microbiological load on the incoming materials; the level of process control
452 achieved for those processes which reduce the level of the microorganism of concern;
453 and the level and variability of the increase of the pathogen of concern during storage and
454 distribution. A food manufacturer can determine where in the process they can have the
455 biggest impact on ensuring that the appropriate proportion of product meets the FSO (i.e.
456 decreasing variability of a lethal process vs decreasing the initial level of the
457 microorganism of concern on the raw materials).
458
459 The following information about the assumptions made with these calculations should be
460 recognized:
461 • All variables are assumed to be log normally distributed. So the log of the
462 variables as used in the FSO equation is normally distributed. This makes also
463 their sum in the FSO equation having a normal distribution. If values have other
464 distributions, Monte-Carlo type calculations are necessary to determine the
465 statistical distribution of the sum. It should be noted, however, that for initial

levels, $\log_{10}$ increase and $\log_{10}$ reduction, a lognormal distribution is often found (and described) in literature, although in actuality the distributions may not precisely meet this assumption they are usually sufficiently close.

- In this example, it was assumed that calculations hold even for low levels. It should be noted that, for instance, a product unit of 100 g with an initial pathogen level of 2 $\log_{10}$ contains, after a 6 $\log_{10}$ inactivation step, a level of -4 $\log_{10}$. This is not a level of -4 $\log_{10}$ in all products, but in reality a level of 1 microorganism in 100 g unit (-2 $\log_{10}$) for only 1% of the units. The other 99% of the units are free of the microorganism. This can, in certain cases, have implications that should be investigated. Because microorganisms are discrete entities, it is important to check that a situation does not arise with less than one microorganism per container or package. If this occurs, Poisson distributions must be considered for the fraction of packages that would contain no microorganisms.
- If no data on standard deviation are available, but min/max-data are present, representing the range where 95% of the data will be, the standard deviation can be estimated by s=0.5*(max-min)/1.96.
- Products with a same level of conformity (equal probability to be above a certain FSO) but different standard deviations of the final level of pathogens, could have a different risk of illness, depending on the dose-response relation.

Both experimental and statistical aspects have been described that can be combined to support the confidence that a process can conform to a set FSO (i.e. validation). The effects of variability in initial level, reduction and/or growth is illustrated and it is shown

how to determine an equivalence in performance, either by the level or the variability in a level. Given the above mentioned assumptions in certain cases this analysis may be needed to be followed up by a more detailed risk assessment.

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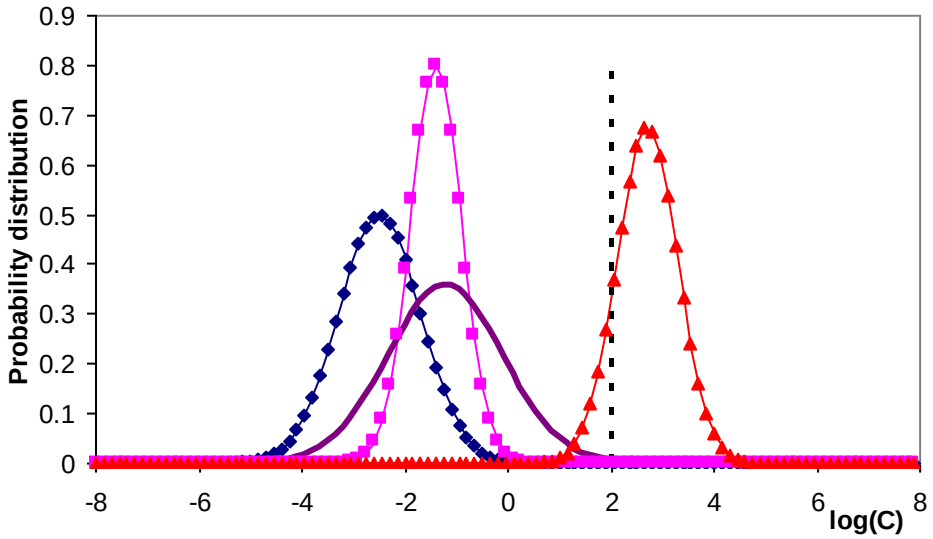

557

558 Figure 1. Probability distribution of the initial level (H_0, ♦), reduction (-ΣR, ■), and

559 increase (ΣI, ▲) of *L. monocytogenes* on fresh cut lettuce and resulting overall

560 distribution (solid line; meaning the distribution of the levels of *L. monocytogenes* in

561 packages of lettuce at the point of consumption), following the input values in Table 2.

562 Proportion of packages that do not meet the FSO (dashed line) is 0.20%.

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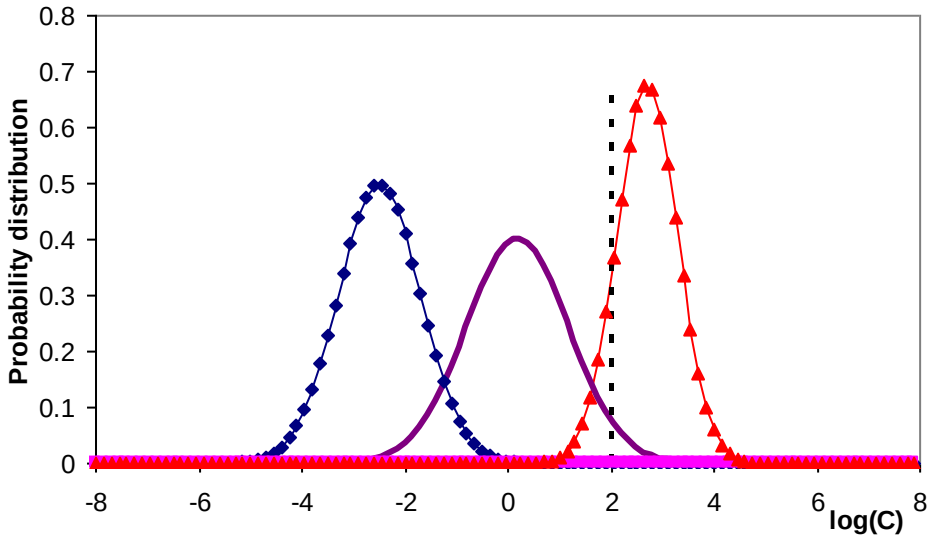

565

566 Figure 2. Probability distribution of the initial level (H_0, ♦), increase (ΣI, ▲) and

567 resulting overall distribution (solid line; meaning the distribution of the levels of *L.*

568 *monocytogenes* in packages of lettuce at the point of consumption) for a process in which

569 the washing step is not effective in reducing the levels of *L. monocytogenes* (ΣR=0),

570 following the input values in Table 3. Proportion of packages that do not meet the FSO

571 (dashed line) is 3.5%.

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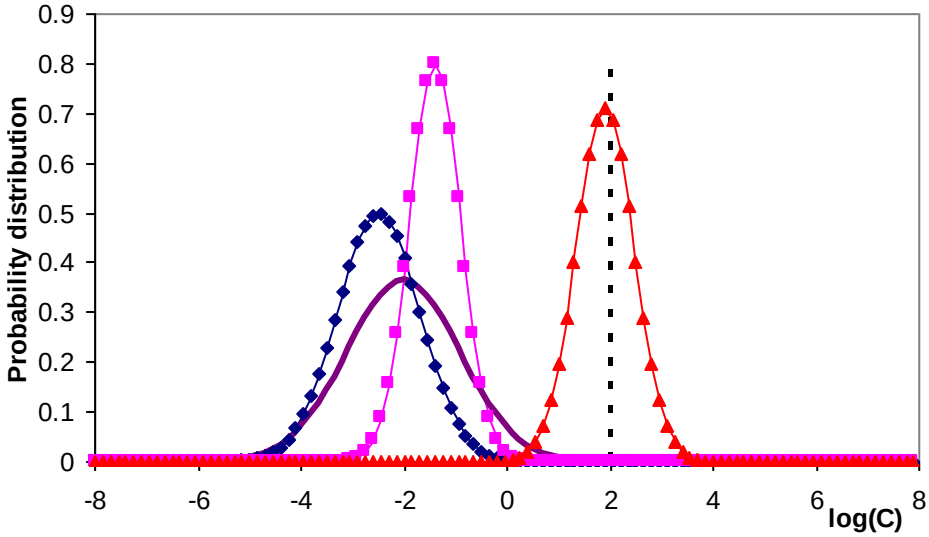

573

574 Figure 3 Probability distribution of the initial level (H_0, ♦), reduction (-Σ*R*, ■), and

575 increase (Σ*I*, ▲) and resulting overall distribution (solid line; meaning the distribution of

576 the levels of *L. monocytogenes* in packages of lettuce at the point of consumption) for a

577 product with a shortened shelf life (see Table 4), therefore the level of growth of *L.*

578 *monocytogenes* in the packaged lettuce (Σ*I*) is decreased. Proportion of packages that do

579 not meet the FSO (dashed lined) is 0.013%.

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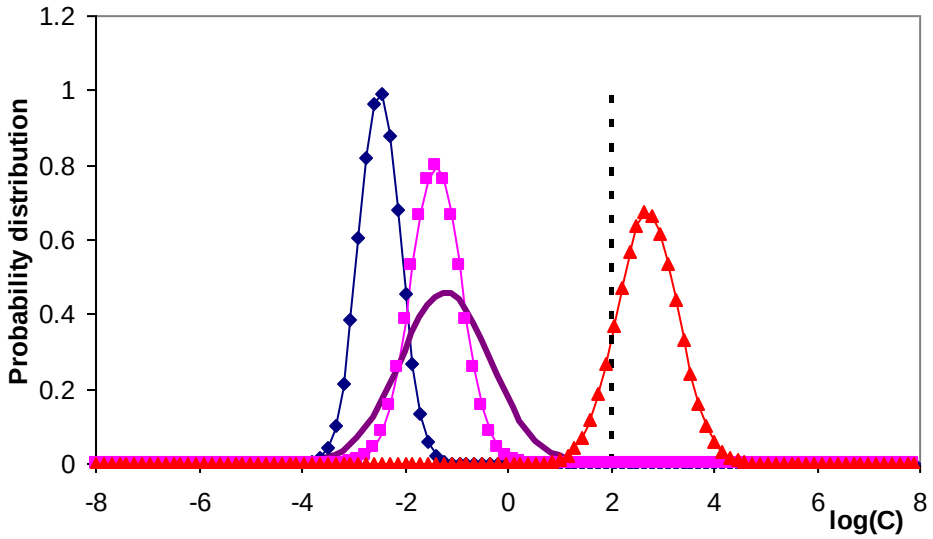

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583 Figure 4. Probability distribution of the initial level (H_0, ♦), reduction (-ΣR, ■), and

584 increase (ΣI, ▲) and resulting overall distribution (solid line; meaning the distribution of

585 the levels of *L. monocytogenes* in packages of lettuce at the point of consumption) for a

586 product with reduced variability of initial levels (H_0) of *L. monocytogenes* on raw

587 materials, following the input values in Table 5. Proportion of packages that do not meet

588 the FSO (dashed line) is 0.012%.

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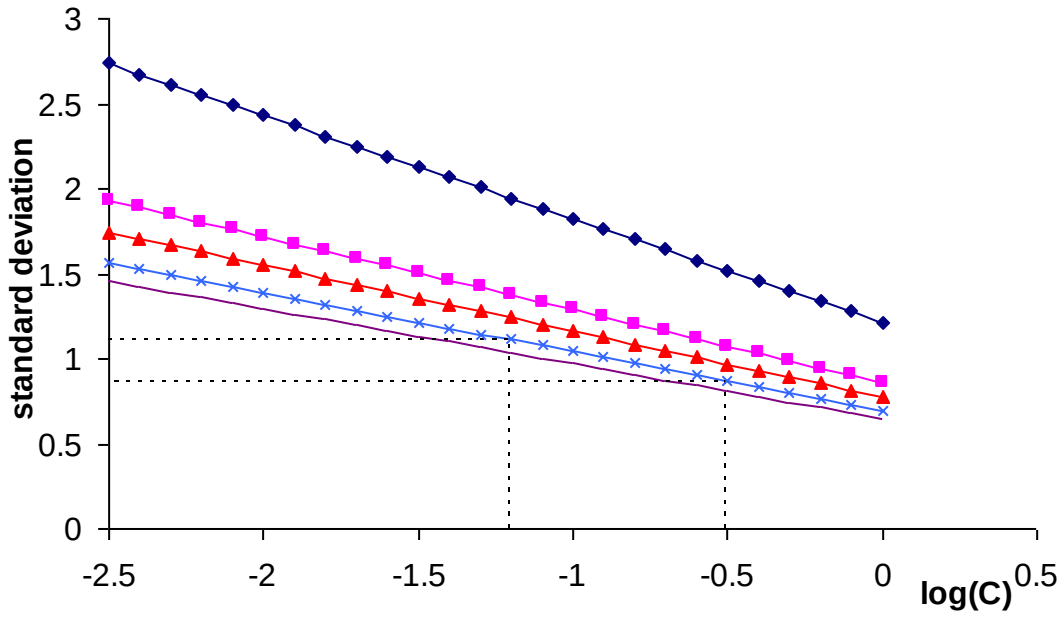

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592 Figure 5. Various combinations of mean log levels, log(C), and standard deviation of the

593 combined distributions for H_0, ΣR and ΣI resulting in a particular proportion of product

594 that does not meet the FSO (in this case FSO=2). The various lines represent different

595 proportions (♦=5%, ■=1%, ▲=0.5%, x= 0.2%, solid line=0.1%) of products not meeting

596 the FSO. The examples from Table 2 and 6 are indicated for a 0.2% level.

597

Table 1. Results of various levels of reduction (ΣR) on the proportion of defective units (P), with standard deviation of the increase step=0.59 ($\log_{10}$ increase normally distributed with standard deviation of 0.59)*

ΣR	H_0-ΣR+ΣI	P (H_0-ΣR+ΣI)>2 (sd=0.59)
0.8	0.1-0.8+2.7=2.0	0.5 (50%)
1.2	0.1-1.2+2.7=1.60	0.25 (25%)
1.77	0.1-1.77+2.7=1.03	0.05 (5%)
2.17	0.1-2.17+2.7=0.63	0.01 (1%)
2.62	0.1-2.62+2.7=0.18	0.001 (0.1%)

*Note the proportion above the FSO can be calculated in Excel by

1-NORMDIST(2,x,s,1),

for example for the last line =1-NORMDIST(2,0.18,0.59,1)=0.001019, so the proportion of being above 2 logs, for a lognormal distribution with log mean 0.18 and standard deviation 0.59 is 0.1%). In this example, H_0 and ΣR have no variation.

Table 2. Results on the proportion of products that do not meet the FSO (packages of fresh cut lettuce calculated to have greater than 2 $\log_{10}$ cfu/g *L. monocytogenes* present at the point of consumption), with various mean and standard deviation values (*s*) for H_0, ΣI and ΣR

	H_0	ΣR	ΣI		Total[1]	
mean $\log_{10}$	-2.50	1.4	2.7		-1.2	H_0-ΣR+ΣI
s	0.8	0.5	0.59		1.112	s=sqrt(s_1^2+s_2^2+s_3^2)
				P(>FSO)	0.20%	

[1] Total is the level of *L. monocytogenes* present in a package of lettuce at the point of consumption

Table 3. The impact of a washing step (ΣR) that does not reduce levels of *Listeria monocytogenes* on lettuce on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective

	H_0	ΣR	ΣI		Total	
mean $\log_{10}$	-2.50	0	2.7		0.2	H_0-ΣR+ΣI
s	0.8	-	0.59		0.994	s=sqrt(s_1^2+s_2^2+s_3^2)
				P(>FSO)	3.5%	

Table 4. The impact of shortening the shelf life of the product from 14 to 7 days, thus reducing the level of growth (ΣI) on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective

	H_0	ΣR	ΣI		Total	
mean $\log_{10}$	-2.50	1.4	1.9		-2	H_0-ΣR+ΣI
s	0.8	0.5	0.56		1.097	s=sqrt(s_1^2+s_2^2+s_3^2)
				P(>FSO)	0.013%	

Table 5. The impact of a reduction in the variability (smaller standard deviation) of the initial levels of *L. monocytogenes* on raw materials (H_0) on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective

	H_0	ΣR	ΣI		Total	
mean $\log_{10}$	-2.50	1.4	2.7		-1.2	H_0-ΣR+ΣI
s	0.4	0.5	0.59		0.8707	s=sqrt(s_1^2+s_2^2+s_3^2)
				P(>FSO)	0.012%	

Table 6. The impact of reducing the variability of the initial levels of *L. monocytogenes* on raw materials (H_0) at the same time as lowering the level of reduction of *L. monocytogenes* during the washing step (ΣR) on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective (compare to Table 2)

	H_0	ΣR	ΣI		Total	
mean $\log_{10}$	-2.50	0.7	2.7		-0.5	$H_0-\Sigma R+\Sigma I$
s	0.4	0.5	0.59		0.8707	$s=\text{sqrt}(s_1^2+s_2^2+s_3^2)$
				P(>FSO)	0.20%	

Table 7 *z* values at various probability levels (one sided test)

Probability level	*z* score
0.05	1.645
0.01	2.326
0.005	2.576
0.002	2.878
0.001	3.090