This is a "Post-Print" accepted manuscript, which has been published in "Food Control".

Please cite this publication as follows:

Zwietering, M.H., Stewart, C.M., Whiting, R.C., International Commission on Microbiological Specifications for Foods (ICMSF) (2010) Validation of control measures in a food chain using the FSO concept. Food Control 21, 1716-1722.

You can download the published version at:
http://dx.doi.org/10.1016/j.foodcont.2010.05.019

1 # VALIDATION OF CONTROL MEASURES IN A FOOD CHAIN
2 # USING THE FSO CONCEPT

3 **Zwietering M.H.[a]*, Stewart, C.M.[b], Whiting R.C.[c], International Commission on
4 Microbiological Specifications for Foods (ICMSF)**

5

6 [a]Laboratory of Food Microbiology, Wageningen University, 6700 EV Wageningen, The
7 Netherlands
8 [b]Silliker Food Science Center, 160 Armory Drive, South Holland, IL 60473, USA
9 [c]Exponent, 17000 Science Drive, Suite 200, Bowie, Maryland 20715, USA

10

11

12

13 * Author for Correspondence:
14 Dr. Marcel Zwietering
15 Laboratory of Food Microbiology
16 Wageningen University
17 6700 EV Wageningen
18 The Netherlands
19 Marcel.Zwietering@wur.nl
20 -31-317-482233

21

22

23 # VALIDATION OF CONTROL MEASURES IN A FOOD CHAIN
24 # USING THE FSO CONCEPT

25 **Zwietering M.H.[a]*, Stewart, C.M.[b], Whiting R.C.[c], International Commission on**
26 **Microbiological Specifications for Foods (ICMSF)**

27

28 [a]Laboratory of Food Microbiology, Wageningen University, 6700 EV Wageningen, The
29 Netherlands
30 [b]Silliker Food Science Center, 160 Armory Drive, South Holland, IL 60473, USA
31 [c]Exponent, 17000 Science Drive, Suite 200, Bowie, Maryland 20715, USA

32

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

43
44
45

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

47

48 * Author for Correspondence:

49 Dr. Marcel Zwietering

50 Laboratory of Food Microbiology

51 Wageningen University

52 6700 EV Wageningen

53 The Netherlands

54 Marcel.Zwietering@wur.nl

55 -31-317-482233

56

57

58

59 **1. Introduction**

60

61 Validation of food processes is defined as establishing documented evidence which
62 provides a high degree of assurance that a specific process will consistently produce a
63 food product meeting its pre-determined specifications and quality attributes (Keener,
64 2006), or as determining if an intervention, when properly applied, will effectively
65 control the microbial hazard(s) (Swanson & Anderson, 2000). So validation is the
66 collection and evaluation of scientific and technical information to determine if the
67 process (treatment), when properly applied, will effectively control the microbiological
68 hazard, or in other words, if the process criteria can reliably deliver a specified
69 performance objective. The overall effectiveness of the control measures should be
70 validated according to the prevalence of microbial hazards in the food of concern, taking
71 into consideration the characteristics of the individual hazards(s) of concern, established
72 food safety objectives/performance objectives and level of risk to the consumer (CAC
73 2007). Validation focuses on the collection and evaluation of scientific, technical and
74 observational information. In order to take full advantage of the flexibility that an outcome
75 based risk management system offers, it is important to be able to demonstrate that the
76 selected control measures actually are capable, on a consistent basis, of achieving the
77 intended level of control. Guidelines for the validation of food hygiene control measures
78 have been proposed by Codex (CAC, 2008). Validation is different from verification and
79 monitoring; verification is used to determine that the control measures have been
80 appropriately implemented, showing that the system is operating as designed, while
81 monitoring is the on-going collection of information on a control measure at the time the
82 control measure is applied to ensure the HACCP system is operating as intended.

83

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

90

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

98

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

106

107 On the other hand, control measures to be validated under the responsibility of regulatory
108 authorities cover all control actions in the system for multiple companies, products and
109 process controls, including consideration of between-lot variability. In this case the
110 validation is targeted at assessing the established POs and FSOs.
111
112 In this paper, the ICMSF equation (ICMSF, 2002) for the prevalence and levels of
113 microorganisms from the initial contamination (H_0), reduction (ΣR), growth and re-
114 contamination (ΣI), and factors influencing these are considered throughout food
115 production until consumption, and in their role in meeting the FSO by the equation H_0 -
116 $\Sigma R + \Sigma I \leq$ FSO. Stochastic aspects of the parameters are taken into account as well as
117 deterministic values. This is illustrated in the following sections with various examples of
118 the use of data to validate one or a series of processes of food production for practical
119 application, including statistical insights.
120
121 **2. Considerations for validation**
122
123 Processes can be validated through the use of predictive modeling, microbiological
124 challenge studies, studies to show that certain limiting parameters (e.g. pH<4.5) are
125 achieved and/or use of default criteria (safe harbors, like 72°C, 15s for pasteurization of
126 milk, or 121°C 20 min. for sterilization). Not all these need to be used, however, often
127 several sources of information can be used together to supply sufficient evidence. When a
128 safe harbor approach is used, it is not normally necessary to conduct validation studies
129 for that process. For example, a safe harbor for milk pasteurization is to deliver a

130 minimum process of 72°C for 15s; this process criterion has already been validated and
131 therefore can be implemented by processors without re-validation of the process. The
132 process would still need to be verified and monitored by the processors.
133
134
135 **3. Validation of control measures**
136
137 When determining the processing criteria (PC) required to achieve a desired PO,
138 generally microbiological studies begin on a laboratory scale, move to a pilot plant scale
139 and then are finally validated on a commercial scale, when possible or necessary.
140 Inactivation kinetic studies can be conducted over a small range of treatments (a unique
141 combination of factors and their levels; for example pH 6.5 and 70ºC) or over a broad
142 range of treatments that would allow for the development of microbiological predictive
143 models. Several good microbiological predictive models are available, including the
144 USDA Pathogen Modeling Programs, which can be found at
145 http://ars.usda.gov/Services/docs.htm?docid=6786 and COMBASE, which can be found
146 at http://wyndmoor.arserrc.gov/combase/. Challenge studies can also be used to
147 determine processing criteria; although they are more limited in scope than models, they
148 are often used as a way of validating the model predictions. Finally, on a commercial
149 scale, challenge studies can be conducted utilizing nonpathogenic surrogate
150 microorganisms; shelf life studies with uninoculated product can also provide useful
151 information for validating a process.
152

153 While microbiological challenge testing can also be used for determining the stability of
154 a product with regards to spoilage over the intended shelf life, the remainder of this
155 discussion will focus on product safety with regards to pathogens relevant to foods.
156
157 In the following sections, each of the terms in the ICMSF equation, the initial
158 contamination (H_0), reduction (ΣR), growth and re-contamination (ΣI), and factors
159 influencing these are discussed sequentially, including data needs, some experimental
160 considerations, and especially effects of their variability.
161
162 *3.1 Determining the initial level (H_0), standard deviation and distribution*
163
164 The design of the food process will determine the importance of incoming material for
165 product safety. The main source of the pathogen of concern may be from a major or
166 minor ingredient, one incorporated in the initial processing steps, or one added later by
167 recontamination. It is important to understand which of the ingredient(s) may harbor the
168 pathogen as well as to understand if there is seasonal effect on the level of the pathogen
169 present [for example the number of lots of ground beef positive for *E. coli* O157:H7
170 increase over the June-October period in the USA (USDA-FSIS, 2009)]. The
171 geographical source of the ingredient may also play a role in the likelihood of whether a
172 certain foodborne pathogen is present in the raw ingredients. If contamination is not
173 avoidable, the goal is to develop specifications and criteria for the incoming material that
174 will limit frequencies and/or levels of contamination and lead to achievement of the final
175 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.

199 (2002), Ross & McMeekin (2003), McKellar & Lu (2004), and Whiting & Buchanan
200 (2007).
201
202 When designing microbial inactivation experiments, kinetic studies measuring changes
203 with time are preferred as they provide more information than end-point measurements.
204 Additionally, kinetic studies offer flexibility and a depth of understanding that is not
205 obtainable via end point measurements alone (Legan et al., 2002). Therefore,
206 experimental points should be selected to allow the true nature of the microbial response
207 to the lethal agent to be determined. The inoculation level should be sufficiently high to
208 demonstrate the performance criteria without the need for extrapolation, if practically
209 possible. Points should be spaced over the time interval to allow any curvature in the
210 response to be described; ideally this typically involves 10-12 points over a 6-7 $\log_{10}$ (or
211 greater) reduction in population size. This implies an inoculation level of at least 10^8-10^9
212 CFU/ ml or g. A zero-time point is critical and equidistant time intervals are often
213 selected, except for very slow inactivation rates where intervals that increase
214 geometrically between samplings are often useful.
215
216
217 *3.2.2 Growth (ΣI)*
218
219 The population of a pathogen will increase during storage periods if the food, storage
220 temperature and packaging conditions support growth. Storage periods may occur for raw
221 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

245 Factors that should be considered when evaluating growth data include the strain(s) used,
246 surrogates, physiological state of the inoculum, method of inoculation, degree of
247 simulation of the experimental or pilot plant conditions to the commercial process,
248 inclusion of all environmental factors in the food (pH, a_w, acid anions) and external
249 factors (temperature, packaging), and inclusion of the spoilage flora. Detailed
250 information on the design and implementation of microbiological challenge studies (also
251 referred to as inoculated pack studies) has been reported by IFT (2001) and Scott et al.
252 (2005).

253

254 *3.2.3 Recontamination (ΣI)*

255

256 If a food process includes pasteurization or another lethal step that eliminates the
257 pathogen, then all of the pathogens present at consumption are the consequence of
258 recontamination. Foods processed to deliver 6 to 8 $\log_{10}$ reduction of the pathogen will
259 result in a very low frequency of contaminated packages after such a process. For
260 example a product containing initially a homogeneous contamination level of 100 cfu/g,
261 in a 100 g package will contain 0.001 cfu/package after a 7 $\log_{10}$ reduction, meaning 1 in
262 1000 packages contaminated with one (or a few) cells. When determining whether such a
263 food meets a PO at a further step or FSO, calculation of the food process begins after the
264 lethal step. The appropriate parameters to consider are the frequency and level of
265 contamination; essentially, they form a new H_0. Little literature data exists for guidance
266 concerning frequencies and levels of recontamination and few applicable models have
267 been developed to estimate the results of recontamination. Sufficient sampling of the

268 specific process at this step or at a subsequent step with a back calculation is the only
269 way to obtain valid data on recontamination. A food process without a lethal step and
270 with several potential points of additional recontamination is difficult to predict.
271 Sufficient sampling of the food after the last point of recontamination is a possible way to
272 validate whether a PO or FSO is being achieved. Another approach to controlling
273 contamination is environmental monitoring and monitoring of food contact surfaces and
274 integrating this information into the sanitation program. Other factors to consider are
275 packaging integrity and proper training on handling practices by employees.
276
277 **4. Validation of FSO compliance, probabilistic aspects: The effect of variability in**
278 **processing on non-conformance to an FSO/PO**
279
280 *4.1 Introduction*
281
282 One way to show compliance to an FSO is by using the ICMSF equation:
283

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

284
285 By combining information from different sources concerning the initial level (H_0),
286 reductions (ΣR) and increases (ΣI) of the microbiological hazard through the food
287 production and distribution chain, it can be determined if the FSO or PO will be reliably
288 met. It can also be determined how variability in the steps in the process/food chain
289 influences the ability to meet the FSO.

290

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

301

302 *4.2 Point-estimate approach*

303

304 In the paper of Szabo, Simons, Coventry & Cole (2003), estimates are made of the initial
305 contamination level of *L. monocytogenes* on pre-cut lettuce, reduction using sanitizing
306 rinses and the increase in levels of the pathogen after packaging and during storage and
307 distribution. For a given initial level of *L. monocytogenes* on lettuce and an expected
308 level of growth (increase) during storage and distribution, the necessary reduction level,
309 in order to achieve a given FSO, can be determined. For example, in Szabo et al. (2003),
310 it is given that for an H_0 of 0.1 $\log_{10}$ cfu/g of *L. monocytogenes* and for a potential
311 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
312 necessary to achieve the set FSO of 2 $\log_{10}$ cfu/g:

313

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

$$0.1 - 0.8 + 2.7 = 2.0$$

314

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

316

317 *4.3 Including variability in the process*

318

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

330

331 *4.4 Including variability in the process for all process stages*

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

334 the distribution of levels of *L. monocytogenes* in packages of fresh cut lettuce at the point
335 of consumption) can be described by a mean value that is equal to the sum of the means
336 of H_0, ΣI, and ΣR. The mean, however, is not a correct indicator of the risk, without
337 representing also the variance. The variance of the total distribution is equal to the sum of
338 the variances (the final standard deviation is the square root of the sum of the squares of
339 the variable standard deviations (Snedecor and Cochran, 1989)). The distributions are
340 represented graphically in Figure 1.
341
342 Given this distribution of outcomes, the proportion of packages of lettuce not meeting the
343 FSO can be determined, which, in this example, is 0.2% (This proportion can be
344 determined from the area under a normal curve that exceeds the FSO using the Excel or
345 similar function, following the procedure as given in the footnote in Table 1).
346
347 *4.5 Ineffective washing step*
348 Assuming that the lettuce washing step is not effective ($\Sigma R = 0$) in reducing the level of
349 *L. monocytogenes* (Table 3, Figure 2), the effect on the overall effectiveness of the
350 process can be determined. We can see that the mean level of *L. monocytogenes* in
351 packages of fresh cut lettuce is higher (from –1.2 to 0.2) and the overall standard
352 deviation of the level decreases (from 1.112 to 0.994) compared to the previous
353 calculation (Table 2). The proportion of packages of lettuce having levels of *L.*
354 *monocytogenes* at the point of consumption that are above the FSO (2 $\log_{10}$ cfu/g)
355 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

379 (standard deviation) in the levels of *L. monocytogenes* present on the lettuce can be
380 obtained by supplier selection, changing supplier specifications, or better input control,
381 the standard deviation of H_0 can be reduced (Table 5, Figure 4; compare with Table 2).
382 By this better process control, the average level of *L. monocytogenes* on the raw materials
383 remains the same, but the final standard deviation goes down, resulting in a lower
384 percentage of packages of fresh cut lettuce that do not meet the FSO (going from 0.2% to
385 0.012%) or, conversely, a larger percentage of product now meets the FSO, comparable
386 to a reduction in shelf life to 7 days (Table 4).
387
388 *4.8 Ability to meet the FSO at the same level of performance by different means*
389 It can also be determined how an equivalent outcome can be achieved (same proportion
390 of the products meeting the FSO), in this instance only 0.2% of packages of fresh cut
391 lettuce not meeting the FSO (see Table 2), by reducing the variability of one of the
392 inputs. For example, if the variability (standard deviation) of the initial levels of *L.*
393 *monocytogenes* on the raw materials is reduced from 0.8 to 0.4, the required level of
394 reduction of *L. monocytogenes* during the lettuce washing step (ΣR) could be decreased
395 from 1.4 to 0.7 while still achieving the same proportion of product that meets the FSO
396 (Table 6).
397
398 *4.9 Relation between log mean value, standard deviation and proportion of products that*
399 *do not meet the FSO (levels of* L. monocytogenes *at the point of consumption are greater*
400 *than the FSO)*

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

406

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

413

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

414

415 In Table 1 the levels of 1.03, 0.63, and 0.18 and with a standard deviation of 0.59
416 correspond to a probability level of 0.05, 0.01, and 0.001 respectively: (2-
417 1.03)/1.645=0.59 (z-value for 0.05 probability level); (2-0.63)/2.326=0.59; (2-
418 0.18)/3.09=0.59

419

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

423

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

424 resulting in a formula that can provide an equivalent change in level following a
425 reduction of the standard deviation.
426 For example, for an FSO set with a confidence level of 99% (meaning that 99% of the
427 product units do confirm to this level), z equals 2.33 resulting in:
428

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

429
430 Therefore, a 0.1 $\log_{10}$ decrease in the standard deviation is equivalent to a 0.233 $\log_{10}$
431 decrease in average level.
432
433 To calculate the difference in equivalent reduction necessary to achieve a 0.2% defective
434 rate, for an H_0 with a 0.8 standard deviation (Table 2) to a H_0 with a 0.4 standard
435 deviation (Table 6) we can perform the following calculation:
436 By reducing the s in H_0 from 0.8 to 0.4, the standard deviation of the overall level will
437 reduce from 1.112 (sqrt(0.8^2+0.5^2+0.59^2), see Table 2) to 0.8707
438 (sqrt(0.4^2+0.5^2+0.59^2) see Table 6), so this translates to a “gain” in log mean of
439 2.878*(1.112-0.8707)= 0.697 logs. Instead of a 1.4 $\log_{10}$ reduction (Table 2), a 0.7 $\log_{10}$
440 reduction is sufficient (Table 6).
441 So how much one could change the mean concentration while retaining the same
442 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

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

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

485

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

489 how to determine an equivalence in performance, either by the level or the variability in a
490 level. Given the above mentioned assumptions in certain cases this analysis may be
491 needed to be followed up by a more detailed risk assessment.
492
493
494 **References**
495
496 CAC (Codex Alimentarius Commission) (2007). *Recommended International Code of*
497 *Hygienic Practice for Egg Products.* CAC/RCP 15. FAO, Rome.
498
499 CAC (Codex Alimentarius Commission) (2008). *Guideline for the Validation of Food Safety*
500 *Control Measures.* CAC/GL 69. FAO, Rome.
501
502 ICMSF (International Commission on Microbiological Specifications for Foods) (2002).
503 *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management.* New
504 York: Kluwer Academic/Plenum Publishers.
505
506 IFT (2001). *Evaluation and definition of potentially hazardous foods. A report by the*
507 *Institute of Food Technologists for the Food and Drug Administration of the U.S.*
508 *Department of Health and Human Services.*
509 http://members.ift.org/NR/rdonlyres/F537AA13-CFDB-420D-94BC-
510 ED763D9C0A4D/0/crfsfssupn2p001007.pdf (Accessed on: 21-12-09)
511

512 Keener, L. (2006). Hurdling new technology challenges: investing in process validation
513 of novel technologies. *Food Safety Magazine*, February/March issue.
514

515 Legan, J.D., Stewart, C.M., Vandeven, M., & Cole, M.B. (2002). Modelling the growth,
516 survival and death of bacterial pathogens in foods. In Blackburn, C. and McClure, P.J.
517 (eds.) *Foodborne Pathogens: Hazards, Risk and Control.* Cambridge, UK. Woodhead
518 Publishing pp. 53-95.
519

520 McKellar, R.C., & Lu, X. (2004). *Modeling Microbial Responses in Foods.* CRC Press,
521 Boca Raton, FL. 343 p.
522

523 Ross, T., & McMeekin, T.A. (2003). Modeling microbial growth within food safety risk
524 assessments. *Risk Analysis* 23: 179-197.
525

526 Scott, V.N., Swanson, K.M.J., Freier, T.A., Pruett, W.P., Jr., Sveum, W.H., Hall, P.A.,
527 Smoot, L.A., & Grown, D.G. (2005). Guidelines for conducting *Listeria monoctogenes*
528 challenge testing of foods. *Food Protection Trends* 25: 818-825.
529

530 Snedecor, G.W., Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State
531 University Press, Ames, IA. 503 pp.
532

533 Swanson, K.M.J., & Anderson, J.E. (2000). Industry perspectives on the use of microbial
534 data for hazard analysis and critical control point validation and verification.

535 *Journal of Food Protection* 63: 815-818

536

537 Szabo, E.A., Simons, L., Coventry, M.J., & Cole, M.B. (2003). Assessment of control

538 measures to achieve a food safety objective of less than 100 CFU of *Listeria*

539 *monocytogenes* per gram at the point of consumption for fresh precut iceberg lettuce.

540 *Journal of Food Protection* 66: 256-264

541

542 USDA-FSIS (2009). *Raw ground beef* – E. coli *testing results*. Available at

543 http://www.fsis.usda.gov/science/2009_Ecoli_positive_results/index.asp (Accessed on:

544 21-12-09)

545

546 Van Gerwen, S.J.C., & Zwietering M.H. (1998). Growth and inactivation models to be

547 used in quantitative risk assessments. *Journal of Food Protection* 61: 1541-1549.

548

549 Whiting, R.C., & Buchanan, R.L. (2007). Predictive modeling and risk assessment. In

550 Doyle, M.P. and Beuchat, L.R. eds. *Food Microbiology: Fundamentals and Frontiers.*

551 3nd Ed. Chapter 45. ASM Press, Washington, D.C. pp. 953-969.

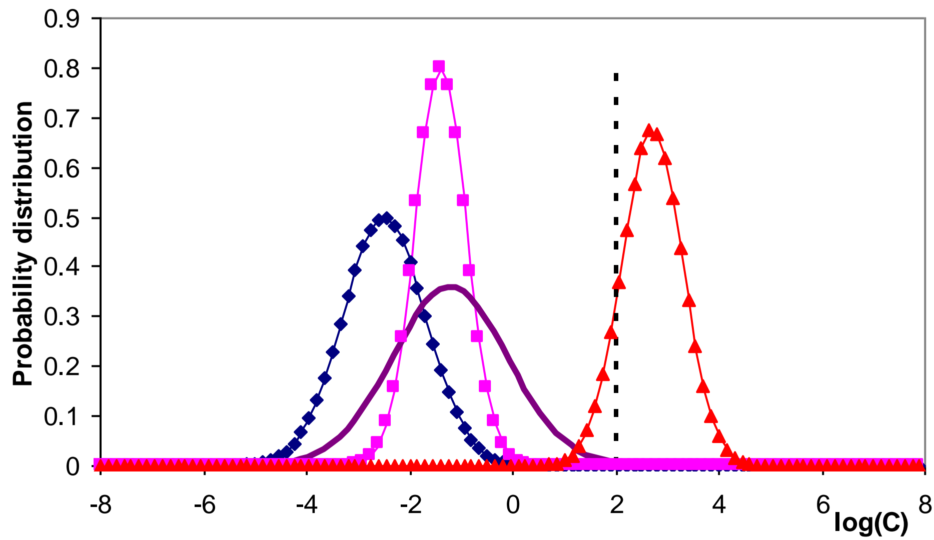
552

553

554

555

556



557

558 Figure 1. Probability distribution of the initial level (H_0, ♦), reduction ($-\Sigma R$, ■), and

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

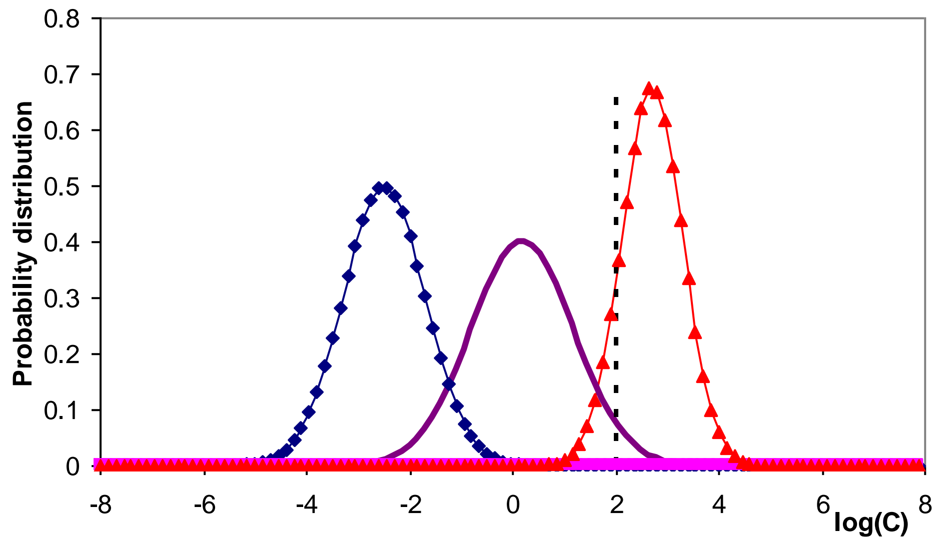
560 distribution (solid line; meaning the distribution of the levels of *L. monocytogenes* in

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%.

563

564



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.*

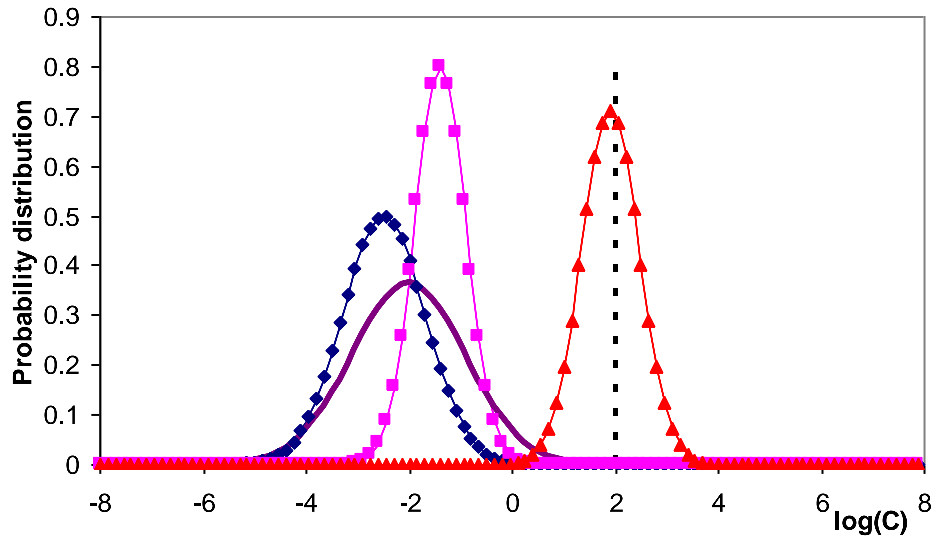
568 *monocytogenes* in packages of lettuce at the point of consumption) for a process in which

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%.

572



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

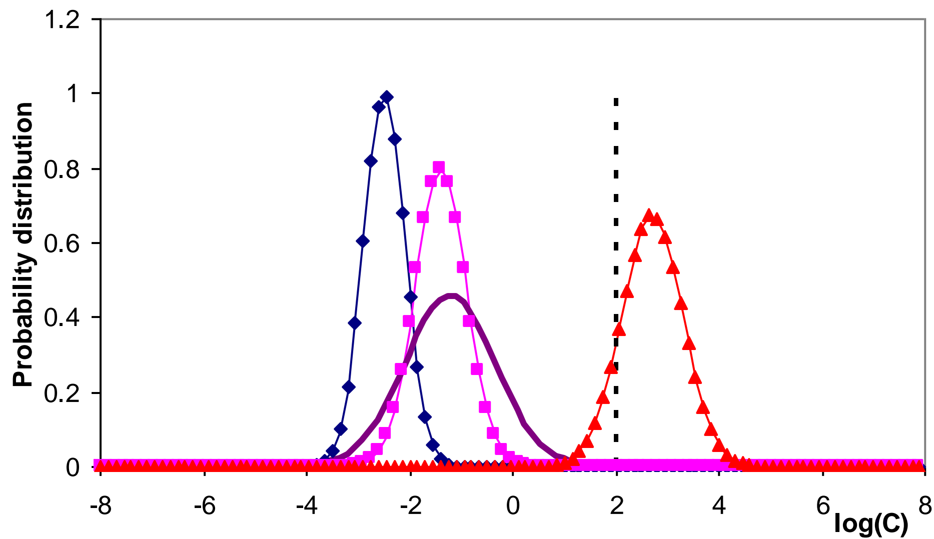
577 product with a shortened shelf life (see Table 4), therefore the level of growth of *L.*

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%.

580

581



582

583 Figure 4. Probability distribution of the initial level (H_0, ♦), reduction ($-\Sigma R$, ■), and

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

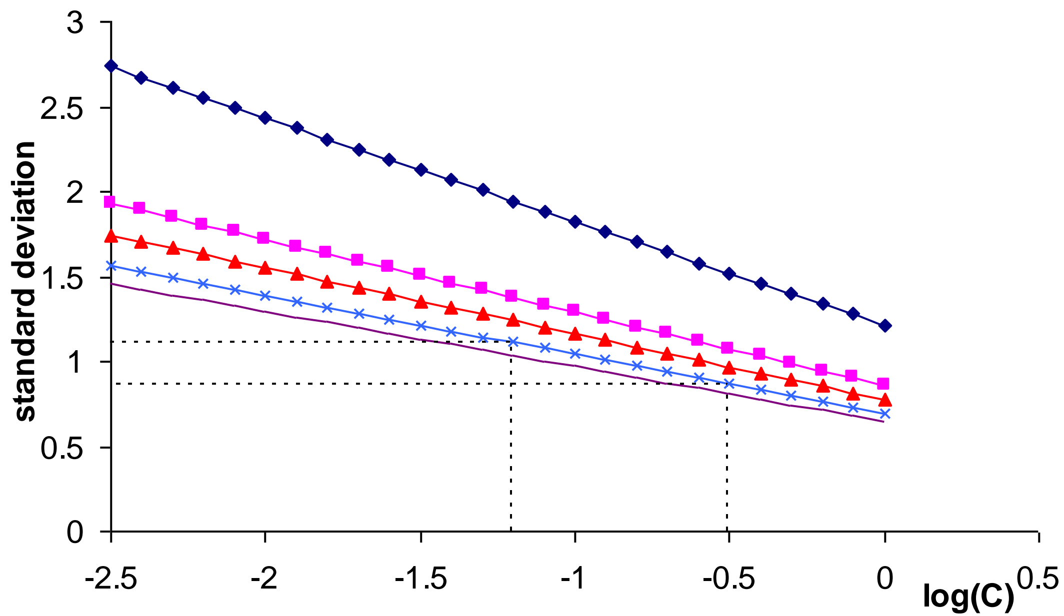
585 the levels of *L. monocytogenes* in packages of lettuce at the point of consumption) for a

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%.

589



590

591

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

598 Table 1. Results of various levels of reduction (ΣR) on the proportion of defective units

599 (P), with standard deviation of the increase step=0.59 ($\log_{10}$ increase normally distributed

600 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%)

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

602 1-NORMDIST(2,x,s,1),

603 for example for the last line =1-NORMDIST(2,0.18,0.59,1)=0.001019, so the proportion

604 of being above 2 logs, for a lognormal distribution with log mean 0.18 and standard

605 deviation 0.59 is 0.1%). In this example, H_0 and ΣR have no variation.

606

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

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

611 [1] Total is the level of *L. monocytogenes* present in a package of lettuce at the point of
612 consumption

613

614

615

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

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

619

620

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

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

624
625
626
627 Table 5. The impact of a reduction in the variability (smaller standard deviation) of the
628 initial levels of *L. monocytogenes* on raw materials (H_0) on the proportion of packages of
629 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	$H_0 - \Sigma R + \Sigma I$
s	0.4	0.5	0.59		0.8707	$s = \text{sqrt}(s_1^2 + s_2^2 + s_3^2)$
				P(>FSO)	0.012%	

630
631

632

633 Table 6. The impact of reducing the variability of the initial levels of *L. monocytogenes*
634 on raw materials (H_0) at the same time as lowering the level of reduction of *L.*
635 *monocytogenes* during the washing step (ΣR) on the proportion of packages of fresh cut
636 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-ΣR+ΣI
s	0.4	0.5	0.59		0.8707	s=sqrt(s_1^2+s_2^2+s_3^2)
				P(>FSO)	0.20%	

637

638

639

640 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

641

642