1 **VALIDATION OF CONTROL MEASURES IN A FOOD CHAIN**

2 **USING THE FSO CONCEPT**

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

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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.

46   **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.

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

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO} \tag{1}$$

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 $\Sigma I = 2.7$ $\log_{10}$ 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

$$H_0 - \Sigma R + \Sigma I = 2.0 \tag{2}$$

$$0.1 - 0.8 + 2.7 = 2.0$$

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

$$s=(2-x)/z=(2-x)/1.645 \tag{3}$$

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

$$\Delta x = z\Delta s \tag{4}$$

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

$$\Delta x = 2.33\Delta s \tag{5}$$

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.

Journal of Food Protection 63: 815-818

Szabo, E.A., Simons, L., Coventry, M.J., & Cole, M.B. (2003). Assessment of control measures to achieve a food safety objective of less than 100 CFU of *Listeria monocytogenes* per gram at the point of consumption for fresh precut iceberg lettuce. *Journal of Food Protection* 66: 256-264

USDA-FSIS (2009). *Raw ground beef* – E. coli *testing results*. Available at http://www.fsis.usda.gov/science/2009_Ecoli_positive_results/index.asp (Accessed on: 21-12-09)

Van Gerwen, S.J.C., & Zwietering M.H. (1998). Growth and inactivation models to be used in quantitative risk assessments. *Journal of Food Protection* 61: 1541-1549.

Whiting, R.C., & Buchanan, R.L. (2007). Predictive modeling and risk assessment. In Doyle, M.P. and Beuchat, L.R. eds. *Food Microbiology: Fundamentals and Frontiers.* 3nd Ed. Chapter 45. ASM Press, Washington, D.C. pp. 953-969.
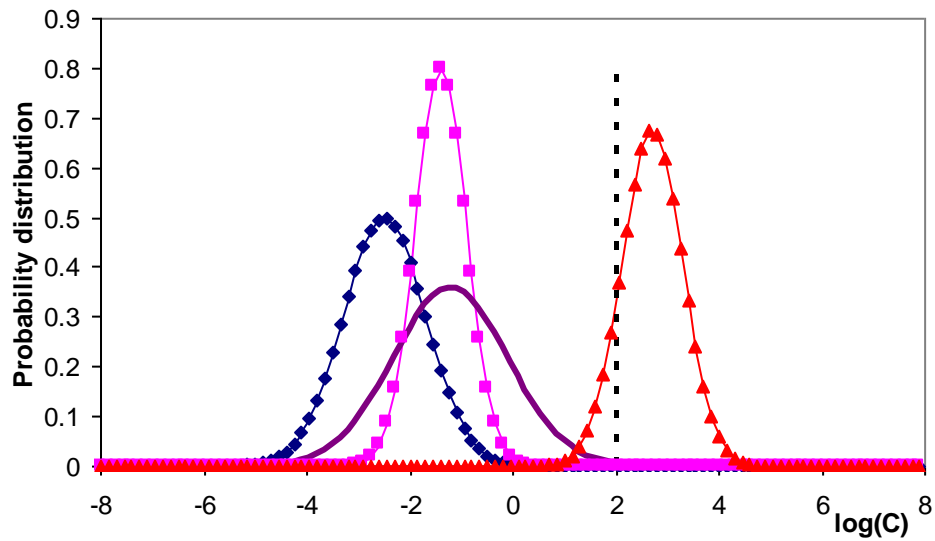
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558 Figure 1. Probability distribution of the initial level (H_0, ♦), reduction (-ΣR, ■), and

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%.
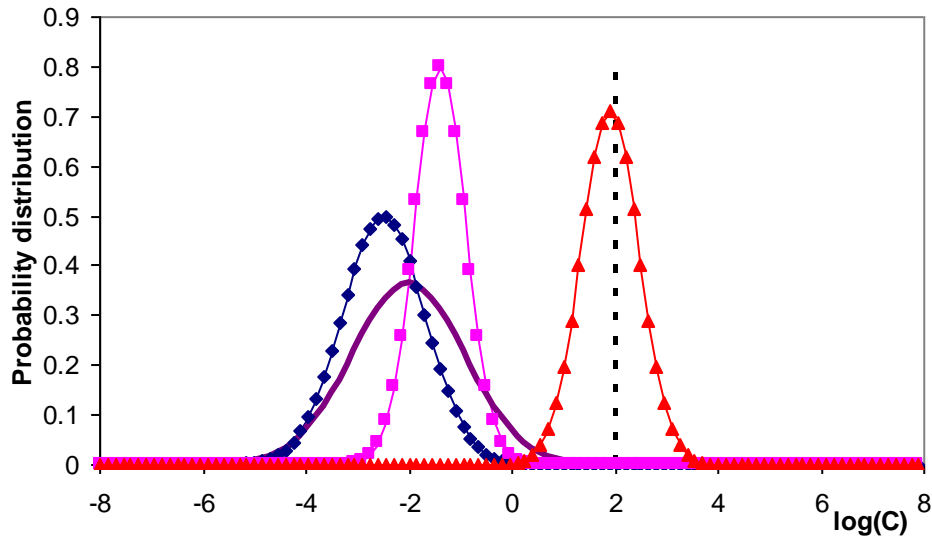
563

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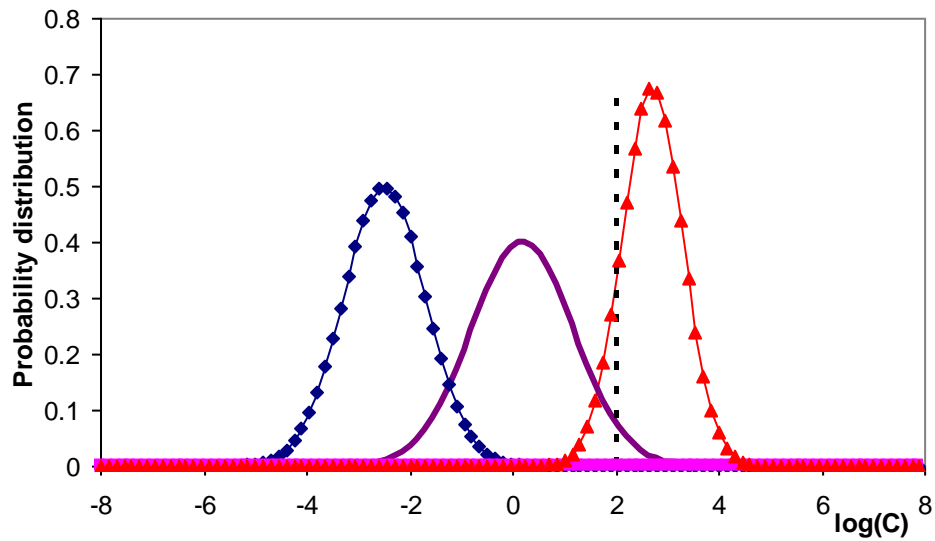
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566   Figure 2. Probability distribution of the initial level (H_0, ♦), increase (ΣI, ▲) and

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* ($\Sigma R=0$),

570   following the input values in Table 3. Proportion of packages that do not meet the FSO
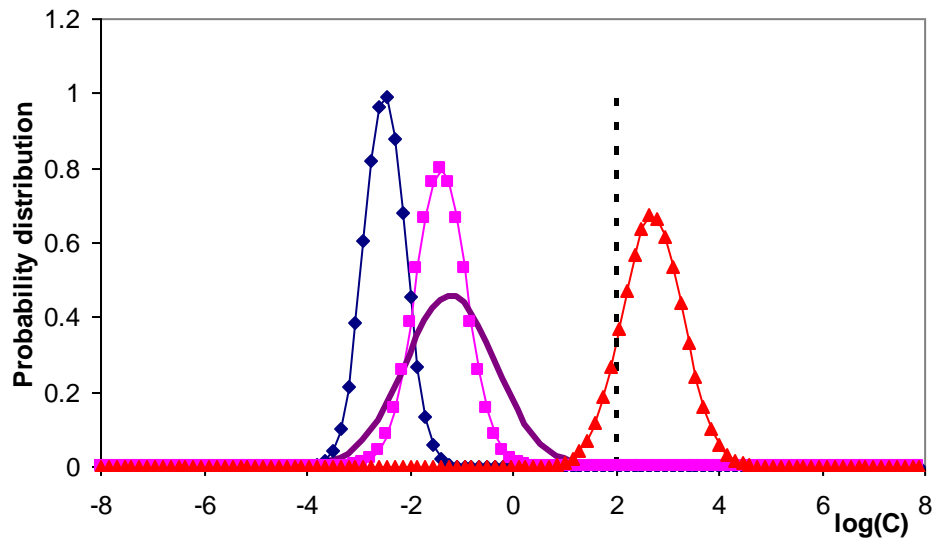
571   (dashed line) is 3.5%.

572

Figure 3 Probability distribution of the initial level (H_0, ♦), reduction (-ΣR, ■), and increase (ΣI, ▲) and resulting overall distribution (solid line; meaning the distribution of the levels of *L. monocytogenes* in packages of lettuce at the point of consumption) for a product with a shortened shelf life (see Table 4), therefore the level of growth of *L. monocytogenes* in the packaged lettuce (ΣI) is decreased. Proportion of packages that do not meet the FSO (dashed lined) is 0.013%.

582

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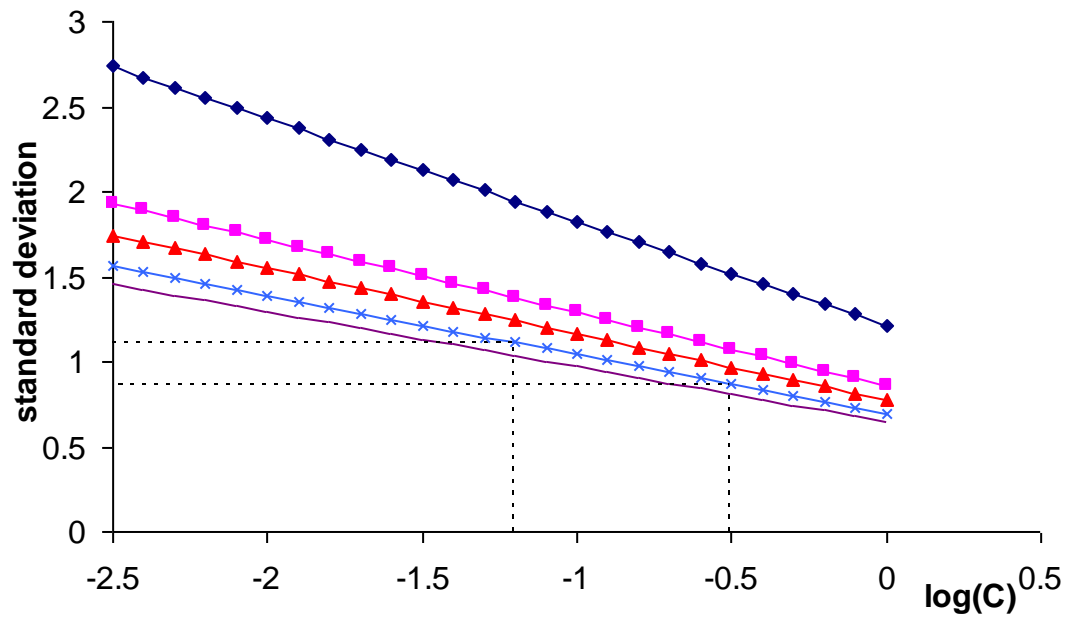
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592   Figure 5. Various combinations of mean log levels, log(C), and standard deviation of the

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

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	$s=\text{sqrt}(s_1{}^2+s_2{}^2+s_3{}^2)$
				$P(>\text{FSO})$	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	$s=\text{sqrt}(s_1{}^2+s_2{}^2+s_3{}^2)$
				$P(>\text{FSO})$	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	$s=\mathrm{sqrt}(s_1^2+s_2^2+s_3^2)$
				$P(>\mathrm{FSO})$	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	$s=\mathrm{sqrt}(s_1^2+s_2^2+s_3^2)$
				$P(>\mathrm{FSO})$	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	$H_0 - \Sigma R + \Sigma I$
s	0.4	0.5	0.59		0.8707	$s = \text{sqrt}(s_1^2 + s_2^2 + s_3^2)$
				$P(>\text{FSO})$	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