A DEMONSTRATION OF THE APPLICATION OF THE *C. PERFRINGENS* RISK ASSESSMENT FOR ESTIMATION OF RISK MANAGEMENT METRICS

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Glossary

ALOP Appropriate Level Of sanitary or phytosanitary Protection. The level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory. (SPS Agreement).

FSO Food Safety Objective. The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP). (Codex Alimentarius Procedures Manual, 16th edition).

ML Maximum Likelihood.

MRM Microbial Risk Management.

PO Performance Objective. The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable. (Codex Alimentarius Procedures Manual, 16th edition).

PC Performance Criterion. The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO. (Codex Alimentarius Procedures Manual, 16th edition).

MC Microbiological Criterion. A microbiological criterion for food defines the acceptability of a product or a food lot, based on the absence or presence, or number of microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot. (CAG/GL 21, 1997).

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1. Introduction

The basic requirements of this project are to demonstrate the use of the *C. perfringens* risk assessment (Crouch and Golden, 2005) and the corresponding risk assessment model (computer code taking account of the variability between food servings, and the uncertainties of measurements) to evaluate a FSO, PO, and corresponding MC consistent with an ALOP. To this end, the risk assessment model has been applied to a single food type, hot dogs, with the assumption that the risk assessment model is an accurate representation for hot dogs.

2. Outline of the demonstration

2.1. What is done

The demonstration proceeds by selecting the type of food to be evaluated, and a (pseudo-)ALOP[1] that drives the whole demonstration. The connection between the risk assessment and the ALOP is demonstrated, together with a method of establishing a FSO corresponding to the ALOP. The FSO is at the point of consumption (by definition and so also by construction), so a PO at a (rather hypothetical) point of food serving production is constructed, and the necessary information to establish a MC applicable at that point is calculated.

2.2. Why it is done

The object is to demonstrate and document the methodology for and practicality of deriving ALOPs, FSOs, POs, and MCs using an available risk assessment. The *C. perfringens* risk assessment (Crouch and Golden, 2005) with its corresponding model is conveniently available, and the author is thoroughly familiar with it (having written both the assessment and the model).

2.3. How it is done

This section summarizes in bullet points how the demonstration proceeds; further details are provided in subsequent sections.

- Run the risk assessment model for the selected food, and determine an upper uncertainty confidence limit on the risk. That upper limit provides the ALOP (expressed in terms of the risk evaluated in the risk assessment). With a chosen confidence, that upper limit exceeds the risk of current practice, so is suitable for an ALOP that is based on current practice (see Sections 2.4 and 6.2).
- Select a suitable metric to measure the FSO. The metric used for the FSO is in theory not unique and the most likely candidates will vary with the risk being evaluated (see Section 7.2). Selection of a suitable metric may require some experimentation with the model, and the final choice may be a matter of practicality.
- Modify the risk assessment model to evaluate and display that metric as well as risk; the risk assessment model may not have been designed to evaluate or to display this metric, so some modification may be necessary to gain access to suitable results.

[1] The definition of an ALOP requires that it be established by a Member of the World Trade Organization for use within its territory. No ALOP has been so established by the U.S. for the food items evaluated, so this document necessarily is using a substitute.

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- Run the risk assessment model to determine the relationship (and its uncertainty) between the FSO metric and risk. This information may be used to specify the FSO in such a way that the ALOP is achieved with the required confidence (see Sections 7.2 through 7.4).
- From the relationship obtained, evaluate a FSO that corresponds to the ALOP with the desired confidence (see Sections 7.5 through 7.7).
- Evaluate a suitable metric for the PO. As for the FSO, there is no unique metric for the PO, so the most likely candidates will vary with the risk being evaluated and may depend on other circumstances such as the practicality of measurement (see Section 8). Again, some experimentation with the risk assessment model may suggest a best candidate.
- Modify the risk assessment model to evaluate and display that metric as well as the FSO metric and risk. Again, as for the FSO, the risk assessment model may not have been designed to evaluate or display the required metric, so may need modification to allow access to it.
- Run the risk assessment model to determine the relationships (and uncertainties) between PO metric, FSO metric, and risk. This information is needed in order to select a value for the PO so that either the FSO or the ALOP is achieved with suitable confidence (see Section 8).
- From the relationships found, evaluate a PO that corresponds to the FSO or ALOP with the desired confidence.
- Select a MC that corresponds to the PO, and evaluate a suitable test plan.[2] This requires taking account of the practicalities of testing and the uncertainties introduced by it (see Section 9).

2.4. Balancing protection against current practice

The presumption throughout this demonstration is that the current food supply is considered sufficiently safe, so that ALOPs can be based on current practices. This corresponds to the presumption made in the context of international trade when evaluating whether any particular standard or requirement imposed on importers differs from those imposed on producers in the importing country. In this context, it balances the desire for protection against industry needs for practicality and equity, by imposing the same constraints on all comers.

3. Lessons learned

The following summarizes some of the lessons learned in this demonstration:

- Evaluating ALOPs corresponding to current practices, and FSOs, POs, and MCs corresponding to such ALOPs, is practical using a suitable risk assessment model.
- A risk assessment model that accounts for both variability between food servings and uncertainties is essential. The *C. perfringens* risk assessment model is such a 2-dimensional model (implemented using Monte Carlo methods) that is suitable.
- Given such a model, the approach described here is eminently practical but not necessarily simple, cheap, or easily understandable.[3]

[2] Generally the metric for the MC is likely to be the same as the metric for the PO, as in this case. Otherwise, an additional modification and run of the risk assessment model would be required to evaluate the relationship between the MC metric and the PO metric.

[3] This report required approximately 200 hours of consultant time, and 300 hours of CPU time on 2.6 GHz Intel P4 CPUs.

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- Preliminary attempts using just the summary results of the *C. perfringens* risk assessment and assumptions about the shape of various distributions (*e.g.* assumptions about lognormal or uniform distribution shapes) were inadequate to allow adequate definition of FSOs or POs. The actual distributions differed substantially from such idealizations, and the results depended critically on those differences.
- The level of detail required for setting ALOPs, FSOs, POs for a particular food type is likely to be higher than required for evaluating a whole industry for one organism; thus more detailed information may be necessary than is available in a whole-industry assessment. In particular, the representativeness of the measurements used and the assumptions made for the whole-industry assessment may be more questionable when applied in detail to particular food types.
- The risk assessment model must be available to be modified, and the analyst performing the modifications needs to be completely familiar with it (including down to the code level, assuming a computer model). For example, it is highly unlikely that a risk assessment model will have been designed to output all the results required during the procedures summarized in Section 2.3; and considerable experimentation may be necessary to evaluate suitable metrics for FSOs and POs.
- If a suitable risk assessment model is available, it is in principle not necessary to evaluate FSOs, although FSOs may be desired or required for other purposes. A direct evaluation of a PO corresponding to the ALOP is straightforward — there does not appear to be any reason to attempt to base the PO on the FSO rather than the ALOP in such circumstances.
- The approach demonstrated here is entirely general, provided a suitable risk assessment is available. For example, the same approach may be used to develop a PO at any point within the food chain; provided only that the risk assessment suitably models the food chain and provides access to that point in the food chain within the model. However, the risk assessment model may impose limitations — the *C. perfringens* model could not be used to evaluate process controls, for example, since it does not model them.
- While the approach demonstrated is general, this demonstration is specific to *C. perfringens*, which has characteristics substantially different from other food-borne disease organisms. Evaluation of FSOs and POs for other organisms will be sufficiently different that drawing general conclusions based on the specific results obtained in this one demonstration (*e.g.* the use of prevalence alone for a PO, see Section 8.1) is not advisable. Further, while the methodology discussed here is general, it may not cover all possibilities — care must be taken in specific cases to adapt and extend as necessary.

4. Background material

This demonstration uses the *Clostridium perfringens* risk assessment (Crouch and Golden, 2005), which was designed for a specific purpose in evaluation of a specific organism. To understand the approaches considered in this demonstration, some information on the organism and the design and results of the risk assessment is pertinent (for further information, see the risk assessment).

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4.1. Summary of pertinent microbiology of *C. perfringens*

C. perfringens is an anaerobic bacterium widely distributed in the environment that forms hardy spores in adverse conditions. The hazard to humans is from high concentrations of vegetative cells in food — when ingested the vegetative cells sporulate, and some types of *C. perfringens* (Type A, CPE+)[4] produce a toxin during sporulation that induces diarhheal illness.[5]

Both vegetative cells and spores contaminate the raw meats and spices used in production of hot dogs. Since hot dogs are Ready-to-Eat foods (RTE), however, they undergo a heat treatment step (also known as the "lethality" step) at a temperature higher than 65°C (150°F) during their production that kills all the vegetative cells of *C. perfringens* present in them. This heat treatment, however, cannot be made severe enough to kill the spores; indeed, a large fraction of the spores are stimulated to germinate into vegetative cells by the heat treatment. Thus immediately after the heat treatment there may still be un-stimulated spores present, together with some spores that will quickly (typically within 1 to 10 hours, depending on temperature) germinate into vegetative cells ready to divide and grow.

C. perfringens vegetative cells grow and divide very rapidly in the right conditions, and conditions are near ideal in warm meat products; growth is favored by relatively high temperatures, with the optimum near 45°C (113°F). Thus after the heat treatment, the hot dogs must be cooled rapidly to temperatures where growth is inhibited — below about 12°C (54°F) prevents growth, and sub-freezing temperatures cause slow death of vegetative cells. The cooling process is known as "stabilization," and various techniques are used in different circumstances to speed cooling. Spores are relatively unaffected by any temperatures in this range, although a few slowly germinate under almost any temperature conditions.

Subsequently, growth of *C. perfringens* vegetative cells in RTE foods depends almost entirely on the storage temperature(s) and time(s) at those temperatures, with the final treatment (*e.g.* eaten raw, heated, or thoroughly cooked) also affecting the number of vegetative cells that get eaten with any serving by increasing their number (slow warming), killing them (cooking at high enough temperature), or both in succession.

4.2. The risk assessment design and some pertinent results

The *C. perfringens* risk assessment (Crouch and Golden, 2005) was commissioned to evaluate the effect on human illnesses of allowing different amounts of growth of *C. perfringens* during the stabilization step in Ready-to-Eat and partially cooked foods containing meat or poultry. It tracked *C. perfringens* vegetative cells and spores in individual servings of RTE and partially cooked foods from their initial production, through the lethality step, the stabilization step, commercial and residential storage, then food preparation, until final consumption.

Since few data are available on conditions in the industry, the production and stabilization steps were treated in a simplified form by evaluating the consequences on human illnesses of assuming fixed amounts of growth during the stabilization step. Many assumptions had to be made about

[4] CPE stands for "*C. perfringens* enterotoxin"

[5] More severe illness can result in rare cases; but the risk assessment does not evaluate such cases.

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the representativeness of the available data for current conditions (such assumptions are listed in the risk assessment) so the absolute results of the risk assessment are debatable; however, the relative effect of the growth during stabilization and other factors affecting illness rates could be obtained, and the uncertainties (assuming representativeness of the data) taken into account.

The risk assessment also made various assumptions about the behavior of *C. perfringens*, in order to interpret the available data and construct the risk assessment. The assumptions included:

- the original contaminating spores are a random mix of distinguishable types of *C. perfringens* (so that Type A, CPE+ spores are a random subset of the original contaminating spores);
- despite the previous assumption, growth is by clonal expansion of (effectively) a single type of *C. perfringens* (*i.e.* the growth rate is identical for all cells originating from the contaminating Type A, CPE+ germinated spores; and all such cells have the same potency to cause diarrhea), so that all final vegetative cells can be considered identical;
- there is no possibility of overgrowth by other organisms (*e.g.* even though Type A, CPE+ cells are a minority, in cases where non-Type A, CPE+ cells were also present in the food, the latter were assumed never to outgrow the Type A, CPE+ cells).

The first two assumptions probably make little difference, since the fastest growing clone would outgrow those arising from other cells (and the final cell counts in cases causing illness are independent of initial cell count, see Section 8 below). The third assumption was partially examined in the risk assessment in the "what if" section (Section 6.5 of the risk assessment).

It was observed that the major (modeled) contributor to human illnesses was storage of RTE or partially cooked foods at elevated temperatures during commercial or home storage. Storage was assumed to occur in refrigerators; however, the measured distribution of temperatures in refrigerators extended to temperatures high enough to allow *C. perfringens* growth (above 12°C); and storage was for sufficient periods that very substantial growth would be inevitable. Thus for the small fraction of servings that were contaminated with *C. perfringens* vegetative cells and that were also stored at relatively high temperatures, *C. perfringens* vegetative cell counts capable of causing human illness could easily be achieved.

5. Selection of food type

5.1. Selection of hot dogs

For the purposes of this demonstration, the object was to select a single RTE or partially cooked food type that has high consumption rate and is easy to use within the *C. perfringens* risk assessment model. Examination of the results of the *C. perfringens* risk assessment (Crouch and Golden, 2005, Table 6.3) shows that food category 1a, corresponding to the single food type "hot dogs" contributed between 15% and 19% of the estimated illnesses (at the maximum likelihood estimate). Food category 2 contributed 55% to 68% of the estimated illnesses, but that food category was very diverse. No other single category, and certainly no other single food type, was predicted to contribute so large a fraction as hot dogs (largely because of the relatively large consumption of hot dogs). Hot dogs were therefore selected for this demonstration.

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5.2. Caveats on the use of the hot dog data and interpreting this demonstration

Examination of the servings selected as "hot dogs" in the input data for the risk assessment model shows a total of 2,099 servings, of which 2,085 are indicated to contain 100% meat. The remaining 14 servings have a recipe that contains 50.6% meat, 4.6% spices,[6] and 44.8% of other components. Running the model with hot dogs only shows that more than half of the illnesses estimated to occur are caused (in the model) by *C. perfringens* originating in spice. The representativeness of the relatively few servings available to characterize the entire hot dog consumption of the U.S. may therefore be a limitation, both because of the small number and because of the limited information apparently available, particularly (in this context) for spice content — *e.g.*, it seems unlikely that only two recipes for hot dogs are actually produced and consumed in the U.S.

The information on *C. perfringens* in spice available for constructing the model was also very limited and probably outdated;[7] and the accuracy of the spice component in recipe information for hot dogs used in the Continuing Survey of Food Intakes by Individuals (1994-1996, 1998), the source of the serving information, is also questionable (these are all problems of representativeness of the available data, listed in Section 4.1 of Crouch and Golden, 2005). There were also insufficient data to separately evaluate the concentrations of *C. perfringens* between different meats, so they were treated together, although, for example, available data showed no observed *C. perfringens* spore contamination in the few beef samples tested in the primary study used (Table 3.3 of Crouch and Golden, 2005). No attempt has been made to separate the various meat components of hot dogs in this demonstration, and the fractions of different meats in the hot dog servings has not been examined. Moreover, this demonstration treats "hot dogs" as a single food commodity, rather than (for example) attempting to separate 100% meat hot dogs from spicy hot dogs.

The problem of the representativeness of available data thus is acute for the food used in this demonstration — the representativeness for hot dogs alone may be substantially lower than for all RTE foods considered in the risk assessment. The absolute results of this demonstration project must therefore be treated cautiously; the entire object of the exercise here is to demonstrate methodology for FSO and PO using the risk assessment, but it would be inappropriate to rely at this stage on the values obtained. All the results obtained throughout this report must be interpreted in this light — it should be considered an exercise demonstrating approaches that may be taken in circumstances corresponding to the assumed inputs, and not necessarily an accurate representation of the hot dog industry.

5.3. Modification of the risk assessment model to use only hot dogs

It is straightforward to modify the *C. perfringens* risk assessment model to evaluate just hot dogs. All that is required is to modify the input file "Food_samples.dat" to contain only servings of hot dogs; or, alternatively, to construct such a modified file, "Hotdogs_only.dat", place it within the data directory, and modify the line

[6] One or more of mustard, cumin, cinnamon, chili, cayenne pepper and black pepper; these were treated together in the risk assessment, because of lack of any data to separate them.

[7] The potential importance of this is demonstrated in Section 9.3.

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```
Food_sample_data='Food_samples.dat';
```
within the module CP_risk.pas to
```
Food_sample_data='Hotdogs_only.dat';
```
The latter approach was used here, since other modifications to the program are also needed to obtain information not previously extracted.[8]

Experience with this project showed that substantial flexibility is required in order to explore various ways of specifying a FSO or PO, in order to extract various pieces of information from various stages of the model. The original model was not designed with such flexibility built-in; various code additions were incorporated to "instrument" the model — the original code was left essentially unchanged, and modified copies of the relevant routines added. The flexibility required to obtain the results shown in this demonstration have been extended to the user interface, so that to extract small variations on the information presented here all that is necessary is to modify new options the control files (with the "instrumented" option selected, the new routines get called in place of the originals, and the additional information is output).

Addition of this instrumentation necessarily slows the program down somewhat when collecting certain types of information (*e.g.* saving the cell counts at each point in the program; tracking both total cells/spores and Type A, CPE+ cells/spores); the original program was partially optimized for speed, since the incidence of illnesses is so low. It should be noted that it was not possible to predict how to modify the program and produce the relevant outputs; what was necessary was first a period of experimentation examining various outputs to discover what is relevant in this case. For other evaluations, even of FSOs or POs for *C. perfringens* for other food types for example, different outputs might be required.

6. Selection of an ALOP

6.1. General considerations

An ALOP (Appropriate level of sanitary or phytosanitary protection) is defined to be "The level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory" (SPS Agreement). For the purposes of microbiological risk management (MRM) in the context of food, the ALOP generally will refer to sanitary protection, rather than phytosanitary protection; and that is the case for this demonstration. In this case, no ALOP (for *C. perfringens* in RTE foods) has been established by the U.S.

6.2. Determination of an ALOP based on current conditions

In principle, establishment of an ALOP could depend on the appropriate level of protection that is considered desirable by a Member within its territory; however, any such ALOP would have to be binding both internally and externally, and Members would have to enforce it within their territory for it to be applicable in international trade. In practice therefore, current conditions within a Member territory are considered to be acceptable, and it would usually be desirable to

[8] The hot dog data are included in the workbook Hotdogs_only.xls in the sheet Data, and the serving size distribution (see Section 8.2) is derived in the sheet Serving_sizes.

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establish an ALOP at as low a level as possible while allowing current practices. Moreover, again in the context of international trade, the current condition in the importing country would generally be presumed to be acceptable there for the purpose of setting an ALOP for imported foods. Therefore the demonstration proceeds by considering how an ALOP might be established as low as possible while corresponding to current conditions in the U.S., assuming that the *C. perfringens* risk assessment accurately reflects those current conditions and describes the uncertainty about current conditions.[9]

Applying the *C. perfringens* risk assessment model to hot dogs alone shows an illness rate of about 3.6 per million hot dog servings, with a fraction of about 5.0 per 1000 servings having a non-zero number of CPE+, Type A *C. perfringens* spores or vegetative cells at the time of consumption (Figure 1),[10] at a growth of 1 $\log_{10}$ during stabilization and at the maximum likelihood estimates.

The following concentrates on calculations at a growth of 1 $\log_{10}$ during stabilization, since that corresponds to the current regulation upper bound for RTE foods, which will be assumed to roughly correspond to current conditions, or at least to conditions that are considered acceptable.

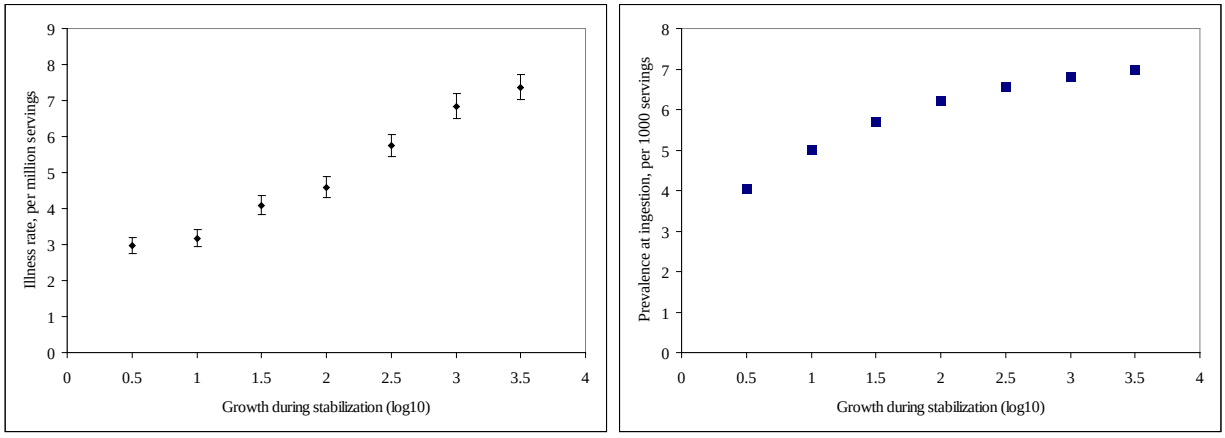

Figure 1. Illness rate and prevalence of live CPE+, Type A *C. perfringens* vegetative cells versus growth during stabilization.

At 1 $\log_{10}$ growth during stabilization, running the *C. perfringens* risk assessment model allows evaluation of the uncertainty distribution (Figure 2). From this distribution, one can read off the lowest illness rate that with any given confidence is currently achieved (insofar as the risk

[9] This corresponds to accepting the representativeness of the data used in the *C. perfringens* risk assessment, applied specifically to hot dogs, as well as accepting the methodology and assumptions used. An alternative approach might be to measure the number of servings of hot dogs and the concurrent number of illnesses due to *C. perfringens* caused by those servings using epidemiological methods. This is currently infeasible in the U.S.

[10] Error bars in Figure 1 correspond to an 80% binomial confidence range due to numerical uncertainty only (Appendix C.2) in a Monte Carlo risk assessment model run with 100,000,000 servings at each growth rate shown. The *C. perfringens* risk assessment model outputs are in workbook output_1.xls, sheet Variability, and the graphs are calculated in the sheet Pictures. This workbook also contains a check, in sheet Pictures, that observed and expected numerical uncertainties agree, using the 30 independent runs of 100,000,000 servings summarized in sheet Growth_1.

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assessment model correctly codifies the uncertainties; but see the detailed discussion in the risk assessment of just this point).

Thus from the results plotted in Figure 2, the 95th percentile point of the uncertainty distribution (z-score of 1.645) is at 13 per million, and the 99th percentile point (z-score of 2.326) is at 21 per million.[11] Thus, insofar as the risk assessment model reflects reality (or is conservative), establishing an ALOP of 21 illnesses per million servings would result in 1% chance (or less) that current conditions fail the ALOP; and establishing an ALOP of 13 illnesses per million servings would result in a 5% chance (or less) that current conditions fail the ALOP.

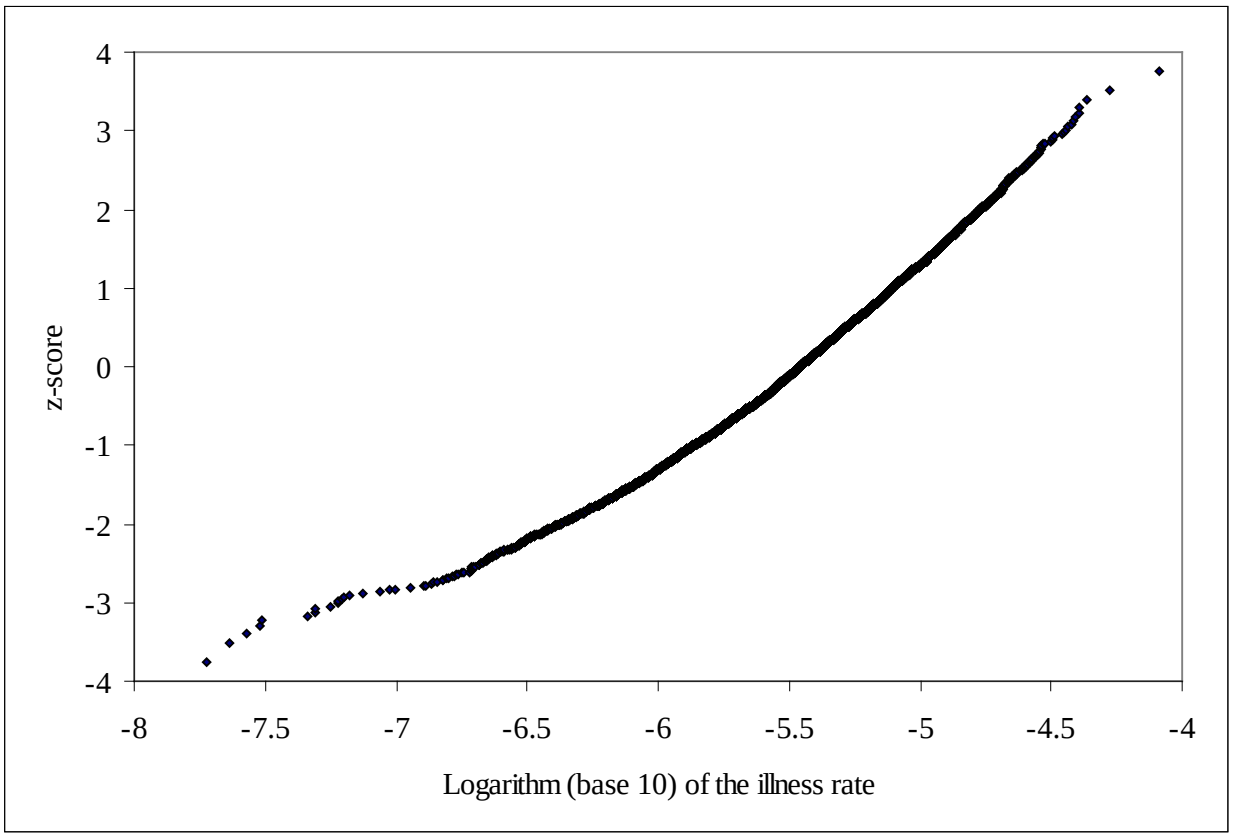

Figure 2. Cumulative uncertainty distribution for the illness rate.[12]

[11] See Appendix C.2 for the evaluation of percentiles and their numerical uncertainties from Monte Carlo results, and Appendix C.1 for information on this type of graph. The numerical uncertainty standard deviations are approximately ±0.3 and ±0.6 per million for the 95th and 99th percentiles respectively.

[12] 7,563 uncertainty iterations of 10,000,000 servings (variability iterations), taking approximately 36 hours CPU time on an Intel P4 at 2.6 GHz. The model outputs are in the workbook FSO_PO.xls, in sheet 7563_run. The same workbook also contains results from model runs with 600 uncertainty iterations of 30,000,000 servings (sheet 600_run) and 7,500 uncertainty iterations of 7,500,000 servings (sheet 7500_run), to evaluate the effects of numerical uncertainties.

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6.3. An aside: a potential negative consequence of establishing an ALOP

Were an ALOP to be established for *C. perfringens* in hot dogs or in other RTE or partially cooked foods, one consequence would likely be substantially increased sampling of such foods for *C. perfringens*. A potential consequence of such sampling would be lower uncertainties in the level and prevalence of *C. perfringens* in the foods, potentially also leading to lower uncertainty in the estimates of illness rates — the uncertainty distribution of Figure 2 would become narrower. In principle, at that point, a lower ALOP could be established at the same confidence level while still ensuring that current conditions meet the lower ALOP.

In practice, it seems unlikely that an ALOP, once established, would be altered absent some deliberate changes in food handling methods. FSOs and POs would be established based on the original ALOP (established when uncertainties were high), but such FSOs and POs would be unnecessarily high because of the original uncertainty inherent in establishing the ALOP.

Thus as uncertainties diminish through future sampling, there is the potential for level or prevalence in foods to increase, while the uncertainty of those levels or prevalences decreases in such a manner that the foods are still demonstrably meeting the original FSOs, POs, and ALOP. The establishment of an ALOP would then have led to a reduction in food safety, since the actual illness rate would increase in this scenario.

For *C. perfringens* in hot dogs, it appears that this potential sequence of events is unlikely. The principal controlling factor is the prevalence of type A, CPE+ *C. perfringens* spores in hot dogs (see Section 8), the uncertainty coefficient of variation for which is about 0.28. The uncertainty coefficient of variation for the illness rate is about 0.94. Entirely removing the uncertainty in initial prevalence of *C. perfringens* would thus only reduce the uncertainly in illness rate by about 4 to 5%,[13] so would have minimal effect on the estimates used above (the 95th percentile estimate of illness rate would be reduced from 13 to 12.5 per million, and the 99th percentile from 21 to 20 per million).

7. Estimating a FSO from the ALOP

7.1. General considerations

The definition of FSO ("the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)") appears to be ill-defined in several ways:

"Concentration" strictly is a mass/unit volume,[14] whereas the most relevant hazard metric may well be different — for example, number/unit volume or number/unit mass or number/serving

[13] Because of the approximate multiplicative nature of the model and the independence of other uncertainties, the uncertainty coefficient of variation in the absence of the initial uncertainty can be estimated as $\sqrt{(0.94^2-0.28^2)} = 0.90$. The evaluation of these uncertainties can be seen in workbook FSO_PO.xls in sheet 7563_run.

[14] The Codex Procedures Manual does not define "concentration" to be anything different. The Manual uses "concentration" in two places explicitly to express a mass/unit mass, which is strictly a mass fraction. Colloquially, "concentration" is also used for mass fraction, mixing ratio (volume/unit volume), number/unit volume, number/unit mass, and in various other ways.

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(*e.g.* for organisms). For *C. perfringens* in hot dogs, the natural metric to use would be number/hot dog, since each hot dog is a natural unit. Since the risk assessment evaluates servings, the natural number to extract from the risk assessment model is number/serving, where a serving size varies with occasion, individual, and (presumably) food supplier.

"Frequency" is similarly ambiguous; frequency is strictly defined to be per unit time, but clearly what is meant is per unit of food (a prevalence); however the unit of food is not specified. For the *C. perfringens* assessment, the natural unit would be prevalence in servings (fraction of servings that are contaminated), or prevalence in hot dogs (fraction of hot dogs that are contaminated); however, it would also be possible to consider evaluation of the prevalence in any particular mass of food (*e.g.* prevalence in 10 gram samples), and this specification would probably be most useful for a MC.

"Frequency and/or concentration" appears to be both ambiguous and to unnecessarily limit the metrics that may be used for the FSO. Taken literally, the definition specifies that the FSO be expressed as one of:

- a maximum frequency
- a maximum concentration
- a "maximum frequency and maximum concentration"
- a "maximum frequency or maximum concentration"
- a maximum "frequency and concentration"
- a maximum "frequency or concentration"

The last has no useful interpretation because frequency and concentration are incommensurate. The fifth is ambiguous in that the combination implied by "and" is undefined. The first four can be assigned meanings, but specify a very limited set of allowable metrics for expression of the FSO. What is required is a more general combination measure of frequency (actually prevalence) and some hazard metric (concentration, mass/serving, number/serving, etc.).

Suppose the ALOP is specified in terms of some response metric R that is derived from the frequency distribution $q(r)$ of responses r for all food servings.[15] Without loss of generality,[16] the response r may be taken to be a probability for the adverse event(s) for which the ALOP is defined, and this restriction is used in what follows.[17]

Then the most common examples for R are

[15] This is the variability distribution for r.

[16] Provided that the probability for the adverse event(s) defining the ALOP is a monotonic function of the response, and this is here assumed.

[17] Graduated responses may always be dichotomized by some threshold for what is considered adverse; and the dose-response relationship may then in principle be discontinuous (a zero to one step function) as a function of the hazard metric. In practice, variabilities between organisms and uncertainties in knowledge will always lead to a continuous probabilistic dose-response relationship for the dichotomous outcome as a function of the hazard metric.

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$$
\begin{aligned}
R &= \max r && \text{(maximum response)} \\
R &= \int r q(r)\, dr && \text{(average response)} \\
R &= \int_{r>0} q(r)\, dr && \text{(prevalence of any response)} \\
R &= \int_{r>r_t} q(r)\, dr && \text{(probability for a response larger than } r_t\text{)}
\end{aligned}
\tag{1}
$$

For a common hazard, or for an acute toxic agent with a well-defined threshold for all members of the population, one may wish to specify an ALOP so that the probability for an adverse event is always sufficiently small for every individual food serving — so the maximum probability for the adverse event arising from the hazard is limited for every serving.[18] For an organism causing a non-life-threatening illness, the average response may be relevant — the ALOP may be specified by the illness rate, expressed for example as the expected number of illnesses per million servings. If the adverse outcome is always life-threatening, it may be appropriate to specify the ALOP purely in terms of the prevalence of the response. A more general approach is to specify the ALOP in terms of the prevalence of servings with probability of response exceeding some threshold.

It seems most natural in this discussion to examine food servings, rather than unit masses of food. Translation of ALOPs or FSOs to normalize them to masses of food would require taking account of the distribution of mass per food serving. Both are done below.

Now suppose the dose-response relation between the response r and a hazard metric h is

$$r = f(h) \tag{2}$$

that is, at hazard metric h there is a probability $f(h)$ for the adverse event to occur (recall from above that the response measure may always be selected as the probability for the adverse event defining the ALOP). Suppose also that the probability of occurrence of hazard metric h among food servings is specified by a probability distribution g.[19] Then the four examples above give, respectively

$$
\begin{array}{lll}
 & \text{ALOP} & \text{FSO} \\
R = & \max r = \max f(h) & \max h \quad \text{if monotonic} \\
R = & \int r q(r)\, dr = \int f(h) g(h)\, dh & \int f(h) g(h)\, dh \\
R = & \int_{r>0} q(r)\, dr = \int_{f(h)>0} g(h)\, dh & \text{Prevalence} \\
R = & \int_{r>r_t} q(r)\, dr = \int_{f(h)>r_t} g(h)\, dh & \text{Generalized prevalence}
\end{array}
\tag{3}
$$

[18] Even for an acute toxic agent with a well-defined threshold, it is unlikely that an ALOP could be set that is guaranteed safe for every member of the population, because of the possibility for allergy or other abnormal sensitivity. The same is true even for food components generally considered non-toxic and nutritious — *e.g.* peanuts.

[19] This is the variability distribution for h.

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The right column shows how to translate these ALOP specifications to an FSO, based on the hazard metric *h*. The first corresponds to using the maximum hazard metric (assuming the dose-response relationship is monotonic) in the FSO. The second shows an expression that cannot be expressed in terms of prevalence and/or maximum hazard metric; the entire distribution for the hazard metric is required, together with the dose-response relationship. The third corresponds to using the prevalence of any non-zero response (or, for a non-threshold dose-response, the prevalence for any non-zero the hazard metric). And the fourth requires a generalized prevalence measure for the hazard metric. In the third and fourth cases, one could re-define the hazard metric to account for the threshold (*e.g.* concentration above a threshold concentration, or more likely quantity per serving above a threshold quantity), in which case the FSO could be defined purely in terms of the prevalence of the re-defined hazard metric. However, such an approach would likely become complicated when attempting to account for variability and uncertainty in any such threshold.

7.2. Use of surrogates

The discussion of Section 7.1 points out that the specification of the ALOP induces a requirement on the functional of the distribution *g* that must be controlled. However, the particular functional *R* required by the ALOP specification may not be readily measurable, so that it may be desired to use a surrogate in its place. For example, it may be strictly required to use the integral

$$R = \int f(h)\, g(h)\, dh \tag{4}$$

to specify a FSO when the ALOP is specified in terms of the expected number of illnesses per serving, but this integral may be difficult to measure. Instead, the FSO may be defined in terms of some surrogate *S* that is easier to measure (*e.g.* prevalence, mean contamination level, maximum contamination level).

The problem with the use of a surrogate *S* is that knowledge of *S* does not imply complete knowledge of *R*, so that introducing a surrogate introduces extra uncertainty. In such a case, specification of the FSO changes from

$$\text{Select} \quad R \quad \text{such that} \quad R < \text{ALOP} \tag{5}$$

to

$$\text{Select the boundaries of} \quad S \quad \text{such that} \quad \Pr(R > \text{ALOP} \mid S) \le \alpha \tag{6}$$

where α is an acceptable level of risk to exceed the ALOP. That is, the surrogate *S* has to be chosen so that the conditional probability for *R* to exceed the ALOP given *S* is less than the acceptable level of risk to exceed the ALOP. The range of values of *S* that are obtained from equation (6) are then those that are compatible with the ALOP and the acceptable level of risk to exceed the ALOP.

Equation (6), rather than equation (5) is actually that most likely to be used, in that usually *R* is not known exactly, so that even in the best case one really obtains a surrogate *S* for *R*, although *S* may just be *R* with uncertainty added. However, equation (6) covers the case for any surrogate, not just the case where the only degree of surrogacy occurs because of uncertainty.

7.3. Selection of levels of risk

The selection of a level of risk in Section 7.2 (the probability α in equation (6)) has to be made with some care, since it may interact with the establishment of an ALOP based on current conditions, as described in Section 6.2, in a way that is not immediately obvious. The method of establishing an ALOP given in Section 6.2 results in some small chance (call it β, chosen as 1% or 5% that current conditions fail to meet that ALOP. If an FSO is now selected in such a way that there is only some small probability α for conditions corresponding to that FSO to fail the ALOP, then if $\alpha = \beta$ the FSO will pretty well conform to current conditions. However, if $\alpha < \beta$ then the FSO may be more stringent than current conditions, while if $\alpha > \beta$ the FSO are likely to be less stringent than current conditions.

7.4. Methodology for evaluation of an FSO from an ALOP using a risk assessment model

Evaluation of an FSO for some surrogate *S* as in equation (6) requires a 2-dimensional probabilistic risk assessment model (typically a Monte Carlo assessment) that accounts for both variability (*e.g.* between food servings, leading to the variability distribution for the hazard metric *h* and response *r*) and uncertainty. Such assessments typically operate by computing the variability distribution for *h* and *r* in an inner loop (with fixed selections from the uncertainty distributions), and the uncertainty distribution in an outer loop (in which different selections are made from the uncertainty distributions), and this approach will be assumed here.[20]

In that case, after constructing the variability distribution for each selection from the uncertainty distributions, the values of *R* and *S* can be computed (since within the risk assessment model all quantities are known). For example, if *R* is given by an average

$$R = \int f(h)\, g(h)\, dh \tag{7}$$

as in one of the examples in equation (3), where *f* is the dose-response function and *g* the variability distribution,[21] the surrogate chosen might be to use in practice a best estimate of the dose-response function

$$S = \int \tilde{f}(h)\, g(h)\, dh \tag{8}$$

Both *S* and *R* may be computed from each variability distribution, and the resulting set of *S* and *R* values obtained from making different uncertainty distribution selections (the uncertainty loop) may be used to construct the confidence region specified by equation (6).

In practice, it will often be assumed that there is a common uncertainty distribution for the ratio of *R* to *S* (in principle, the uncertainty distribution for *R* could depend on the value of *S* in more complex ways) so this ratio may be computed for each uncertainty iteration, allowing construction of the uncertainty distribution for this ratio. Confidence limits on the ratio then allow construction of the region of S specified by equation (6). That is the approach taken here.

[20] The nomenclature used here will correspond to Monte Carlo analyses; but the same ideas may be used in the less common instances where analytic methods are used for either the variability distribution, the uncertainty distribution, or both.

[21] Both of these will be constructed conditional on the values selected from the uncertainty distributions.

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For Monte Carlo assessments, with finite numbers of samples for both variability and uncertainty loops, some care has to be taken in choosing the numbers of such samples. The integrals in equations (7) and (8), for example, would usually be approximated by summations over the variability samples; and no matter what method is used the result would have an associated numerical uncertainty due to the finite number of samples. Similarly, the finite number of samples in the uncertainty loop leads to numerical uncertainty in locating the confidence limit implicit in equation (6). Such numerical uncertainties are discussed further in Appendices C.2 through C.4.

7.5. Evaluation of FSO for *C. perfringens* — preferred specification

The ALOP for *C. perfringens* evaluated in Section 6 corresponds to the second example given in Section 7.1. Strictly, evaluation of an FSO corresponding to that ALOP must take account of the full distribution of hazard metric (in this case, the number of *C. perfringens* type A, CPE+ vegetative cells in a serving) and the dose-response curve. In fact, in this case, the FSO can and should be stated in some such form as:

$$\int f(h)\, g(h)\, dh \le \text{ALOP} \tag{9}$$

where $g(h)$ is the frequency distribution for servings with hazard metric h (number of type A, CPE+ vegetative cells per serving), and $f(h)$ is the dose-response curve (the probability of illness for ingestion of h cells of a random *C. perfringens* type A, CPE+ clone). This is a requirement on the whole distribution $g(h)$ for the hazard metric, not just "concentration" or prevalence. To take account of the uncertainty in the dose-response curve, the specification becomes more complex, in the form:

$$\Pr\left(\int f(h)\, g(h)\, dh > \text{ALOP}\right) \le \alpha \tag{10}$$

where α is the acceptable level of risk to exceed the ALOP (*e.g.* 1% to 5%). Since f is supposed not known exactly, equation (10) does not give an explicit practical method of evaluation; but one approach would be to define

$$\begin{aligned} F &= \int f(h)\, g(h)\, dh \\ S &= \int \tilde{f}(h)\, g(h)\, dh \end{aligned} \tag{11}$$

where the tilde (~) indicates maximum likelihood (ML) estimate, so that S is computable from observations. Then knowledge of the uncertainty distribution round the MLs (obtainable from the same data that provided the ML estimates), allows computation of the set $\{S\}$ specified by equation (6), that is

$$\{S\} \quad \text{such that} \quad \Pr(F > \text{ALOP} \mid S) \le \alpha \tag{12}$$

the boundaries of which set would be the FSO specification. In a Monte Carlo, the method of doing this would be to compute each expression given in equation (11) in each uncertainty iteration, calculate the ratio F/S (using the assumption that the distribution of this ratio is independent of the value of S), and save those values. The resultant distribution of the ratio F/S may then be used to calculate the boundary of $\{S\}$ such that equation (12) is satisfied.[22]

[22] It may also be necessary to take account of numerical uncertainty in the evaluation of F and S in each uncertainty iteration, see Appendices C.2 through C.4.

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This discussion really presumes that $g(h)$ can be specified in some useful parametric form,[23] so that the set $\{S\}$ of equation (12) can be expressed in terms of those parameters. For example, if the shape of $g(h)$ were known to be lognormal, then it would be possible[24] to evaluate the integrals in equation (11) in terms of the median and geometric standard deviation (since those two parameters would then completely define g), and then the boundary of the set $\{S\}$ in equation (12) would be defined by some inequality or inequalities relating those parameters to the acceptable level of risk α.

7.6. Evaluation of FSO for *C. perfringens*; maximum concentration is not sufficient

No simple statistics (such as maximum concentration or prevalence) of the distribution $g(h)$ are strictly sufficient in this case to adequately define a FSO, because specification of any such metric does not necessarily correlate (under all circumstances) with the frequency of illnesses (which is the desired metric for the ALOP).

To illustrate this point, Figure 3 shows the maximum likelihood (ML) estimate for cumulative distributions for numbers of vegetative cells initially present in the food servings (just prior to 10-fold growth during stabilization), and at the time of consumption (Appendix C.1 describes the scales used for this graph). It can be seen in Figure 3 that the initial numbers of cells are approximately lognormal (with a slightly shorter tail, indicated by the upward curvature), whereas the final number of cells have a distribution that is difficult to describe compactly, but clearly has a very long right tail (at high vegetative cell numbers).

For *C. perfringens*, the predicted maximum number of cells in a serving is governed by the maximum vegetative cell concentration that *C. perfringens* can grow to in the food examined (in reality, it would be the maximum that would not be thrown out as evidently contaminated[25]). The risk assessment demonstrates that growth to such high concentrations is quite likely for a small fraction of food servings, because of the failed status of a small fraction of consumer refrigerators.

[23] The same need not be true of the estimates, although in practice such estimates probably would be defined by a limited number of parameters. However, the estimates could be expressed in terms of different parameters — whatever is needed to allow evaluation of S.

[24] This could still require numerical evaluation of the integrals, so that the resultant inequality/inequalities would be specified numerically rather than analytically.

[25] The possibility of such discard is not considered in the main part of the risk assessment, although it is examined in a "what if" scenario. It is not examined here.

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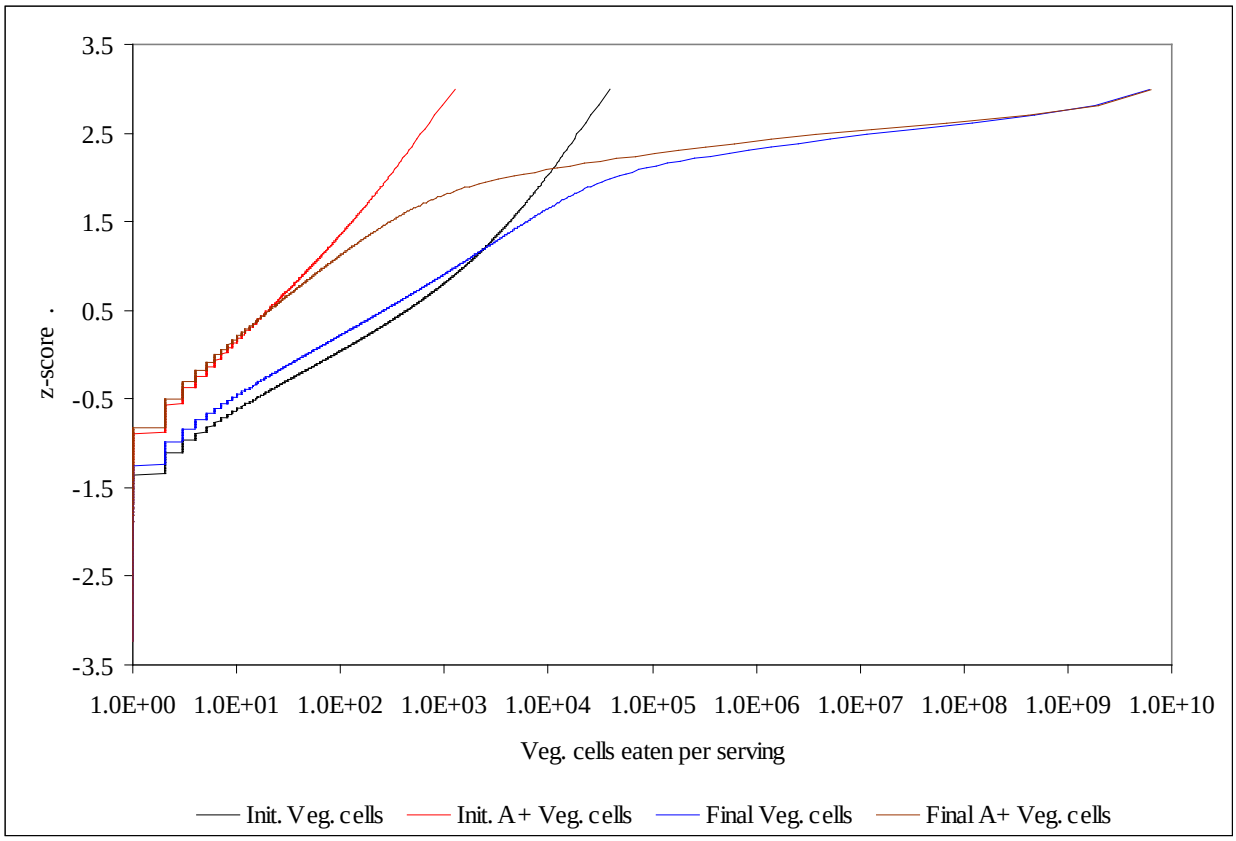

Figure 3. Cumulative distributions for vegetative cell numbers (separately for total *C. perfringens* and Type A, CPE+).[26]

The small fraction of food servings so affected provide the long tail of the distributions seen in Figure 3. The importance of this long tail may be seen in Figure 4 of large consumed cell counts (>10^8 cells/serving, at and to the right of the extreme right hand end of Figure 3), where servings causing illnesses (in this model run of 100,000,000 servings) are plotted in a distinct color. The selection of the cutoff cell count is such that Figure 4 contains approximately 97.4% (340/349) of the predicted illnesses, but only 1.88×10^{-5} (1 in 53,000) of the total servings.

[26] These distributions were obtained using a 100,000,000 serving model run. Results are in workbook Output_2.xls in sheet "Cell numbers".

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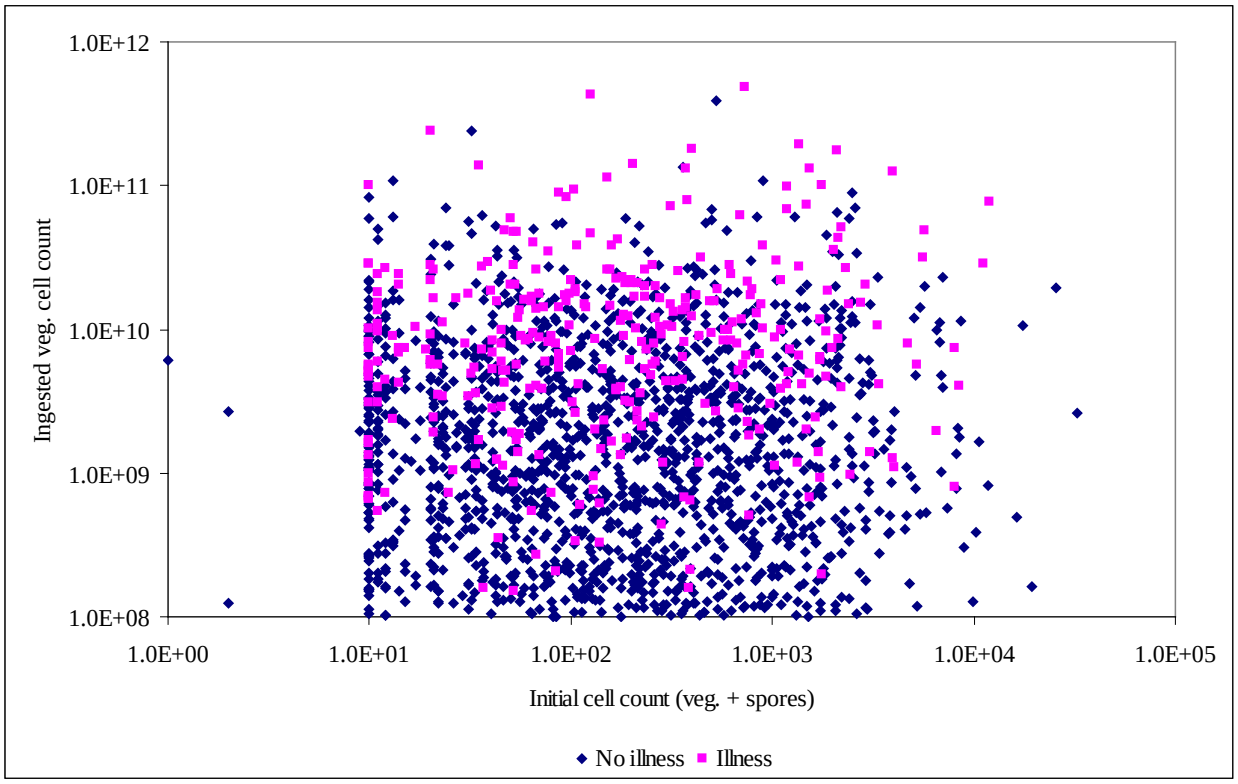

Figure 4. Scatterplot of the ingested veg. cell count versus the initial (veg. + spore) cell count for Type A, CPE+ cells; all 1878 cases with $>10^8$ cells per serving in 10^8 servings.[27,28]

Thus almost all the illnesses are due to the extreme right hand end of the distribution in Figure 3, with the great majority of that distribution being irrelevant; only the top 0.002% of the distribution contributes significantly.

Evaluation of the distribution $g(h)$ shown in Figure 3, and particularly the important part of that distribution ($>10^8$ cells/serving, shown later in Figure 8) in this case requires the numerical approach of the risk assessment. Attempting to approximate the risk assessment model steps using simple analytic distribution shapes for variability distributions (*e.g.* by assuming lognormality of the cell number distribution), or using simple parameters (like geometric mean and standard deviation) for the whole distribution, would fail to correctly capture the critical part, the extreme upper tail of the final distribution. Although the particular details of this problem may be specific to *C. perfringens*, such details require separate evaluation in every case.[29]

[27] The assumed initial growth of a factor of 10 during stabilization results in the sharp boundary at the left at 10 cells, with the few cases with less than 10 cells being servings with initially no vegetative cells but with a few spores that germinate during subsequent storage. This sharp boundary is an artifact of the growth approximation used in the model — a deterministic growth model is used, rather than the correct stochastic one; this approximation should have essentially no effect on the desired illness predictions, and was adopted for speed and simplicity.

[28] The model run output is in workbook Output_2.xls, sheet "High Cell Numbers".

[29] It is possible that an analytic approach concentrating on extreme values combined with prevalence might work. No investigation of such an approach has been attempted.

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7.7. Evaluation of FSO for *C. perfringens*; using prevalence as a surrogate

The number of illnesses produced by *C. perfringens* in the food servings examined is evidently directly proportional to the prevalence of *C. perfringens* in those food servings at the consumer, *all other things being equal*. Indeed, this should always be true for any food-related risk.

However, specifying a FSO in terms of prevalence requires the *assumption* that all other things would remain equal,[30] in this case that changes in prevalence could be achieved without changing the distribution of cell counts in the servings still containing *C. perfringens*. It is not clear that this assumption holds. The risk assessment model itself demonstrates that only small changes in the number of illnesses can be achieved by modifying the allowed growth of germinated spores during stabilization after the lethality step of production. Figure 1 shows the changes in illness rate and prevalence, and they are clearly not proportional over the full range shown, so that changes in the distribution of cell counts must occur. However, directly modifying the prevalence would require different strategies, presumably designed to change the prevalence in the incoming raw material or elsewhere in the food supply chain. The effect of such strategies (with a fixed growth during stabilization) on the distribution of *C. perfringens* at the consumer is not known, and the risk assessment is not designed to evaluate such questions.[31]

However, if one makes the *assumption* that the distribution would not change, a FSO may be evaluated in terms of prevalence either of type A, CPE+, *C. perfringens* in the final food servings, or alternatively, in terms of total *C. perfringens* in the final food serving. The latter requires the additional assumption that the fraction of type A, CPE+*C. perfringens* would not alter as the prevalence alters, and introduces a further uncertainty in the specification (the uncertainty of that fraction).

The evaluation of a FSO under such conditions using the risk assessment model is relatively straightforward. It is necessary to account for both the uncertainty in the dose-response assessment and, at fixed prevalence, the uncertainty in the distribution of numbers of vegetative cells in servings at the time they are eaten. In the risk assessment model, this simply requires evaluating the uncertainty distribution for the ratio of the illness rate (or expected rate, see Appendix B) to the prevalence. The ratio can be obtained for both type A, CPE+, and for total *C. perfringens*. From this uncertainty distribution, and using the *assumption* of proportionality between illness rate and prevalence, the value of the FSO can be obtained at any particular uncertainty percentile by reading off the ratio of illness rate to prevalence and then computing from this ratio the prevalence that corresponds to the chosen ALOP (which is specified as an illness rate). This sequence of operations corresponds to determination of the set $\{S\}$ in equation (6): here the surrogate S is the prevalence of *C. perfringens* (either type A, CPE+ or total). The assumption of proportionality between F and S ensures that all uncertainties are correctly taken into account by evaluation of the distribution of the ratio of expected illness rate to prevalence,

[30] Alternatively, this assumption could be replaced by a proof. Strictly speaking, the assumption required is that a correctly weighted integral of the uncertainty distribution for number of illness at fixed prevalence is invariant as the prevalence changes.

[31] In the case of *C. perfringens* contamination, it is not clear that any risk assessment designed for such a task could be constructed at the moment, in view of the poor understanding of the origins of the contamination and lack of any obvious way to directly change the prevalence once the food is contaminated.

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and provides a one-to-one correspondence between percentiles on this distribution and the corresponding boundary of $\{S\}$ (prevalence) — indeed, $\{S\}$ is a line segment, the upper boundary of which corresponds to risk level α.

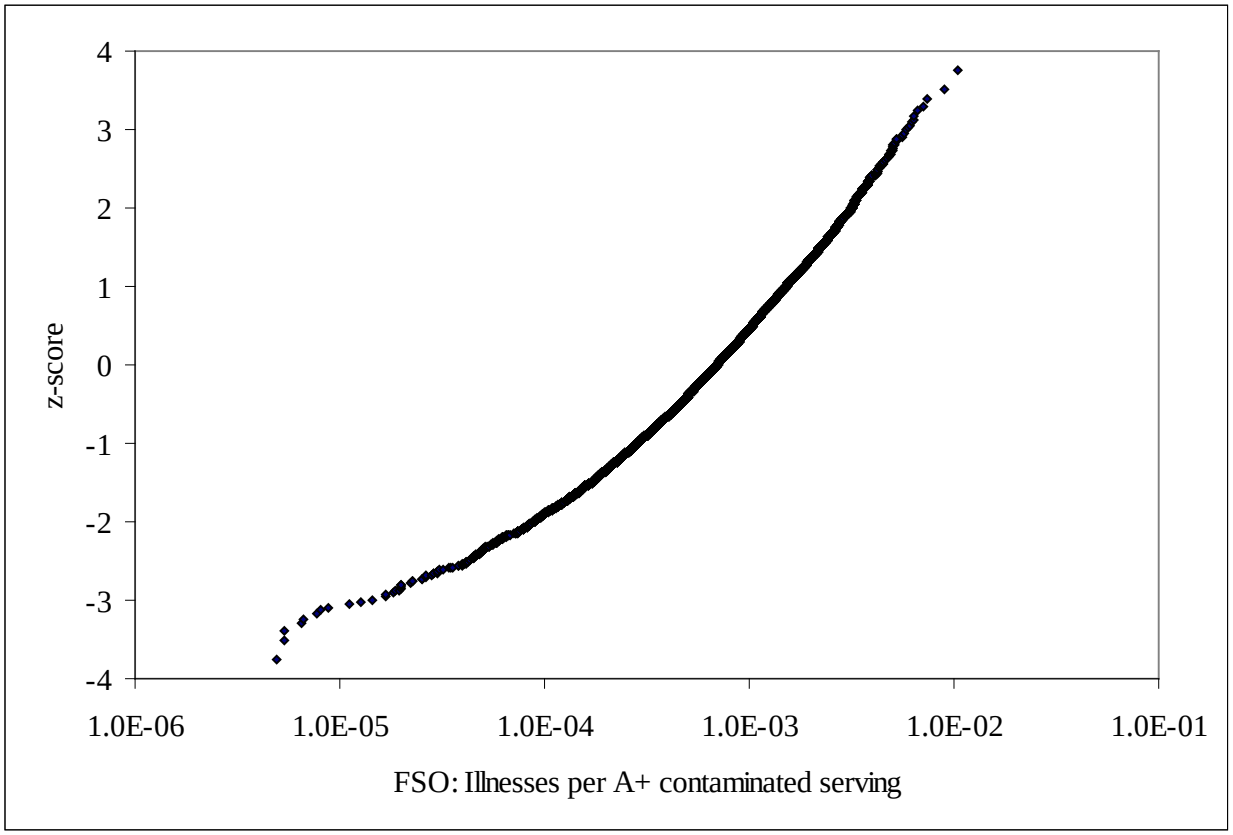

Figure 5. Uncertainty distribution for the ratio of illness rate to vegetative *C. perfringens* type A, CPE+ contamination rate at consumption.

Figure 5 shows the uncertainty distribution for the ratio of illness rate to *C. perfringens* type A, CPE+ vegetative cell contamination rate at consumption.[32] The 95th percentile of this is at approximately 2.5×10^{-3}, and the 99th percentile is at approximately 3.8×10^{-3} illnesses per contaminated serving.[33] In a similar manner, Figure 6 shows the uncertainty distribution for the ratio of illness rate to *C. perfringens* total vegetative cell contamination rate at consumption. The 95th and 99th percentiles on these distributions are 1.2×10^{-3} and 1.8×10^{-3} illnesses per contaminated serving, respectively. Taking these two percentiles, and the two options for ALOP, gives FSOs as shown in Table 1.

The discussion of Section 7.3 should be borne in mind when examining Table 1. The ALOP of 21 illnesses per million servings was designed so that there was 1% chance or less that current conditions exceeded that ALOP. Selecting a FSO so that there is only 5% chance the ALOP is

[32] This is literally at consumption, so it includes the effect of any cooking that was done.
[33] The numerical uncertainty standard deviation in these values is one unit in the last significant digit or less.

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exceeded (the 95th level of certainty) probably corresponds to an FSO that is less stringent than current conditions. Conversely, for an ALOP of 13 illnesses per million servings (5% chance or less that current conditions exceed that ALOP) an FSO selected at the 99th level of certainty will probably be more stringent than current conditions. The two choices with $\alpha = \beta$ in the notation of Section 7.3 result in nearly identical FSOs, and should correspond approximately to current conditions.

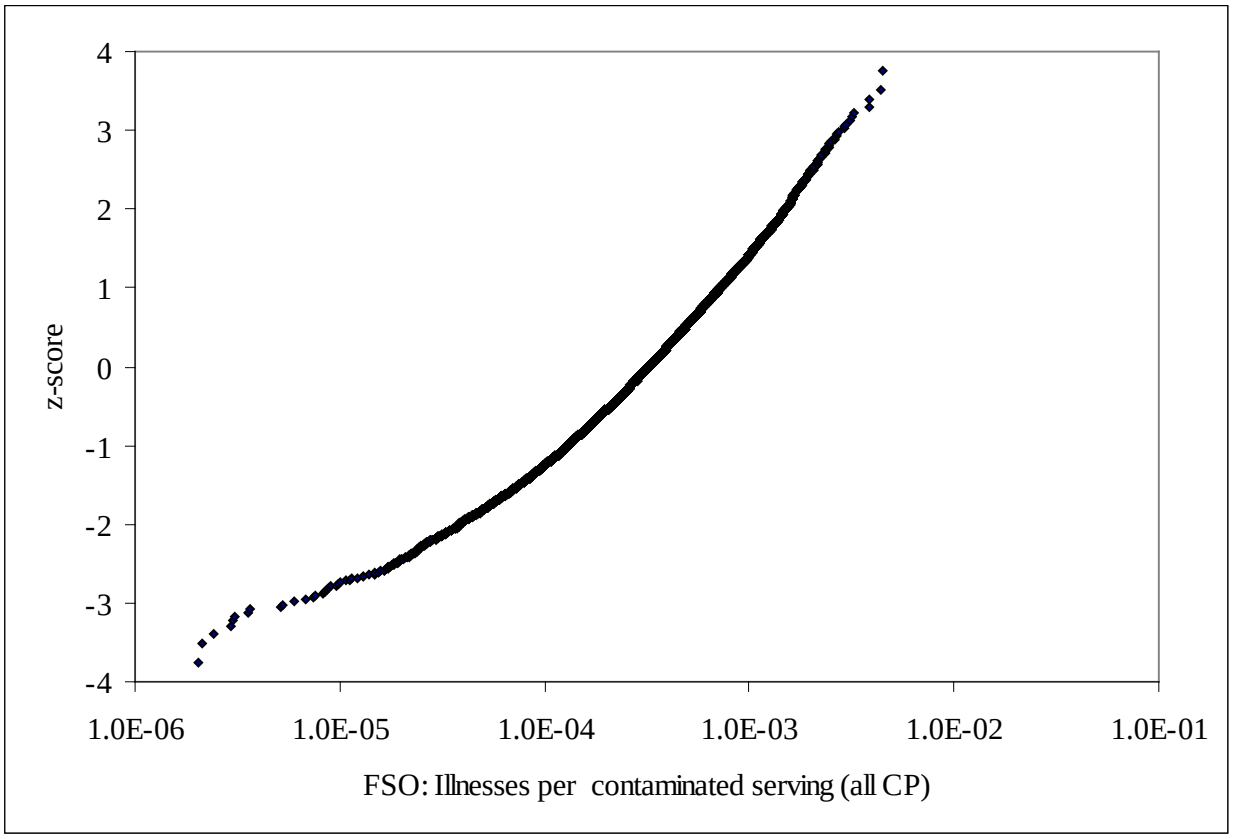

Figure 6. Uncertainty distribution for the ratio of illness rate to total vegetative *C. perfringens* contamination rate at consumption.

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Table 1. FSOs for the two ALOP selections and two levels of certainty

ALOP Illnesses per million servings	Level of certainty	FSO: Prevalence in hot dog servings of:	
		Type A, CPE+ *C. perfringens*	Total *C. perfringens*
21	95th	0.85%	1.80%
	99th	0.55%	1.16%
13	95th	0.53%	1.12%
	99th	0.34%	0.72%

8. Evaluation of a PO for *C. perfringens* post-stabilization

8.1. A PO based on servings

The discussion of Section 7 for evaluation of a FSO can be extended to the evaluation of a PO at an earlier point in the production of hot dogs. The selected point for this demonstration is immediately after the stabilization step, prior to any storage at (or transport from) the production location. For evaluation of a PO, some surrogate for the required functional *R* (see Section 7.2) must necessarily be used, since prior to the point of consumption the distribution $g(h)$ does not exist (except in the trivial case that there is no change in the distribution of hazard metric between the point of application of a PO and the point of consumption).

For *C. perfringens*, the risk assessment model allows evaluation of the relation between the distribution of numbers of cells per serving just after stabilization and just before consumption. Figure 7 shows this relationship for total *C. perfringens* for the 11,242 servings with non-zero vegetative cell number at consumption in a sample of 1,000,000 servings.[34] A similar picture is obtained for type A, CPE+ *C. perfringens* (necessarily so, since there is nothing specific to this sub-type between these two points of the model). For relatively low final vegetative cell counts (below about 10^6 cells/serving) there is a reasonable correlation between initial and final cell count. However, such low cell counts are very unlikely to produce illness. Most illnesses are caused by cell counts higher than 10^8 cells/serving (Figure 4). Both Figure 4 and Figure 7 show that there is practically no correlation between initial and final numbers of cells for this extreme range — the distribution of cell counts $>10^8$ cells/serving is independent of the initial cell counts.

The lack of correlation of high final cell counts (hence illnesses) with initial cell counts is emphasized by Figure 8, showing the distributions of cells/serving for type A, CPE+ cells where there were more than 10^8 cells/serving at consumption (in a 100,000,000 sample, evaluated at the ML estimates for all parameters). The distributions are identical for an initial cell count <300 cells/serving and for ≥300 cells/serving, for both illness-causing and non-illness-causing

[34] The model results for this run are in workbook Output_2.xls, in sheet "input-output".

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servings.[35] To complete these observations, Table 2 shows that the probability for a serving initially contaminated with vegetative cells to also be contaminated with vegetative cells just prior to consumption is not strongly dependent on initial cell count (the 1–9 initial cell count range consists of spores only).

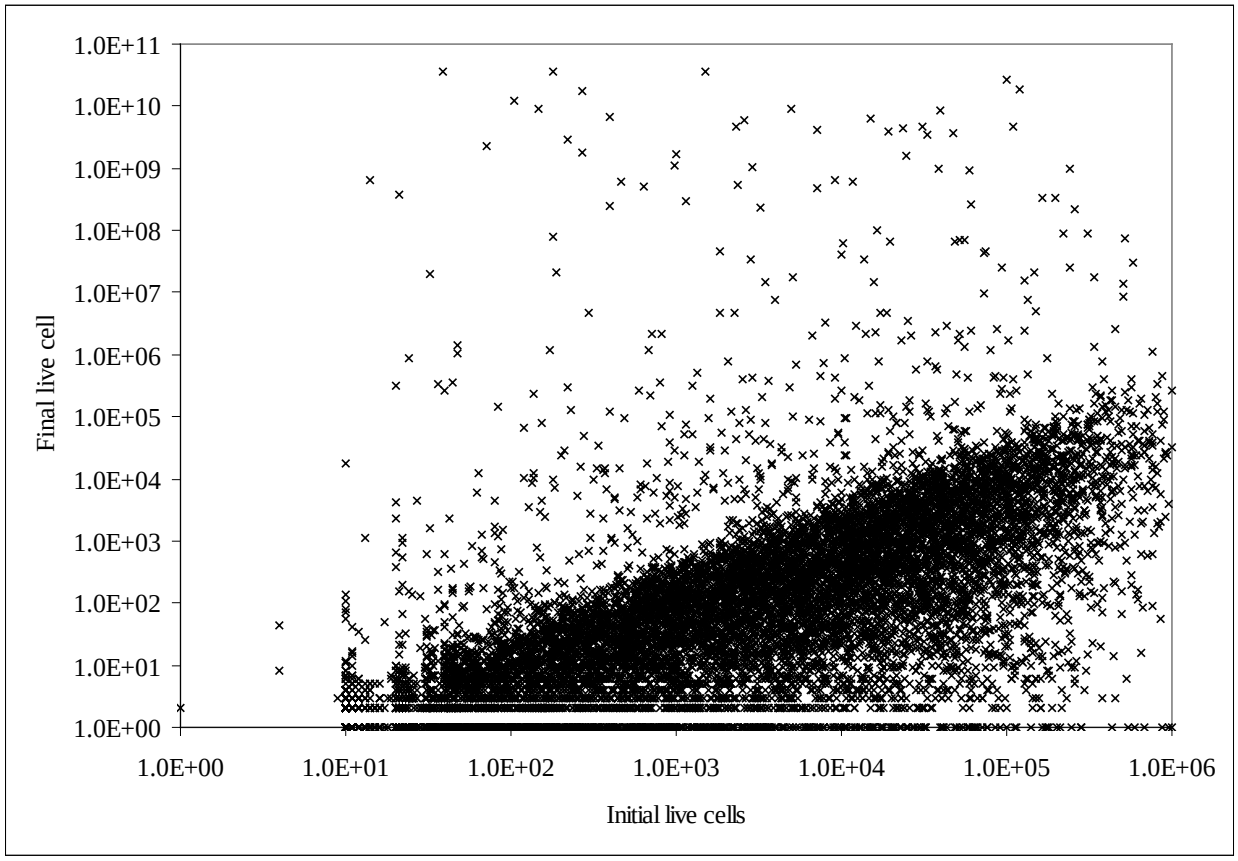

Figure 7. Relationship between initial live cells (spores + vegetative cells) and final vegetative cells, for non-zero final vegetative cells.

[35] The slight visual difference between <300 and ≥300 cell/serving distributions is most likely due to pure chance. Kolmogorov-Smirnoff and Kuiper statistics (Press *et al*., 1992) for differences in distribution indicate all differences have $p > 0.3$.

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Table 2. Fraction of servings producing non-zero final cell count

Initial live cell count (spores + vegetative cells)	1–9	10–99	100–999	1,000–9,999	10,000–99,999	100,000–999,999	>1,000,000
Initial number of Servings	1585	4804	5059	5471	4972	1644	75
Final number of servings with non-zero cell count	4	1690	2505	3022	2961	1013	47
Surviving Fraction	0.0025	0.35	0.50	0.55	0.60	0.62	0.63
Total 23,610 servings with initial live cells (vegetative + spore) in a 1,000,000 serving simulation							

Thus for the evaluation of a PO, using prevalence as a surrogate is probably about the best that can be done, since almost any other characteristic of the distribution of cells/serving just after stabilization appears to be irrelevant to the number of illnesses caused.

The approach is exactly the same as for the FSO (the same Monte Carlo iterations can be used; all that is necessary is to save the prevalence at the relevant point in the model, in this case just after stabilization). It is assumed that the illness rate is proportional to the prevalence in servings, and the uncertainty distribution for the ratio of illness rate to prevalence calculated (that is, the illness rate per contaminated serving). For type A, CPE+ *C. perfringens* cells (vegetative + spores) this gives 95th and 99th percentiles of the distributions of illnesses per contaminated serving of 8.9×10^{-4} and 13.8×10^{-4} illnesses per contaminated serving respectively (Figure 9).[36] For total *C. perfringens* cells it gives 95th and 99th percentiles of the distributions of illnesses per contaminated serving of 5.5×10^{-4} and 8.3×10^{-4} illnesses per contaminated serving respectively (Figure 10).[37] Applying to the ALOP gives the POs shown in Table 3 for vegetative cells + spores of *C. perfringens* just after stabilization (the values for the POs are higher than the corresponding values for the FSOs in Table 1 primarily because of the effect of cooking of a large fraction of the hot dogs consumed — such cooking will almost always kill most or all of any *C. perfringens* present).

[36] The numerical uncertainty standard deviation in these value is 4 units or less in the least significant digit shown.
[37] The numerical uncertainty standard deviation in these value is 3 units or less in the least significant digit shown.

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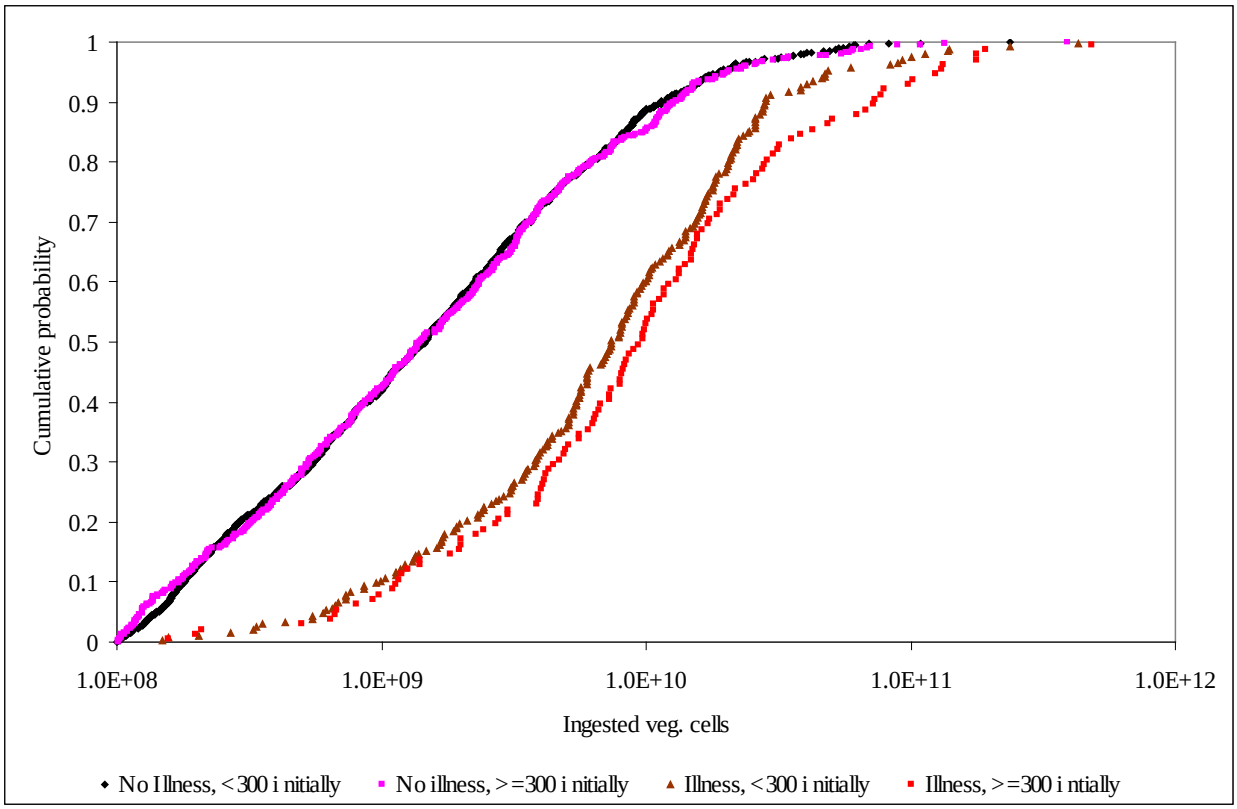

Figure 8. Distribution of final vegetative cell numbers/serving (type A, CPE+) for $\geq 10^8$ cells/serving.

The discussion of Section 7.3 should be taken into account when examining Table 3; the same phenomena can be expected as described at the end of Section 7.7 for the FSO; and indeed, the two cases where $\alpha = \beta$ in the notation of Section 7.3 result in nearly identical POs that should correspond approximately to current conditions.

Table 3. POs for the two ALOP selections and two levels of certainty

ALOP Illnesses per million servings	Level of certainty	PO: prevalence in hot dog servings of:	
		Type A, CPE+ *C. perfringens*	Total *C. perfringens*
21	95th	2.36%	3.84%
	99th	1.52%	2.52%
13	95th	1.47%	2.39%
	99th	0.95%	1.57%

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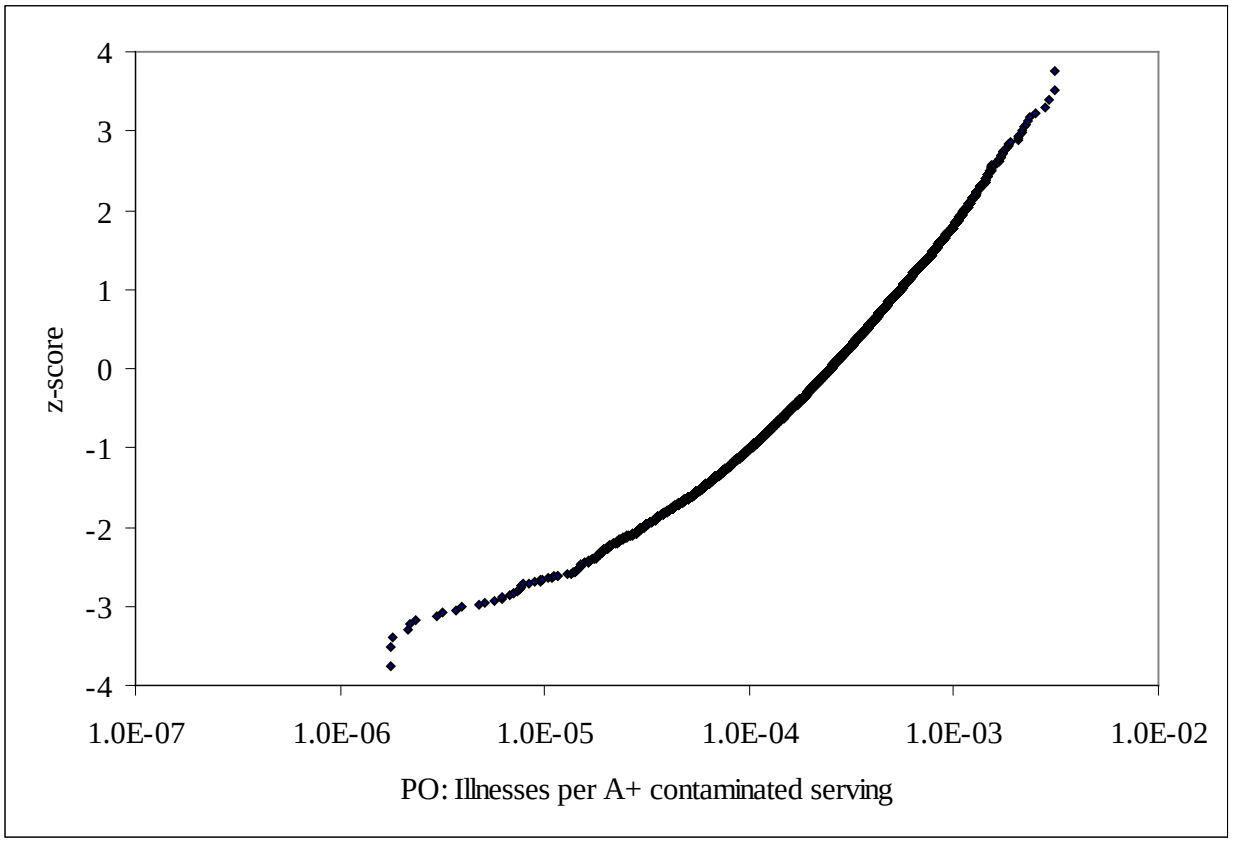

Figure 9. Uncertainty distribution for the ratio of illness rate to *C. perfringens* contamination rate by type A, CPE+, vegetative cells+spores just after stabilization.

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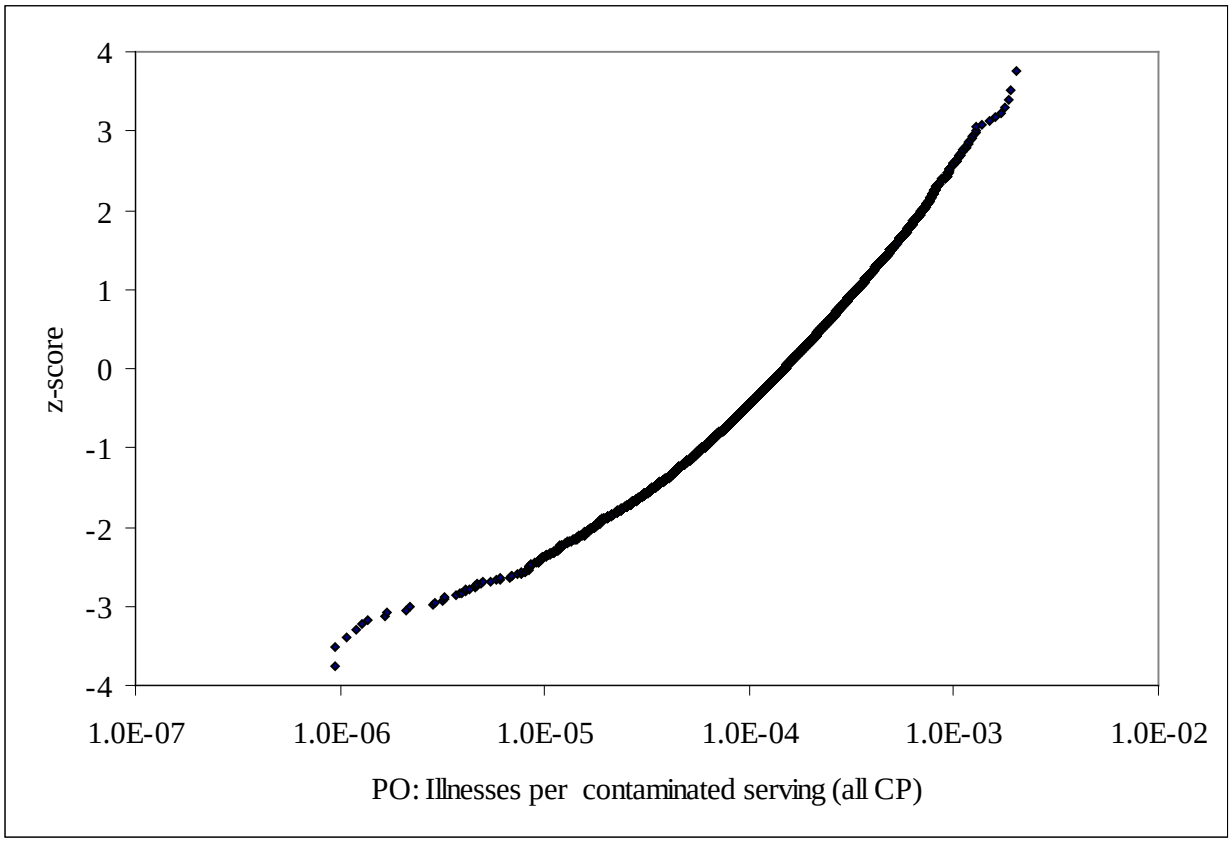

Figure 10. Uncertainty distribution for the ratio of illness rate to *C. perfringens* contamination rate by total vegetative cells+spores just after stabilization.

8.2. A PO based on a fixed food mass

The preceding discussion has been based on the prevalence in hot dog servings. This is a natural unit of exposure for the risk assessment and corresponds to what people are exposed to. However it does not represent a PO that is particularly practical for implementation of a MC, since it would require random sampling and analysis of sample weights chosen from the distribution of hot dog serving size, and detection of both ungerminated spores and vegetative cells[38] within that whole sample. The distribution of hot dog serving size is shown in Figure 11. The majority of serving weights appear to be clustered at or near 2 and 4 ounces (56.7 and 113 grams); indeed, the claimed major retailer of hot-dogs in the U.S. sells 2 or 4 ounce beef hot-dogs.[39]

For evaluation of sampling plans, the actual sample weight that is analyzed for cells is required. For example, for the original measurements on which the risk assessment is largely based 50 g

[38] Table 2 indicates that the spore component contributes negligibly, and the risk assessment confirms that. The vegetative cells at this point of the process have arisen by germination from spores in the original meat or spices.

[39] See http://www.7-eleven.com/newsroom/funfacts.asp — "7-Eleven sells close to 100 million fresh-grilled hot dogs every year, more than any other retailer in America" and http://www.7-eleven.com/products/products_index.asp.

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meat samples were selected, but effectively only 1/3 g of that sample was actually plated (the sample was diluted with an additional 100 mL, and 0.1 mL of the result plated — Kalinowski *et al*., 2003). The only important quantity is the actual amount of originally-sampled material that is analyzed for *C. perfringens*, taking account of any dilution or concentration steps; the originally-selected sample size is irrelevant. For this demonstration, it will be assumed that the sampling procedure can measure vegetative cells in 1 g of sample.

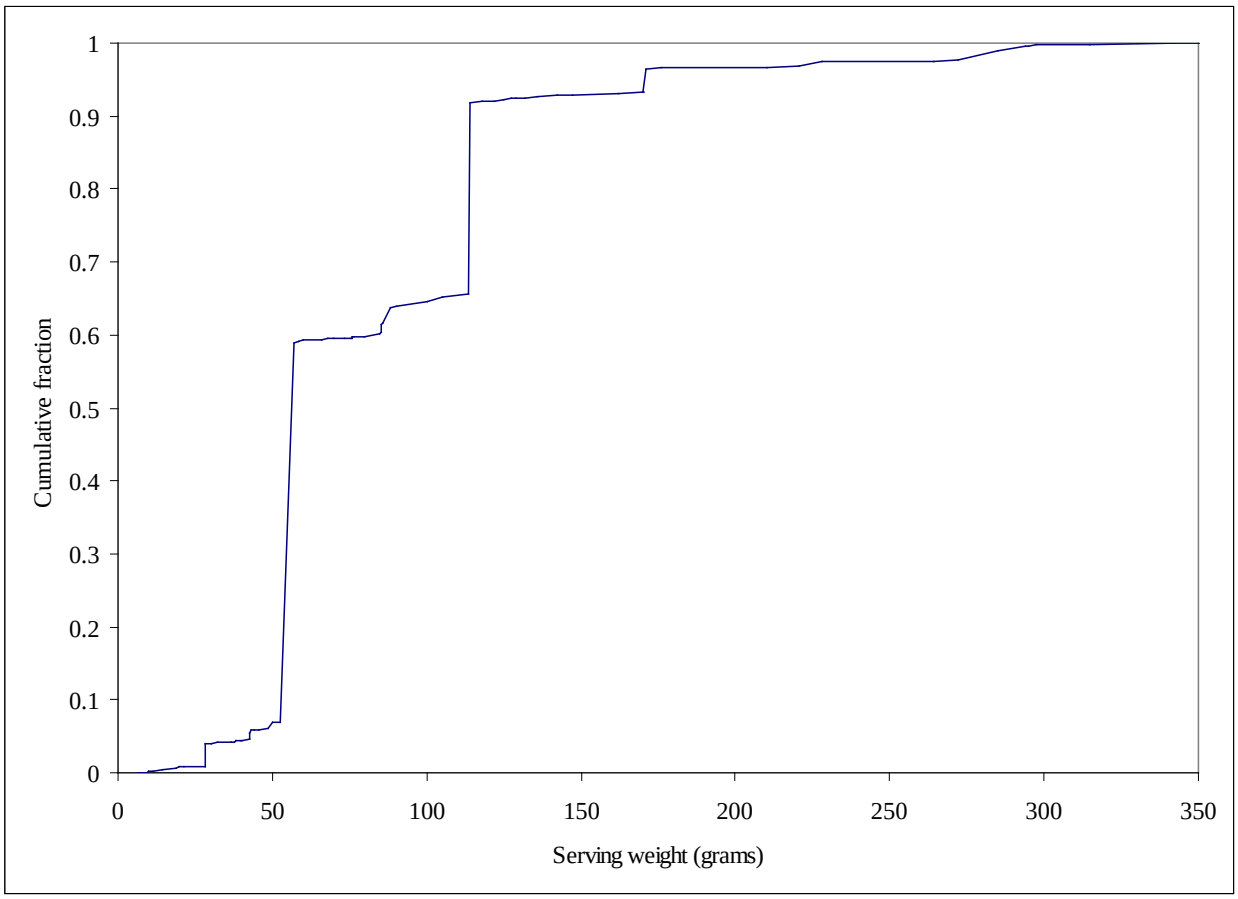

Figure 11. Cumulative distribution for hot dog serving weight.

For the evaluation of a PO, therefore, it is assumed that the illness rate is proportional (but with uncertainty) to the vegetative cell prevalence in 1 g samples, and the distribution of the ratio of illness rate to prevalence in 1 g samples calculated within the risk assessment model just as for the prevalence in servings in Section 8.1. This requires adding a component to the risk assessment model that calculates *C. perfringens* incidence in such a 1 g sample, in parallel to the calculation for a whole serving. This could be done either by sub-sampling from the serving size evaluated (provided the serving sizes exceeds the measurement size — the smallest hot dog serving is 6.29 g), or by independently evaluating (using the same concentrations) another 1g "serving" within the same uncertainty loop of the Monte Carlo procedure. For this demonstration, the first procedure (sub-sampling) was implemented, since it is slightly faster.

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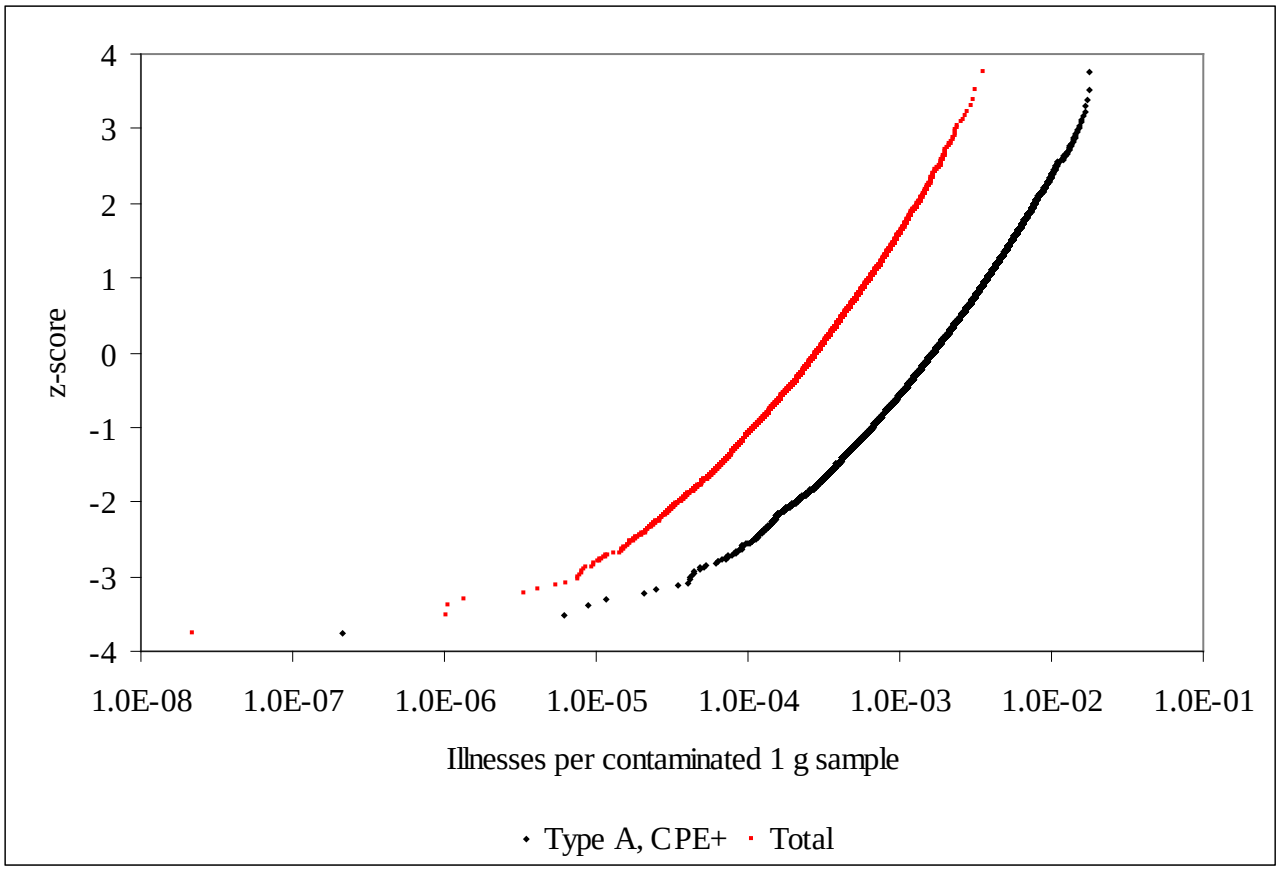

Figure 12. Uncertainty distribution for the ratio of illnesses to vegetative-cell contaminated 1 g samples.[40]

Figure 12 shows the uncertainty distribution for the ratio of illnesses to contaminated 1 g samples (*i.e.* illness rate divided by prevalence of vegetative cells in 1 g samples). The 95th and 99th percentiles of these distributions are, for Type A, CPE+: 6.2×10^{-3} and 9.1×10^{-3} illnesses per contaminated sample respectively; for total *C. perfringens*: 1.1×10^{-3} and 1.6×10^{-3} illnesses per contaminated sample respectively. From these, POs may be derived as before for the prevalence in 1 g samples, as shown in Table 4.

[40] 7,500 uncertainty iterations of 10,000,000 servings (variability iterations), taking approximately 36 hours CPU time on an Intel P4 at 2.6 GHz. The model results are shown in workbook PO_fixed.xls, sheet Run_10_2. As a check, three such runs were performed and compared with three runs of 7,500 uncertainty iterations of 7,500,000 servings (see workbook MC_runs.xls). No substantial differences were found.

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Table 4. POs (for 1g samples) for the two ALOP selections and two levels of certainty[41]

ALOP Illnesses per million servings	Level of certainty	PO: vegetative cell prevalence in 1 g samples of:	
		Type A, CPE+ *C. perfringens*	Total *C. perfringens*
21	95th	0.34%	2.00%
	99th	0.22%	1.29%
13	95th	0.21%	1.24%
	99th	0.13%	0.80%

Again, the discussions of Section 7.3 and the end of Section 7.7 apply.

Note: it can be seen that there is a large difference between the columns for Type A, CPE+ and Total *C. perfringens* in Table 4, but a much smaller difference in Table 1 and Table 3. This is due to the extreme nature of the concentration distribution of *C. perfringens* estimated in the risk assessment. For example, using the concentration distribution for total *C. perfringens* in meat, the prevalence varies slowly for samples above 1 g; from 1.3% at 100g, to 1% at 10 g, and to 0.7% at 1 g sample size. Below 1 g, the prevalence drops more sharply; at 0.1 g it is 0.3%, at 0.01 g it is 0.09%. Thus from the average serving size of 84 g down to the assumed sample of 1 g, the prevalence of total *C. perfringens* changes from 1.3% to 0.7%, about a factor two difference. For type A, CPE+, about 0.6% of total *C. perfringens*, the corresponding change is from 0.5% prevalence to 0.06% prevalence, a factor of almost 10. These differences largely explain the changes between Table 4 and Table 1/Table 3.

8.3. Relation of PO to current conditions

If the ALOP is based on current conditions (Section 6.2), then the PO is necessarily also based on current conditions, albeit indirectly. As pointed out in Section 7.3, some care is needed to ensure that estimating a FSO or PO from the ALOP does not result in a value that is too stringent. In the particular case studied here, the original measurements upon which the risk assessment is based correspond almost exactly to the location for which the PO is evaluated. In such circumstances, an alternative approach would be to estimate a PO directly from available measurements of current conditions. That approach would ensure (with any required confidence) that current conditions do not violate the PO; although the risk assessment model would still be required to evaluate what is the corresponding ALOP.

9. Evaluation of a MC for *C. perfringens* at the plant gate

9.1. Relation of PO and MC

The PO evaluated in Section 8 is a microbiological specification that contributes to an ALOP (see the Glossary). To meet such a specification requires an acceptability criterion, a MC, for a food product or food lot. A MC consists of (CAG/GL 21-1997, §2.1):

[41] Numerical uncertainties (standard deviation) in the values in the penultimate column of Table 4 are about 1 in the last digit of the values shown, and in the last column are about 3 in the last digit of the values shown

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- a statement of the microorganisms of concern and/or their toxins/metabolites and the reason for that concern;
- the analytical methods for their detection and/or quantification;
- a plan defining the number of field samples to be taken and the size of the analytical unit; and
- microbiological limits considered appropriate to the food at the specified point(s) of the food chain.

In addition, the MC should also state (CAG/GL 21-1997, §2.2):

- the food to which the criterion applies;
- the point(s) in the food chain where the criterion applies; and
- any actions to be taken when the criterion is not met.

Further, "when applying a microbiological criterion for assessing products, it is essential, in order to make the best use of money and manpower, that only appropriate tests be applied to those foods and at those points in the food chain that offer maximum benefit in providing the consumer with a food that is safe and suitable for consumption" (CAG/GL 21-1997, §2.3).

A statement of the first two components of the MC is omitted here; the reason for concern with *C. perfringens* is described in detail in the risk assessment (Crouch and Golden, 2005), and the analytical methods are assumed to correspond to those used by Kalinowski *et al.* (2003). [42] The appropriate microbiological limit is the PO obtained in Section 8. The food evaluated is hot dogs, immediately before leaving the production facility, and it is assumed that appropriate corrective actions will be taken if the MC is not met.

In the following discussion, it should be recalled that the PO has, for *C. perfringens*, been evaluated in terms only of the prevalence of *C. perfringens*, for the reasons discussed in Section 8.1. In evaluations of other organisms, it might also be appropriate to take account of the level of the organism in food in designing sampling plans to demonstrate or evaluate compliance with the PO, or the effect of process controls in achieving the PO.

9.2. Design of a sampling plan

Evaluation of a MC now comes down to designing a test program that can detect the prevalences shown in Table 4 with acceptable false positive and negative rates. The discussion here is primarily concerned with the sample sizes required, in order to evaluate the feasibility of any such MC. Sampling of hot dogs is assumed at random,[43] with 1 g of any sample actually analyzed (this is the size of the analytic unit; see also Section 9.3). For this demonstration it will further be assumed that the detection rate for vegetative cells is 100%. For lower detection rates,

[42] These included plating to enumerate the number of viable cells in approximately 1/3 gram of sample, and subsequent confirmation of identity as *C. perfringens*.

[43] The hot dogs are assumed to be a random sample of all hot dogs produced; in particular, to mix spicy and non-spicy hot dogs randomly in the same stream. In practice this would never happen. The importance is shown in Section 9.3; but see also the caveats of Section 5.2.

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the distribution of counts of vegetative cells would have to be taken into account, and the detection efficiency for different numbers of cells evaluated experimentally.[44]

Following the discussion of Section 7.3 and the end of Section 7.7, the appropriate selection from Table 4 are prevalences 1.3% for total *C. perfringens*, or 0.22% for type A, CPE+ *C. perfringens*. Detection of type A, CPE+ *C. perfringens* first requires detection as *C. perfringens*, with additional tests for type A, CPE+; so would be less efficient than just detecting total *C. perfringens*.

The false positive and negative rates allowable for the MC would likely depend on circumstances, depending on the stringency required to demonstrate compliance. The design of a sampling plan is standard, and requires specification of the increment in prevalence to be detected with some specified (high) probability, and specification of the (low) probability to falsely flag a non-compliance.

Suppose that any prevalence of s or higher is to be detected with probability P_d or more, while any prevalence equal to or less than the PO of p must be declared non-compliant with probability P_n or lower. Then for the most efficient sampling plan it is required to find the smallest N and an R, $0 \le R \le N$ such that:

$$\sum_{r=R}^{N} \binom{N}{r} s^s (1-s)^{N-r} \ge P_d$$
$$\sum_{r=R}^{N} \binom{N}{r} p^s (1-p)^{N-r} \le P_n \qquad (13)$$

For example, choosing

$p = 0.013$ (the PO)
$s = 0.026$ (double the PO)
$P_d = 0.9$
$P_n = 0.1$

gives the minimum number of samples N as 727, with $R = 14$ or more indicating non-compliance.

9.3. Size of the analytic unit

Sections 8.2 and 9.2 examine the case of 1g samples. This mass was chosen because it is likely close to the largest practical mass that may be plated reasonably economically in the manner described by Kalinowski *et al*. (2003). It is possible that some larger mass could be efficiently analyzed, since all that is required is prevalence (rather than the counts obtained by Kalinowski *et al.*, 2003). For example, a larger mass of food could be incubated to amplify the number of *C. perfringens* vegetative cells, and a small subsample then tested. Such a procedure would require some experimental verification of its specificity and sensitivity, since current selective media do

[44] This cannot be evaluated theoretically, since the reasons for non-detect probably would not be independent for multiple cells within a sample. The experiments of Kalinowski *et al.* 2003, and Taormina *et al*. (2003) suggest, but do not prove, high efficiency for detection even of single vegetative cells.

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not entirely eliminate other anaerobic spore-forming organisms that might outgrow any *C. perfringens* initially present.[45]

The effect of varying the sample mass analyzed can be approximately estimated from the distribution of contamination assumed in the risk assessment. Figure 13 shows the maximum likelihood estimates for prevalence of total *C. perfringens* in various sample masses of the meat or meat and spice mix used for hot dogs, according to the estimates in the risk assessment (Crouch and Golden, 2005), as a function of sample mass from 0.1 to 1000 g. It can be seen that there is not a huge variation with sample mass — for a variation of a factor of 10,000 in sample mass, the prevalence varies by a factor of about 5 (from 0.004 to 0.022 for the "Combined" curve in Figure 13). For the more practical range, from about 1 g to 100 g the prevalence varies from about 0.008 to 0.018,[46] and the corresponding PO could be expected to vary in about the same fashion.

The "Combined" curve in Figure 13 corresponds to the mix of 100% meat with spicy hot dogs observed in the food survey on which the risk assessment was based, and thus to the "food type" treated in this demonstration. In practice, it would presumably be possible to separately sample 100% meat hot dogs and spicy hot dogs — they could be considered separate food types, with separate FSOs, POs, MCs and possibly even different ALOPs.[47] Figure 13 shows that, based on the information used in the risk assessment, spicy hot dogs would have much higher prevalence of *C. perfringens*, and hence would contribute disproportionately to the risks (but see the caveats of Section 5.2).

[45] Such interfering organisms were present in the method used by Kalinowski *et al.* (2003); see the risk assessment (Crouch and Golden, 2005, Table 3.3, footnote c) for details.

[46] These are maximum likelihood estimates for current conditions, so at 1g are lower than the POs of Table 4 since the latter (should) correspond to upper bounds under current conditions (see Sections 7.3 and 8.3).

[47] If they were considered different food types, ALOPs were adopted to correspond to current conditions, and the information in the risk assessment is accurate (but see Section 5.2), then they would necessarily have substantially different ALOPs. If they are not considered different food types, and the MC derived here were applied to a production line producing just spicy hot dogs, then the spicy hot dog production line would fail the MC under current conditions (again, assuming the risk assessment information is accurate at such a disaggregate level).

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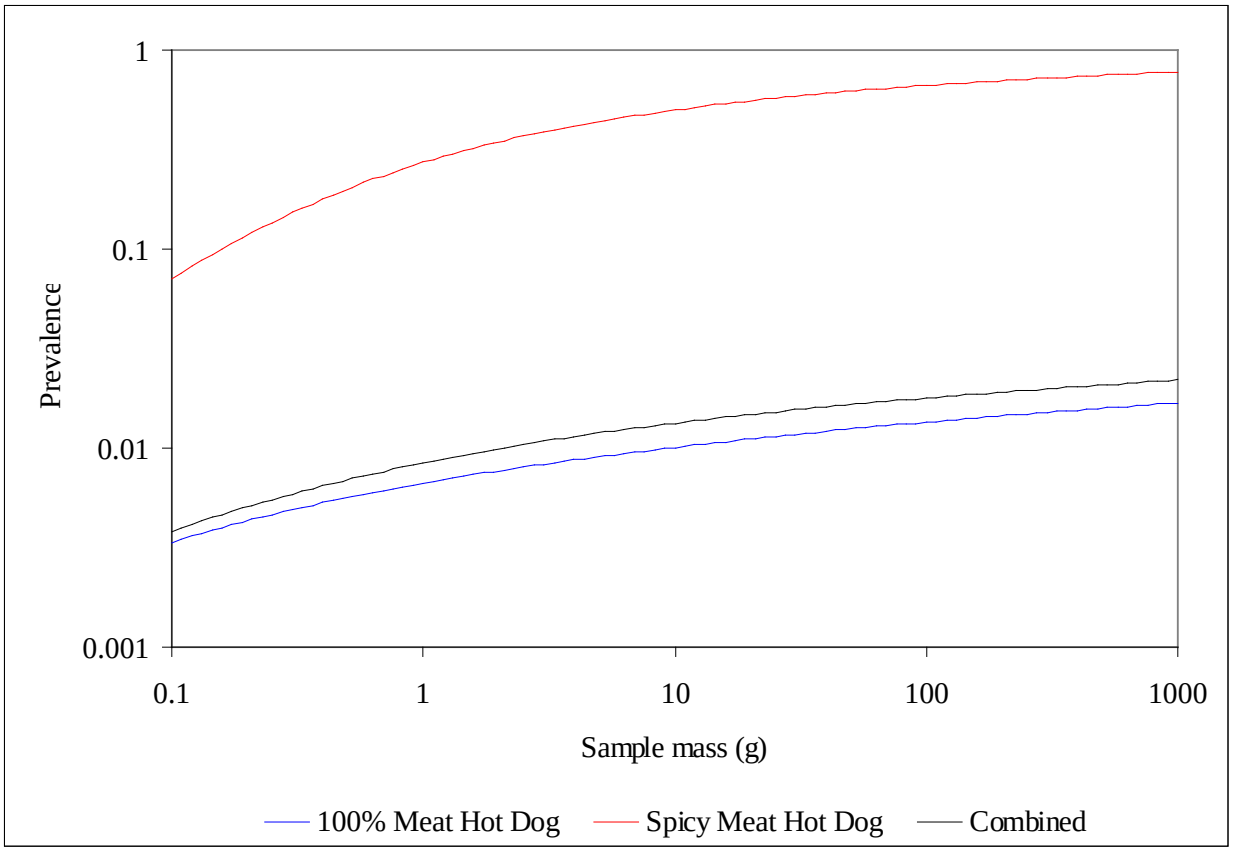

Figure 13 Prevalence of *C. perfringens* in various sample sizes of hot dogs.[48]

10. General references

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[48] These prevalence curves are calculated in the workbook Prevalence.xls, sheet Prevalence, based on the parameters given in the *C. perfringens* risk assessment.

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Appendix A. A short history of the Guidelines for Microbiological Risk Assessment and Microbiological Risk Management (in particular the terms ALOP, FSO, PO, PC)[49]

[References for this Section are provided in chronological order in Section A.3]

A.1. Guidelines for Risk Assessment

In December 1994, the 27th Session of the Codex Committee on Food Hygiene (CCFH) called for a draft of guidelines "on the application of the principles of Risk Assessment and Risk Management to food hygiene including strategies for their application" (ALINORM 95/13, paras. 95–100). The call was motivated by:

- recommendations from the March 1991 FAO/WHO Conference on Food Standards, Chemicals in Food and Food Trade,
- discussion of a paper from 1993 (ALINORM 93/37) on risk assessment procedures used by the Codex and its subsidiary and advisory bodies,
- the call in June 1994 by the 41st Session of the Executive Committee of the Codex Alimentarius Commission (CAC) for a FAO/WHO joint expert consultation for advice on harmonized definitions for use in risk analysis, and the development of risk assessment methodology and decision-making criteria.[50]

The draft guidelines were to be based on the advice provided by the joint expert consultation.

The Joint Expert Consultation took place in March 1995, providing a Report (FAO/WHO, 1995) that summarized the deliberations. The report proposed definitions for various terms used in risk analysis (defined as Risk Assessment, Risk Management, and Risk Communication). It then concentrated primarily on issues of Risk Assessment for chemical agents and biological agents in food, discussed the importance of uncertainty and variability characterization, and made recommendations generally and specifically for chemical hazards, biological hazards, and with respect to uncertainty and variability.

In June 1995, the 42nd Session of the Executive Committee of the Codex Alimentarius Commission (ALINORM 95/4, paras. 25–28) considered the Report of the Joint Expert Consultation and endorsed the recommendations in principle (noting that one recommendation had already been addressed). The Executive Committee noted the concentration on Risk Assessment by the Joint Expert Consultation. The Committee therefore recommended further work on Risk Management, risk communication, and definition of the roles and responsibilities of the different bodies involved in the risk analysis process, and called for specific policy guidelines for risk analysis. The Executive Committee also recommended that the definitions propounded by the Joint Expert Consultation (with various modifications) be circulated for government comment.

In July 1995, the 21st Session of the Codex Alimentarius Commission (ALINORM 95/37, paras. 27–30) also considered the Report of the Joint Expert Consultation and endorsed its recommendations in principle, largely followed the recommendations of the Executive

[49] This is a bare bones summary; it omits many interactions between various other Codex and non-Codex activities. The summary has been derived solely from the available documentation, so may mislead as to events that are inaccurately documented or through misinterpretation.

[50] References are provided in ALINORM 95/13.

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Committee, and agreed that the Report and recommendations should be examined by relevant Codex committees.

In December 1995, the 28th Session of the CCFH (ALINORM 97/13, paras. 51–58) took note of the previous deliberations. The Committee called for the development of a discussion paper that included a preliminary framework to incorporate risk analysis, including principles and guidelines specific to the work of the Committee.

In October 1996, the document "Principles and guidelines for the application of microbiological Risk Assessment; (Prepared by the United States)" (CX/FH 96/10[51]) was considered as Agenda Item 8 by the 29th Session of the CCFH (ALINORM 97/13A, paras. 35–39) under the heading "Guidelines on the application of principles of Risk Assessment and Risk Management to food hygiene including strategies for their application". The document was advanced to Step 3 of the Procedure[52], and included as "Proposed Draft Principles and Guidelines for the Conduct of Microbiological Risk Assessment" in an Appendix (ALINORM 97/13A, Appendix IV). The proposed draft summarized general principles for microbiological Risk Assessment, and (separately) guidelines for application; there was no discussion of Risk Management beyond noting that it should be functionally separate from Risk Assessment. According to later discussion (ALINORM 99/13, para. 68) Annex 2 to CX/FH 96/10 was subsequently incorporated in the guidelines for risk management (see below).

At the 30th Session of the CCFH in October 1997 (ALINORM 99/13, paras. 21–27), a modified draft document (ALINORM 99/13, Appendix IV) "Proposed draft principles and guidelines for the conduct of microbiological Risk Assessment" was advanced to Step 5 of the Procedure. Once again, this document concentrated on Risk Assessment, again noting that Risk Management should be functionally separate.

At the 31st Session of the CCFH in October 1998 (ALINORM 99/13A, paras. 19–34), various modifications to Agenda Item 3 (CX/FH 98/3,[53] "Draft principles and guidelines for the conduct of microbiological Risk Assessment") were made, and the modified document (ALINORM 99/13A, Appendix II) was advanced to Step 8 of the Procedure.

At the 23rd Session of the CAC in June/July 1999 (ALINORM 99/37, para. 114), the Commission adopted the Draft Principles and Guidelines for the Conduct of Microbiological Risk Assessment at Step 8. The Principles and Guidelines are now officially part of the Codex (CAC/GL-30).

A.2. Guidelines for Risk Management (in particular the terms ALOP, FSO, PO, PC)

The 29th Session of the CCFH in October 1996 (ALINORM 97/13A, para. 66) agreed to initiate work to develop recommendations for the management of microbiological hazards for foods in

[51] No copy of CX/FH 96/10 has been located.
[52] Codex procedures are described in the Procedural Manual, available at http://www.codexalimentarius.net/web/procedural_manual.jsp
[53] No copy of CX/FH 98/3 has been located.

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international trade, and this was approved by the 22nd Session of the Codex Alimenatarius Commission (ALINORM 97/37, para. 130 and Appendix IV).

In January 1997, a joint FAO/WHO expert consultation in risk management was held, producing a report (FAO/WHO, 1997) that (inter alia) provided some risk management definitions, enunciated 8 general principles of food safety risk management, and summarized the current risk management practices in the Codex Alimentarius Commission, its subsidiary bodies, and advisory expert committees. The consultation recommendations included a call for the CAC to give high priority to the development and adoption of recommendations for the risk management of microbial hazards in food.

The 30th Session of the CCFH in October 1997 (ALINORM 99/13, paras. 68–73) examined as Agenda Item 11 the paper "Recommendations for the Management of Microbiological Hazards for Foods in International Trade" (CX/FH 97/10,[54] with comments). It was noted that this incorporated Annex 2 from the 1996 proposed Risk Assessment document (CX/FH 96/10, see above), together with an Annex from another document intended to provide guidance on sampling plans. It was noted that other Codex committees were continuing work on Codex-wide policies on Risk Analysis. The Committee agreed to circulate the working document for comments, in light of the outcome of the FAO/WHO Consultations on Risk Assessment (FAO/WHO, 1995) and Risk Management (FAO/WHO, 1997).

At the 31st Session of the CCFH in October 1998 (ALINORM 99/13A, paras. 77–87), Agenda Item 10 was a "Discussion paper on recommendations for the management of Microbiological hazards for foods in international trade" (CX/FH 98/10[55]), that introduced concepts such as "Food Safety Objective (FSO)" and "Risk Profile." This agenda item was discussed in conjunction with Agenda Item 3, the "Draft principles and guidelines for the conduct of microbiological Risk Assessment." There was an extensive discussion on the meaning of "Food Safety Objectives" and other terms and principles. Ultimately the Committee decided to change the title of the paper to "Principles and Guidelines for the Conduct of Microbiological Risk Management" to be consistent with the Risk Assessment document, and called for a redraft of the paper for circulation at Step 3 of the Procedure.

At the 32nd Session of the CCFH in December 1999 (ALINORM 01/13, paras. 91–111), Agenda Item 8 was the "Proposed draft principles and guidelines for the conduct of Microbiological Risk Management at Step 3" (CX/FH 99/8, together with comments; and Appendix IV of ALINORM 01/13). The paper included definition and discussion of "Food Safety Objectives (FSO)," and a draft section on the "Precautionary Principle". The section on FSOs was unchanged from previous drafts, but it was stated that this concept was under discussion by other Codex Committees. There was extensive discussion, particularly on the Precautionary Principle, and the Committee decided to circulate the document at Step 3 for government comments, with a redrafting for the next session.

[54] No copy of CX/FH 97/10 has been located.
[55] No copy of CX/FH 98/10 has been located.

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At the 33rd Session of the CCFH in October 2000 (ALINORM 01/13A, paras. 83–95), the "Proposed draft principles and guidelines for the conduct of microbiological risk management" were Agenda Item 6 (CX/FH 00/6), a re-drafted version taking account of previous comments. Among other changes, the re-draft "tried to make progress on the subject of Food Safety Objectives," and offered a new version. After considerable discussion by the Committee the document was returned to Step 3 with several proposals for development. These included the sending out of a Circular Letter to obtain views on and examples of Food Safety Objectives, and the request to take account of the WHO Kiel Expert Consultation on the Interaction between Assessors and Managers of Microbiological Hazards in Foods (WHO/SDE/PHE/FOS/007, 2000) in development of the text on Risk Profile.

Agenda item 7 (CX/FH 01/7, July 2001) for the 34th Session of the CCFH was a re-drafted "Proposed draft principles and guidelines for the conduct of microbiological risk management." This laid out a definition for Appropriate Level of Protection (ALOP) corresponding to that used by the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement, http://www.wto.org/english/docs_e/legal_e/15-sps.pdf, Annex A).[56] It also gave a distinct definition for Acceptable Level of Risk (ALR); but subsequently (Section 5.2.1.1) claimed this to be synonymous with ALOP[57] and equivalent to Tolerable Level of Risk (TLR; not explicitly defined). These terms had been used in previous drafts and discussions without explicit definition. Food Safety Objective (FSO) is also defined as "The level of microbiological hazard that is tolerable in a food at a specified point along the food chain in order to provide the appropriate level of public health protection." "Performance criterion" was defined as "the required outcome of one or more control measures at a step or combination of steps that contribute to assuring the safety of a food." In addition, other terms used throughout the document were defined (where available, definitions were identical to those in the Procedural Manual of the CAC, 11th edition, or other elements of the Codex). There is a substantial discussion of FSO, pointing out that an FSO is a measure of hazard, whereas the corresponding ALOP is a measure of risk. Thus an ALOP reflects a particular countries' public health goals, while the FSO converts that ALOP into parameters that can be controlled by food producers and monitored by government agencies (*e.g.* an ALOP might be expressed as a negligible probability for illness occurring due to a particular [microbiologically-produced] toxin in food; whereas the corresponding FSO might correspond to a defined amount of the illness-causing toxin in the food). The FSO should contain four components — the food of concern, the hazard of concern, the ALOP, and the corresponding value of the hazard in food. FSOs are supposed to be quantitative and verifiable, although not necessarily directly verifiable by end-product testing (*e.g.* they might be verifiable by a time/temperature protocol based on a performance criterion).

[56] The SPS agreement, Article 5, places limitations on the selection of an ALOP by a member country that necessarily modify the definition; Section 5.2.1.1 of the draft document implies that such limitations apply also to the ALOP of the draft document, at least for countries signatory to the international trade agreements of the World Trade Agreement. However, Section 5.2.1.1 introduces considerations that do not necessarily correspond to the definition of the SPS agreement (*e.g.* the requirement that "Decisions on ALOP/ALR should be determined primarily by human health considerations").

[57] The SPS agreement also considers ALOP and ALR to be equivalent, but does not formally define the latter.

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At the 34th Session of the CCFH (ALINORM 03/13, October 2001, paras. 99–128) the Committee extensively discussed the document. There was disagreement on whether the ALOP/ALR should always be scientifically justified,[58] and left this point to further consideration by the drafting group. There was also disagreement over the point in the food chain where a FSO should be applied; as a compromise the Committee decided to adopt a definition of FSO as "The maximum frequency and/or concentration of a [microbiological] hazard in a food at the time of consumption that provides the appropriate level of health protection [(ALOP)]," and requested the drafting group to expand on the differences and relationship between FSOs and performance criteria.

Agenda item 6 (CX/FH 03/7, December 2002) for the 35th Session of the CCFH was a re-drafted "Proposed draft principles and guidelines for the conduct of microbiological risk management (at Step 3 of the Procedure)." The definition of ALOP was unchanged (and still corresponded to the SPS agreement definition), although there was clarifying text indicating that for the purpose of the guidelines an ALOP referred to a level of protection of human health established for a foodborne microbiological hazard (the SPS agreement definition is more general). The definition of FSO was that adopted at the 34th Session of the CCFH, while the definition of "Performance criteria" was modified to state "microbiological outcome" in place of just "outcome." The discussion of FSO (Section 5.2.2.1) generally corresponded in meaning to that of CX/FH 01/7, but was more concisely stated and incorporated the explicit requirement of the Committee's compromise (34th session) that the FSO correspond to the point of consumption. Interestingly, the requirement that FSOs be quantitative and verifiable was omitted, and the possibility of non-quantitative FSOs left in (Section 6.2.1 explicitly recognizes this). Verifiability was left as an open question; "FSOs are seldom verifiable as regulatory standards . . . need to be translated by the competent authority to performance, process or microbiological criteria further up the food chain, . . ." — the method for this translation was not specified.

At the 35th Session of the CCFH (ALINORM 03/13A, January-February 2003, paras. 78–98) the draft was discussed point by point. Further evaluation of many points was requested, including the points within the food chain at which FSOs should be applied, taking account other Codex committees discussions on the same problem (although CCFH had the lead role). The Committee recognized that the SPS agreement definition of ALOP had precedence, but that "application of the term within the remainder of the text" was the responsibility of the CCFH. The document was returned to Step 2 for circulation, comment and further consideration.

Agenda item 6 (CX/FH 04/6 January, 2004) for the 36th Session of the CCFH was the again re-drafted "Proposed draft principles and guidelines for the conduct of microbiological risk management (at step 3 of the procedure)." Many definitions were referred to the Codex Procedural Manual, other Codex guidelines, or the WTO (for ALOP). The only definitions specific for these principles were:[59]

[58] The Committee does not seem to have here realized that introducing such additional constraints may change the definition of the term, so that it would not necessarily conform to the SPS agreement, rather than just specializing to a specific situation. This potential oversight was corrected at the 35th Session.

[59] The first definition is of no interest here.

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Risk manager: A representative of a government at a national level or regional level or a representative of an international organization who has the responsibility for MRM.

Food Safety Objective (FSO): The maximum frequency and/or concentration of a [microbial] hazard in a food at the time of consumption that still provides the appropriate level of protection.

Performance Objective (PO): The maximum frequency and/or concentration of a [microbial] hazard in a food at a specified step in the food chain before time of consumption that will ensure the achievement of an FSO or ALOP, as applicable.

Performance Criterion (PC): The desired effect in the frequency and/or concentration of a microbial hazard(s) in a food that must be achieved by the application of one or more control measures to meet or contribute to meeting a PO or a FSO.

At Section 4, the WTO definition of ALOP is specialized (for the purpose of the guidelines) as being for foodborne microbiological risks. FSOs (Section 6.2.1) are said to be "established by the competent authority" and "generally need to be translated by the competent authority and/or food businesses to PO, PC, or microbiological criteria (MC) at earlier stages in the food chain." POs (Section 6.2.2[60]) were introduced here for the first time, apparently meet the demand for an FSO-like measure at earlier points in the food chain. It was stated that "POs are not intended to be verified by analytical means;" but then "POs, unlike FSOs, are intended to be measurable and verifiable."[61] Typical verification would be by establishment of a microbiological criterion (MC)[62] "that would provide a statistically based means for verifying within a stated degree of confidence that the PO is not being exceeded." Accordingly, "a MC will have to be more stringent that the PO or PC [or FSO] upon which it is based in order to assure that the PO is being met with a specified level of confidence." A MC might be based on measurement of a related parameter, *e.g.* indicator organisms.

At the 36th Session of the CCFH (ALINORM 04/27/13, March-April 2004, paras 63–90) there was again extensive discussion. The Committee agreed on a broader definition of FSO (beyond just microbiological hazards), and to forward the definitions of FSO, PO, and PC to the Codex Committee on General Principles for endorsement and adoption by the Commission. The document was returned to Step 2 for revision, with subsequent circulation for comments at Step 3 before the next session.

At the 27th Session of the CAC (ALINORM 04/27/41, June-July 2004, para. 16 and Appendix II), the Commission accepted the definitions of FSO, PO, and PC on an interim basis. The definitions accepted and incorporated in the Procedural Manual were:

[60] This section is incorrectly numbered 6.3.2 in the document, but occurs between Sections 6.2.1 and 6.2.3.
[61] The only other uses of "analytical" in the document as meaning by measurement (as opposed to theoretical analysis) indicate that these two statements are contradictory.
[62] See "Principles for the Establishment and Application of Microbiological Criteria for Foods", CAC/GL 21 (1997) URL: http://www.codexalimentarius.net/download/standards/394/CXG_021e.pdf. The establishment of a MC includes definition (*inter alia*) of analytical methods, sampling plans, microbiological limits, and the number of analytical units that should conform to the limits.

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Food Safety Objective (FSO): The maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP).

Performance Objective (PO): The maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before the time of consumption that provides or contributes to an FSO or ALOP, as applicable.

Performance Criterion (PC): The effect in frequency and/or concentration of a hazard in a food that must be achieved by the application of one or more control measures to provide or contribute to a PO or an FSO.

Agenda Item 6 (CX/FH 05/37/06, December 2004) for the 37th Session of the CCFH was another re-draft of "Proposed draft principles and guidelines for the conduct of microbiological risk management (MRM) (at Step 3 of the Procedure)." All definitions except for "Risk Manager" were now incorporated in the Procedures Manual, other Codex guidelines, or (for ALOP) the SPS Agreement. The general discussion of Section 4 indicates that microbiological risk management (MRM) options selected [to manage a particular hazard] should be "scientifically justifiable, proportionate to the risk identified and not more restrictive of trade or technological innovation than required to achieve the ALOP." Section 6 provides an extended discussion of MRM options separately for the Codex and for Countries, including selection of FSOs, POs, PCs, and MCs. It goes on to provide a description of the concepts and interrelationships between FSO, PO, PC, and MC. Section 6.2.3 on Performance Criteria introduces two new terms, Process Criteria and Product Criteria, defined to mean:

Process Criterion: parameters of a control measure that if properly applied have been established as meeting, either alone or in combination with other control measures, a performance criterion.

Product Criterion: a physical or chemical attribute of a product that if properly applied as a control measure has been established as meeting, either alone or in combination with other control measures, a performance criterion.

Annex III provides very short examples of where FSO, PO, PC, MC, Process Criteria, and Product Criteria could (or could not) be used for a pasteurized perishable product, a raw fermented product, and for a raw product kept at a temperature in which the hazard could grow.

At the 37th Session of the CCFH (ALINORM 05/28/13, March 2005, paras. 100–133 and Appendix III), the proposed guidelines were introduced as being ready for advancement to Step 5 of the Procedure, provided that Annex III be further elaborated at a different pace to the main body to provide guidance on using the definitions of FSO, PO, PC, MC, Process Criteria, and Product Criteria in practice. After discussion and modifications, the Committee agreed to forward the proposed guidelines to the 28th Session of the CAC for adoption at Step 5, and to return Annex III to a Working Group at Step 2 for further comments and re-drafting, taking account of the planned FAO/WHO expert consultation on the application of Risk Assessment to Risk Management.

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The 28th Session of the CAC (ALINORM 05/28/41, July 2005, para. 71 and Appendix VI) adopted the proposed guidelines at Step 5 and advanced them to Step 6.

In April of 2006 the FAO/WHO joint expert consultation on the "The Use of Microbiological Risk Assessment Outputs to Develop Practical Risk Management Strategies: Metrics to improve food safety (FAO/WHO, 2006) took place in Kiel, Germany.

Following the request of the CCFH at its 37th session to expand Annex III ("Examples of the use of food safety objectives, performance objectives, process and product criteria"), the Working Group produced a substantially expanded re-draft (CX/FH 06/38/4-Add.1, September, 2006). The Working Group found that a detailed consideration of examples would be needed, so they developed one detailed example. However, "the fully articulated example was highly technical and required extensive explanatory information; and it proved almost impossible to achieve the detailed that the risk assessors felt was necessary to understand the subject adequately while at the same time achieving the brevity requested by the risk managers" (the example was 27 pages long, including diagrams). The Working Group thus also produced a second version "that focuses on the framework, processes, data needs and review criteria that a competent authority should consider when it establishes food safety risk management metrics that are linked via a quantitative microbiological risk assessment to public health outcomes," and requested guidance from CCFH (the second version was 18 pages, including diagrams).

At the 38th Session of the CCFH (ALINORM 07/30/13, December 2006, paras. 23–26, 37–81, 82–85, 234–241, Appendix IV) the CCFH was informed of the joint FAO/WHO expert meeting on "The Use of Microbiological Risk Assessment Outputs to Develop Practical Risk Management Strategies: metrics to improve food safety." Some concern was expressed that the interpretation of ALOP within the expert meeting was not consistent with the definition used in the SPS Agreement; and the representative of WHO indicated that additional clarification would be provided. The CCFH also considered the "Draft principles and guidelines for the conduct of microbiological risk management" (ALINORM 05/28/13, Appendix III, at Step 6) and its relation to Annex III (CD/FH 06/38/4-Add.1, "Examples of the use of food safety objectives, performance objectives, process and product criteria" at Step 3). After extensive discussion the two documents were kept separate and, during section-by-section examination and modification of the first (taking account comments received), the Committee agreed to forward the resulting draft (ALINORM 07/30/13, Appendix IV) to the CAC for final adoption at Step 8. The Committee noted that comment and other material omitted from the principles and guidelines document, together with Annex III, consisted of two parts — "one dealing with examples and approaches for utilizing quantitative microbiological risk assessment techniques to link the stringency of control measures to hygiene outcomes and a second dealing with metrics to achieve the desired level of public health protection." The Committee therefore established a set of "Terms of Reference for the development of an Annex[63] for the development of Draft Guidelines for the Establishment and Use of Risk Management Metrics for the Management of Microbial

[63] Note: this Annex now becomes Annex II, since one Annex of the principles and guidance document (ALINORM 07/30/13, Appendix IV) was deleted during discussion.

Food Safety Hazards,"[64] and expected the work could be completed in 2009 for adoption by the CAC in 2010.

At its Thirtieth Session in July 2007, the CAC (ALINORM 07/30/REP, para. 39 and Appendix IV) adopted the "Principles and Guidelines for the Conduct of Microbiological Risk Management" (CAC/GL 63).

Agenda Item 8 for the 39th Session of the CCFH (CX/FH 07/39/8, August 2007) was a complete redraft of Annex II (see footnote 63), now re-titled "Guidance on Microbiological Risk Management Metrics." Risk Management Metrics were defined according to the FAO/WHO joint expert meeting (FAO/WHO, April 2006) as "quantitative expressions that indicate a level of control at a specific step in a food safety risk management system." The document provided a general overview of the use of such risk management metrics in risk management of microbiological hazards, including a protocol for consideration "as a means of ensuring the principles for microbiological risk management lead to transparent, informed decisions."

At the 39th Session of the CCFH (ALINORM 08/31/13, October-November 2007, paras. 126–146 and Appendix IV), Annex II was discussed, slightly modified, and advanced for final adoption by the 31st Session of the CAC at Step 5/8 (omitting steps 6 and 7). The Committee requested the FAO/WHO to develop a practical manual on the implementation of metrics; although the Representative of FAO cautioned that completion would take some years because of the need to gain practical experience in the application of the metrics at a national level.

A.3. References for Appendix A (in chronological order of document dates)

URLs link to English versions of the documents, in PDF format where available. In many cases, substituting "f" or "s" for the terminating "e" of the URL will retrieve corresponding documents in French or Spanish respectively. HTML versions of some of these documents are available: for ALINORMs from http://www.codexalimentarius.net/web/archives.jsp?lang=en (substitute =FR or =ES in place of =en for French or Spanish versions of the web site), for other documents through subdirectories of ftp://ftp.fao.org/codex/ (for many documents, versions in French and/or Spanish are available through these links).

ALINORM 93/37. (1993). Hathaway, S.C. Risk assessment procedures used by the Codex Alimentarius Commission, and its subsidiary and advisory bodies. 27 pp. [Available as fiche or TIFF images from the FAO Online Catalogue, http://www4.fao.org/faobib/, accession number 341301, Job number Z9858, Fiche No. 341247-301.]

ALINORM 95/13. FAO/WHO (December, 1994). Distribution of The Report of the 27th Session of the Committee on Food Hygiene. URL: http://www.codexalimentarius.net/download/report/56/al95_13e.pdf

[64] The terms of reference were set out in a conference room document CRD 22, modified "taking into account comments made at the current session" (ALINORM 07/30/13, para. 240). The distribution of CRDs is "limited to those Members and observers present at the meeting," (Information Guide for First-Time Delegates to a Codex Session, URL: ftp://ftp.fao.org/codex/Information_for_delegates/Info_leaflet_en.pdf) so no written copy of the terms of reference appears to be available.

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FAO/WHO (March, 1995). Application of Risk Analysis to Food Standards Issues, a Joint FAO/WHO Expert Consultation, Geneva, Switzerland, 13-17 March 1995. WHO/FNU/FOS/95.3. URL: http://www.who.int/entity/foodsafety/publications/micro/en/march1995.pdf

ALINORM 95/4. FAO/WHO (June, 1995). Report of the Forty-Second Session of the Executive Committee of the Codex Alimentarius Commission. URL: http://www.codexalimentarius.net/download/report/492/al95_4e.pdf

ALINORM 95/37. FAO/WHO (July, 1995). Report of the Twenty-First Session of the Joint FAO/WHO Codex Alimentarius Commission. URL: http://www.fao.org/docrep/meeting/005/v7950e/v7950e00.htm

ALINORM 97/13. FAO/WHO (December, 1995). Report Of The Twenty-Eighth Session Of The Codex Committee On Food Hygiene,. URL: http://www.fao.org/docrep/meeting/005/w0124e/w0124e00.htm

CX/FH 96/10. Principles and guidelines for the application of microbiological risk assessment; (Prepared by the United States) (English). Codex Committee on Food Hygiene, Sess. 29, Washington, DC (USA), 21-25 Oct 1996 / Joint FAO/WHO Food Standards Programme, Rome (Italy) , 1996 , 15 p. (Available as fiche or TIFF images from the FAO Online Catalogue, http://www4.fao.org/faobib/, as Accession No: 371171, Report No: FAO-ESN--CX/FH/96/10 , Fiche No: 371169-171.]

ALINORM 97/13A. FAO/WHO (October, 1996). Report Of The Twenty-Ninth Session Of The Codex Committee On Food Hygiene. [Incorrectly labeled 97/31A on the document itself]. URL: http://www.fao.org/docrep/meeting/005/w3700e/w3700e00.htm

FAO/WHO (January, 1997). Risk management and food safety: Report of a Joint FAO/WHO Consultation. FAO Food and Nutrition Paper 65. URL: ftp://ftp.fao.org/docrep/fao/w4982e/w4982e00.pdf

ALINORM 97/37. FAO/WHO (June, 1997). Report of the Twenty-Second Session of the Codex Alimentarius Commission.. URL: http://www.codexalimentarius.net/download/report/517/Al97_37e.pdf

ALINORM 99/13. FAO/WHO (October, 1997). Report Of The Thirtieth Session Of The Codex Committee On Food Hygiene. URL: http://www.codexalimentarius.net/download/report/112/Al99_13e.pdf

ALINORM 99/13A. FAO/WHO (October, 1998). Report Of The Thirty-First Session Of The Codex Committee On Food Hygiene. (discussion at paragraph 87). URL: http://www.codexalimentarius.net/download/report/113/Al9913ae.pdf

CAC/GL-30 (1999). Principles and guidelines for the conduct of microbiological risk assessment. URL: http://www.codexalimentarius.net/download/standards/357/CXG_030e.pdf

CX/FH 99/8 (October, 1999). Proposed Draft Principles and Guidelines for the Conduct of Microbiological Risk Management (*Document prepared by France*). URL: ftp://ftp.fao.org/codex/ccfh32/FH99_08e.pdf

ALINORM 01/13. FAO/WHO (December, 1999). Report Of The Thirty Second Session Of The Codex Committee On Food Hygiene. URL: http://www.codexalimentarius.net/download/report/114/Al01_13e.pdf

WHO/SDE/PHE/FOS/007 (March 2000). The Interaction between Assessors and Managers of Microbiological Hazards in Food: Report of a WHO Expert Consultation in collaboration with The Institute for Hygiene and Food Safety of the Federal Dairy Research Center and The Food and Agriculture Organization of the United Nations. URL: http://www.who.int/entity/foodsafety/publications/micro/en/march2000.pdf

CX/FH 00/6 (July, 2000). Proposed draft principles and guidelines for the conduct of microbiological risk management (At Step 3 of the Procedure). URL: ftp://ftp.fao.org/codex/CCFH33/fh00_06e.pdf

ALINORM 01/13A. FAO/WHO (October, 2000). Report Of The Thirty Third Session Of The Codex Committee On Food Hygiene. URL: http://www.codexalimentarius.net/download/report/115/Al0113ae.pdf

CX/FH 01/7 (July, 2001). Proposed draft principles and guidelines for the conduct of microbiological risk management (At Step 3 of the Procedure). URL: ftp://ftp.fao.org/codex/ccfh34/fh01_07e.pdf

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CX/FH 03/7 (December, 2002). Proposed draft principles and guidelines for the conduct of microbiological risk management. URL: ftp://ftp.fao.org/codex/ccfh35/fh03_07e.pdf

ALINORM 03/13A. FAO/WHO (January-February, 2003). Report of the Thirty-Fifth session of the Codex Committee on Food Hygiene. URL: http://www.codexalimentarius.net/download/report/117/Al0313ae.pdf

CX/FH 04/6 (January, 2004). Proposed draft principles and guidelines for the conduct of microbiological risk management (at step 3 of the procedure). URL: ftp://ftp.fao.org/codex/ccfh36/fh04_06e.pdf

ALINORM 04/27/13. FAO/WHO (March-April, 2004). Report of the Thirty-Sixth session of the Codex Committee on Food Hygiene. URL: http://www.codexalimentarius.net/download/report/615/al04_13e.pdf

ALINORM 04/27/41. FAO/WHO (June-July, 2004). Report of the Twenty-seventh Session of the Codex Alimentarius Commission. URL: http://www.codexalimentarius.net/download/report/621/al04_41e.pdf

CX/FH 05/37/06 (December, 2004). Proposed draft principles and guidelines for the conduct of microbiological risk management (MRM) (at Step 3 of the Procedure). URL: ftp://ftp.fao.org/codex/ccfh37/fh37_06e.pdf

ALINORM 05/28/13 (March, 2005). Report of the Thirty-Seventh Session of the Codex Committee on Food Hygiene. URL: http://www.codexalimentarius.net/download/report/638/al28_13e.pdf

ALINORM 05/28/41 (July, 2005). Report of the Twenty-eighth Session of the Codex Alimentarius Commission. URL: http://www.codexalimentarius.net/download/report/644/al28_41e.pdf

FAO/WHO (April 2006). The Use of Microbiological Risk Assessment Outputs to Develop Practical Risk Management Strategies: Metrics to improve food safety. Report of a Joint FAO/WHO Expert Meeting. Kiel, Germany, 3–7 April, 2006. URL: ftp://ftp.fao.org/ag/agn/food/kiel.pdf or http://www.who.int/entity/foodsafety/publications/micro/MRA_Outputs.pdf

CX/FH 06/38/4-Add.1 (September, 2006). Annex III: Examples of the use of Food Safety Objectives, Performance Objectives, Process and Product criteria at Step 3. URL: ftp://ftp.fao.org/codex/ccfh38/fh3804ae.pdf

CX/FH 06/38/3 (November, 2006). Progress Report on the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) and Related Matters. URL: ftp://ftp.fao.org/codex/ccfh38/fh38_03e.pdf

ALINORM 07/30/13 (December, 2006). Report of the Thirty-Eighth Session of the Codex Committee on Food Hygiene. URL: http://www.codexalimentarius.net/download/report/671/al30_13e.pdf

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CX/FH 07/39/8 (August, 2007). Principles and Guidelines for the Conduct of Microbiological Risk Management: Annex II: Guidance on Microbiological Risk Management Metrics – at Step 4. URL: ftp://ftp.fao.org/codex/ccfh39/fh39_08e.pdf

ALINORM 08/31/13 (October-November, 2007). Report of the Thirty-Ninth Session of the Codex Committee on Food Hygiene. URL: http://www.codexalimentarius.net/download/report/686/al31_13e.pdf

Appendix B. Monte Carlo simulation of illnesses and illness rates

The Monte Carlo procedure used in the risk assessment (Crouch and Golden, 2005) is designed to sample the distribution of illness rate (number of illnesses per serving). To this end, it simulates the number of illnesses in a fixed (large) number of servings, and the numerical uncertainty in this rate is obtained simply because the number of illnesses is Poisson distributed about its expected value. By "numerical uncertainty" here is meant that uncertainty due to using only a finite number of samples in the Monte Carlo simulation.

With a sufficient number of samples, it is possible to obtain a slightly better estimate of the expected illness rate; that is, an estimate with slightly lower numerical uncertainty.

For each serving (numbered $i = 1,2, ..., n$), the Monte Carlo procedure estimates a probability p_i for an illness due to that serving (by applying the dose-response curve to the number of type A, CPE+ vegetative cells in the serving), then samples for illness from the serving [illness from the serving is a Bernoulli random variable, that is a (0,1) random variable taking the value 1 with probability p_i] and counts the resulting illnesses.

The probability for any random serving to produce an illness is thus $E(p_i)$ where the expectation is over all servings (so this expectation is independent of serving) and the total number of illnesses in a large number n of servings is just Poisson[65] with expected number $nE(p_i)$ and variance also $nE(p_i)$. Thus in the Monte Carlo procedure, the number of illnesses obtained in the simulation is taken as an estimate of the expected number, and the numerical uncertainty (standard deviation) is estimated as the square root of the number of illnesses obtained.

An alternative approach to estimating $nE(p_i)$ is available, and that is to average over the values p_i obtained in the variability loop of the Monte Carlo procedure and calculate the variance of these values to estimate the uncertainty in the expected number of illnesses (both such calculations are standard).

The disadvantage of the latter method, and the reason it was not used in the risk assessment, is that the uncertainties in the estimates so obtained depend strongly on higher moments of the distribution of the p_i. Since these probabilities depend on the numbers of cells per serving, this distribution is also extremely right-tailed (see Figure 3 and Figure 8 for the distribution of cells/serving), so the uncertainties in the mean and standard deviation estimates can be extreme.

A hypothetical example should make this plain. Consider the possibility that most servings have zero probability for illness (because they contain no *C. perfringens* vegetative cells), 3% of them have probability 10^{-5} for causing illness, and 10^{-5} (1 in 100,000) of them have probability 0.999 of causing illness (because they have so many *C. perfringens* vegetative cells). The true probability of illness per serving is then $(0.999 \times 10^{-5} + 10^{-5} \times 0.03) = 1.029 \times 10^{-5}$ with 97% of the probability coming from the very rare but highly contaminated servings.

[65] From outside the Monte Carlo program, that program acts as a black box producing servings, each one delivered with an equal, small, chance of causing illness; and there are a large number of servings involved. These together are sufficient to imply Poisson statistics in the number of illnesses.

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Unless there are sufficient samples in the Monte Carlo evaluation to catch the rare servings that are highly contaminated, the uncertainty estimates for the mean probability of illness will be substantially too small. In this case, for example, a Monte Carlo with 10^5 servings has about 50% chance of seeing a highly contaminated serving. If it does not, the estimated illness probability will be about 0.03×10^{-5}, since the Monte Carlo sample will obtain about 3,000 servings each with 10^{-5} probability for illness, and 97,000 with zero probability of illness. The resultant estimates would be 0.03×10^{-5} probability for illness, with estimated numerical standard deviation of 0.0005×10^{-5}, both far too low.

For the *C. perfringens* risk assessment at the uncertainty MLE, 10^8 servings give an estimated number of illnesses of about 350, with a corresponding numerical uncertainty of ± 18.7 at 1 standard deviation. Evaluating the mean of the distribution of p_i gives a similar mean estimate, with standard deviation of about ± 9.5. Examination of the upper tail of the distribution shows that the Monte Carlo samples include at least tens of servings with the maximum possible number of cells/serving, indicating that in this case the standard deviation obtained from the distribution of p_i may well be reasonably accurate. Thus for 10^8 servings, averaging the probabilities gives an uncertainty of the mean probability about ½ the Poisson uncertainty of counting illnesses. The same can be expected for any number of servings, since both uncertainty estimates scale with the inverse square root of the number of servings, provided that the number is high enough to contain sufficient at the extreme right tail of the distribution. Empirical tests indicate that as few as 5×10^6 servings generally appears to be adequate; and the uncertainty estimates remain about 0.5 to 0.75 of the Poisson uncertainties.[66]

[66] It is straightforward to prove that the uncertainty (standard deviation) estimates obtained by averaging the p_i must be less than the Poisson $\sqrt{n}$, with the smallest reduction obtained for extreme distributions of p_i (those with p_i taking values 0 and 1 only).

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Appendix C. Numerical considerations in Monte Carlo assessments

C.1. Graphical plots of distributions

Many of the figures in this demonstration are empirical distributions obtained from Monte Carlo simulations. In this case, the form of the model for *C. perfringens* growth is mostly multiplicative, so that the expected shapes of the distributions are roughly lognormal, since that is what is expected from the multiplication together of multiple values almost independently of the shapes of the distributions for the original values. To show the full range of such near-lognormal distributions, it is convenient to graph them on scales on which lognormal distributions would be straight lines — such scales even out the distributions and allow showing all parts of them without too much distortion or loss of detail.

The graph that shows lognormal distributions as straight lines plots the logarithm of the quantity versus the z-score, defined as the inverse normal of the cumulative probability. That is, for a quantity X that has a cumulative distribution $P(x)$ (*i.e.* $\text{Prob}(X<x) = P(x)$), plotting $\ln(x)$ or $\log_{10}(x)$ versus $\Phi^{-1}(P)$ will give a straight line if P is lognormal, where Φ is the standard cumulative normal probability function, and

$$P = \Phi(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x} \exp\left(-t^2/2\right) dt \quad \Leftrightarrow \quad x = \Phi^{-1}(P) \tag{14}$$

For a Monte Carlo assessment (or for any finite number of random samples), the true cumulative distribution $P(x)$ is not available exactly, only an approximation obtained by putting the samples in numerical order and counting what fraction of the samples are below any particular value x. This approximation introduces a distortion into the graph; but that distortion can be minimized (Cunnane, 1978) for an ordered set of samples $x_1 \le x_2 \le ... \le x_n$ by plotting

$$\ln(x_i) \quad \text{versus} \quad \Phi^{-1}\left(\frac{i - 3/8}{n + 1/4}\right) \tag{15}$$

This is the type of plot used in this demonstration; the "z-score" for any sample from the Monte Carlo simulation is obtained by computing the inverse normal given in equation (15).

C.2. Numerical uncertainty

Monte Carlo assessments are used to evaluate random samples from distributions that are convolutions of the distributions of the input variables. Quantities of interest are usually approximated by statistics of these random samples *e.g.* the mean, standard deviation, or a percentage point. Such statistics are subject to sampling uncertainties that typically scales as $1/\sqrt{N}$ where N is the number of Monte Carlo samples,[67] and this sampling uncertainty is what is termed numerical uncertainty here. In principle, it may be made as small as desired by increasing the number of Monte Carlo samples, although in practice it may be difficult or impractical to calculate sufficient samples to make the numerical uncertainty completely negligible.

[67] In principle it may be possible to generate samples on a lattice that ensures the sampling uncertainties scale as 1/N, but the method of generating such a lattice is only known in special cases. In the typical case where samples are generated completely randomly, the $1/\sqrt{N}$ scaling applies.

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C.2.1. Example: mean and standard deviation

Consider a Monte Carlo procedure that returns sample values p_i, i = 1, 2, ..., N from the distribution of some variable p, as in Appendix B. Then estimates of mean and standard deviation of p are

$$m = \frac{1}{N}\sum_{i=1}^{N} p_i$$
$$s^2 = \frac{1}{N}\sum_{i=1}^{N}\left(p_i - m\right)^2 \tag{16}$$

as usual, and the numerical uncertainty (standard deviation) in m is given by $s/\sqrt{N}$.

C.2.2. Example: percentage points

A less well known example is the evaluation of percentage points of the distribution of p. Suppose it is desired to estimate the α fractile (100α percentage point) of the distribution. A point estimate is obtained by re-numbering the p_i in order so that

$$p_1 \le p_2 \le p_3 \le \ldots \le p_N \tag{17}$$

and then choosing

$$p_{[\alpha N]} \tag{18}$$

as the estimate for the α fractile, where [•] represents the nearest integer function. Recalling that each observation is equally likely (for a straightforward Monte Carlo), an estimate of numerical uncertainty for this fractile can be obtained. The probability to obtain exactly n samples smaller than the α fractile is given by the binomial distribution

$$\Pr(n) = \binom{N}{n}\alpha^n\left(1-\alpha\right)^{N-n} \sim \mathrm{N}\left(\alpha\mathrm{N}, \sqrt{\mathrm{N}\alpha\left(1\text{-}\alpha\right)}\right) \tag{19}$$

where the approximation applies for large N, and N(μ,σ) is a normal distribution with mean μ, standard deviation σ. Thus the ±1 standard deviation numerical uncertainty for the α fractile is approximately

$$p_{\left[\alpha N - \sqrt{N\alpha(1-\alpha)}\right]} \quad \text{to} \quad p_{\left[\alpha N + \sqrt{N\alpha(1-\alpha)}\right]} \tag{20}$$

for large N (provided also that αN and $(1-\alpha)N$ are also large). This is the form that is most useful, since it can be obtained directly from the sorted Monte Carlo samples; however, the numerical uncertainty can also be seen to scale with $1/\sqrt{N}$ by seeing that this range is approximately

$$F\left(\alpha\right) \pm \sqrt{\frac{\alpha\left(1-\alpha\right)}{N}}\, f\left(\alpha\right) \tag{21}$$

where F is the cumulative distribution of p and $f = F'$.

The same approach as just described can be used to estimate (for large N, αN and $(1-\alpha)N$) that the value $p_{[\alpha N]}$ corresponds to a true fractile β, where

$$\beta = \alpha \pm \sqrt{\frac{\alpha\left(1-\alpha\right)}{N}} \tag{22}$$

gives a central estimate and 1 standard deviation uncertainty range.

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C.3. The effect of numerical uncertainty in the variability loop

In many applications, including estimation of an ALOP, FSO, and PO, what is required is evaluation of the uncertainty distribution for some quantity that is a function of a variability distribution. The variability distribution is approximated by a finite number of samples within a Monte Carlo variability loop, with the required quantity evaluated from that finite number of samples. As pointed out in Appendix C.2, this will usually result in a numerical uncertainty that is inversely proportional to the square root of the number of samples in the variability loop.

The uncertainty distribution is then obtained by an outer, uncertainty loop of the Monte Carlo; and some interesting statistic of the uncertainty distribution obtained from the Monte Carlo uncertainty samples. However, what is actually obtained are samples from the convolution of the true uncertainty distribution and the numerical uncertainty distributions[68] of the internal variability loop. Thus either the latter must be sufficiently small to be ignored (compared with the true uncertainty distribution), or some way of taking both into account must be used to estimate information from the uncertainty samples. In the main part of this demonstration, the number of samples has generally been selected so that numerical uncertainties are small enough to be ignored; Appendix C.4 gives an example of the second approach.

C.4. Application to the *C. perfringens* assessment

Evaluation of the illness rate due to *C. perfringens* in hot dogs illustrates the sizes of numerical uncertainties, and also shows how the numerical uncertainty may be reduced by suitable assumptions.

Using 100 uncertainty iterations of 5,000,000 servings[69] (variability iteration) gives the empirical distribution in Figure 14 for illness rate. With only 100 uncertainty iterations, the empirical 95th and 99th percentiles have high numerical uncertainty — applying equation (20) selects the 93rd to 97th highest estimates as a 1 standard deviation range for the 95th percentile, corresponding to 1.2×10^{-5} to 2.1×10^{-5} (12 to 21 per million, best estimate 18 per million), and the 98th to 100th highest estimates as a 1 standard deviation range for the 99th percentile, corresponding to 2.1×10^{-5} to 2.7×10^{-5} (21 to 27 per million, best estimate 21 per million). Obviously with so few uncertainty samples the numerical uncertainty is substantial, perhaps too wide for practical use; and the irregular shape of the empirical distribution in this region also prompts caution in using these estimates.

It should also be borne in mind that the numerical uncertainty within the variability loop (the 5,000,000 servings) has been propagated into these estimates. The number of illnesses estimated ranged from 1 to 134 in the 100 uncertainty loops, with corresponding uncertainties ranging from approximately 100% (at the left end of Figure 14) to 10% (at the right end of Figure 14) based on Poisson statistics[70] (the relative uncertainty standard deviation is just $1/\sqrt{n}$ for a Poisson observation of n). Translated into logarithmic standard deviations, these correspond to about 0.8 down to 0.1. However the variability shown in Figure 14 corresponds to a logarithmic standard

[68] The numerical uncertainties of the statistic obtained from the variability iteration may vary with the true uncertainty, hence the plural distributions.

[69] This takes approximately 10 minutes on a PC with an AMD3200 processor.

[70] See Appendix B

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deviation of about 0.8 (see below) so the additional variation due to the numerical uncertainty of the individual points should be small at the upper end of the distribution (the uncertainties add roughly in quadrature, and $0.8^2 + 0.1^2 \approx 0.81^2$), although it could affect the lower end substantially.

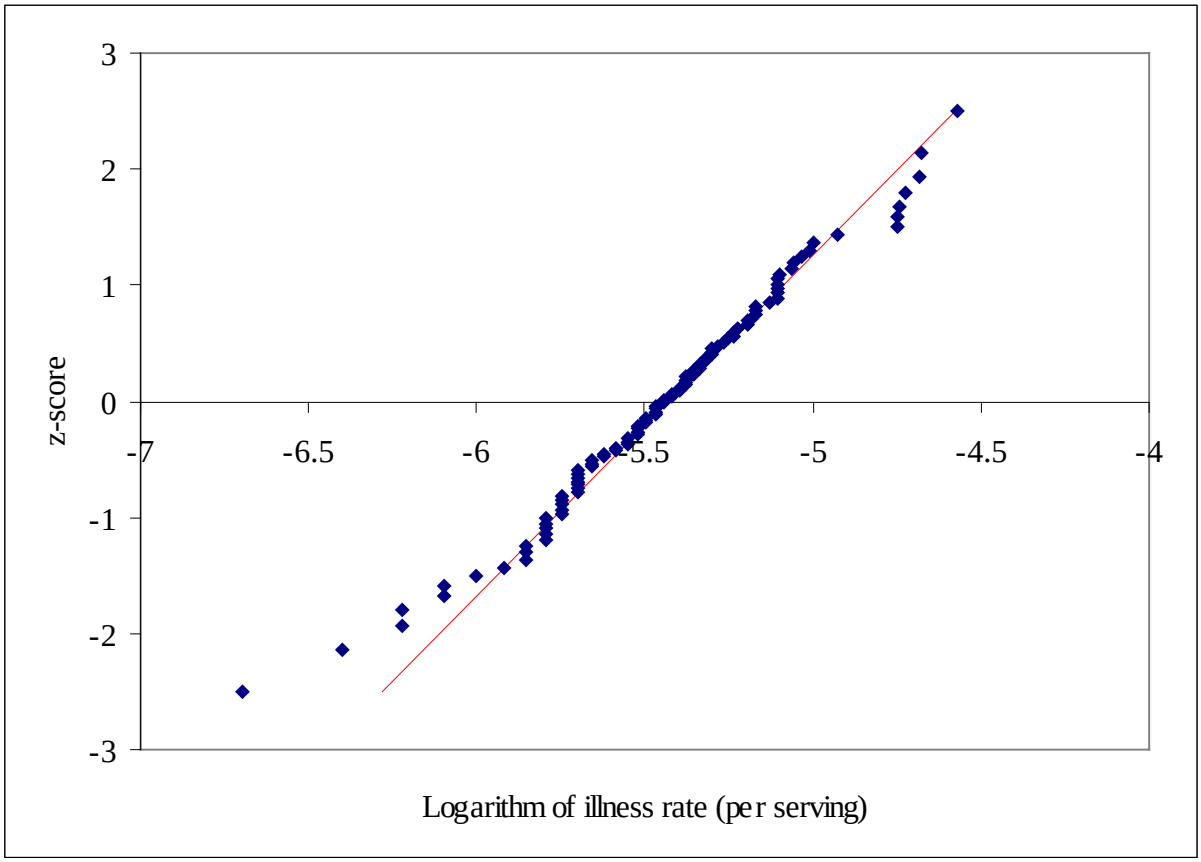

Figure 14 Illness rate computed using 100 iterations of 5,000,000 servings, with ML fitted lognormal distribution.[71]

A possible improvement in the numerical uncertainty estimates can be obtained if it is taken into account that the largely multiplicative structure of the risk assessment suggests that the uncertainty distribution is likely a form close to lognormal. Indeed, the distribution in Figure 14 is indistinguishable from lognormal, the standard deviation of the natural logarithm of the rate being about 0.8. Fitting a single lognormal to the 100 uncertainty samples (taking account of the Poisson numerical uncertainty of each point) gives maximum likelihood estimates for the 95th and 99th percentiles of 13.5 per million and 23 per million respectively. The precise loglikelihood used in this case was

[71] Results and calculations for this model run are in workbook Output_1.xls, sheet Uncertainty_1.

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$$J = \sum_{i=1}^{n} \ln\left(\frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} dt \exp\left(-\frac{(t-\mu)^2}{2\sigma^2} \right) \frac{e^{r_i t} \exp\left(-e^t\right)}{r_i^{r_i} e^{-r_i}} \right) \quad (23)$$

where n is the number of uncertainty iterations, r_i is the number of illnesses[72] predicted in the i^{th} uncertainty iteration, and μ and σ are the mean and standard deviation of the logarithm of the illness rate. Using likelihood methods then gives numerical uncertainties (at 1 standard deviation) of 1.8 per million and 4 per million at the 95th and 99th percentiles, repectively. These are somewhat better (in having smaller uncertainties) than the estimates obtained directly from the Monte Carlo results; but come at the cost of an assumption about the shape of the distribution. In fact, as seen in Figure 2, this assumption is incorrect — the lower end of the distribution deviates significantly from the lognormal shape when more Monte Carlo samples are taken, and the effect of such deviations would have to be examined separately in every case.

A similar technique could presumably be used with a more general shape for the distribution. For example, a suitably constrained polynomial could be used rather than a straight line, or a LOESS technique applied. However, such approaches have not been pursued here, since it is easier to run the Monte Carlo sufficient times to obtain adequately small uncertainties.

C.5. Reference for Appendix C

Cunnane, C. (1978). Unbiased plotting positions — a review. *J. Hydrology* 37:205–222.

[72] For this run, the technique of Appendix B to get smaller uncertainty estimates for the illness rate was not used.

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Appendix D. Changes in the *C. perfringens* Risk Assessment Model Program

D.1. Introduction

The setup and running of the *C. perfringens* risk assessment model program is described in Appendix E of the risk assessment (Crouch and Golden, 2005). The modifications to the program that were needed for this demonstration are described in this appendix.

D.2. Correction

One error was noted in the program, and has been corrected (the correction is noted in the code as "correction: Added "Nearest" on 1/29/08" in a comment in the file CP_risk.pas). This error was in the calculation of a value that was not used in the risk assessment, so has no effect on the results and conclusions of the risk assessment.

The routine that calculated the number of vegetative cells in a serving at the time of eating (method `next_sample` of class TFoodServing) failed to round that number to the nearest integer. Many of the food servings where all cells were killed by cooking, for example, returned a tiny but positive value because the estimated number of cells was a very small fraction (which would round to zero) after applying the exponential die-off caused by cooking (*e.g.* typically the "number of cells" was less than 1×10^{-10}). Such small values did not change the estimated illness rates (since the probability of illness in such cases was so small).

However, the calculated prevalence was incorrect, because the prevalence was obtained by counting the number of servings with a non-zero result. Such an error, uncorrected, would have affected the results of this demonstration.

D.3. Modifications to the inputs

D.3.1. Setup and running the program

The setup and running of the program is essentially unchanged from the description of the risk assessment, Appendix E.1. The change in data requirements is described in Section 5.3, that is: the input file "Food_samples.dat" has been replaced with "Hotdogs_only.dat", and the line

```
Food_sample_data='Food_samples.dat';
```

within the module CP_risk.pas has been modified to

```
Food_sample_data='Hotdogs_only.dat';
```

All other input files are identical except for the control file, which has been augmented.

D.3.2. Structure of the control file

The control file described in the risk assessment, Appendix E.2, has been augmented. The same six keyword-value pairs as described there must still be present, but the following additional four keyword-value pairs are now also required and are described below:

```
Instrumented        1
Cell_count          1e6
Keep_zeros          0
Measured_mass       1 g   # Unit of mass required
```


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"Instrumented" This has the value 0 or 1. If the value is 0, the program behavior is exactly the same as before (subject to the correction described in Section D.2), and the following three keyword-value pairs are ignored. If the value is 1, additional information is calculated and recorded, as described in Section D.4

"Cell_count" If Uncertainty_loops=1 then all servings with vegetative cell count exceeding the value given for Cell_count are recorded, as well as illness causing ones, and subsequently details of these servings are printed (see the risk assessment, Appendix E.3.2, and Section D.4.4).

"Keep_zeros" This has the value 0 or 1. If Uncertainty_loops=1 then if keep_zeros=1, then information on all the servings is retained and printed; if keep_zeros=0 then information only on those servings with non-zero vegetative cell count is retained and printed. This is used to extract the complete variability distributions for multiple end-points calculated in the program, to allow correlation analysis; rather than just the individual distributions without correlation information.

"Measured_mass" This is the value of the sample mass that is evaluated, in addition to individual servings. It is assumed in the program that the sample mass is less than or equal to the minimum serving mass in the input servings file (hotdogs_only.dat); if this is not true, a warning will be printed every time a smaller serving is sampled. For hot dogs, the minimum serving mass was 6.29 g. It would be possible to modify the program so that sample sizes larger than the minimum serving size can be specified, but this was not necessary for this demonstration.

D.4. Modifications to the outputs

D.4.1. The case Instrumented=0

If the keyword Instrumented is assigned the value 0 in the control file, then the output of the program is unchanged (see the risk assessment, Appendix E.3), except that the number of servings with non-zero vegetative cell counts will now be different because of the correction described in Section D.2.

D.4.2. The case Instrumented=1, Command Box (screen) output

The command box screen output while the program is running (risk assessment Appendix E.3.1) is slightly modified. There are now 8 numbers output at the end of each variability loop, instead of seven. These 8 numbers are, in order:

1. Growth during stabilization, (this is the summary growth value specified in the sensitivity parameter file, see risk assessment Appendix E.4.2),
2. Uncertainty loop number,
3. number of servings with non-zero vegetative cells at the time of being eaten (out of the total servings = variability_loops for this uncertainty loop),
4. number of illnesses occurring for this uncertainty loop,
5. number of cases for this uncertainty loop where contamination was detected (and the food thrown out); this occurs only in "what if" situations (see below),
6. mean estimate for the number of illnesses (see Appendix B)

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7. standard deviation estimate for the mean estimate (see Appendix B)
8. difference in time counter value from the beginning of the program to the time this line was output, in seconds (note: the time counter increments for a maximum of one day, then starts over, so runs over 1 day can give negative numbers here; you would have to add the number of days to get the correct time).

D.4.3. The case of `Instrumented=1`, `Uncertainty_loops=1`, Output file

In this case, the output file is very similar to that described in the risk assessment Appendix E.3.2. As before, this is an ASCII text file containing tab-delimited values on each line, with each line separated by a carriage-return, line-feed pair. For each uncertainty loop and value of growth during stabilization, first a line is output with the following 12 numbers:

Growth	Growth during stabilization, (this is the summary growth number specified in the sensitivity parameter file, see below),
Non-zero	Number of servings in this uncertainty loop that had non-zero veg. cells at the time of being eaten
# cutoff	Number of servings with detected contamination (and the serving discarded); this occurs only in "what if" situations (see below),
# ill	Number of illnesses
Mean	The mean estimate for the number of illnesses (see Appendix B)
SD	standard deviation estimate for the mean estimate (see Appendix B)
Measured A+	The number of samples (of mass `Measured_mass`) positive for type A, CPE+, vegetative cells, just post-stabilization.
Measured tot	The number of samples (of mass `Measured_mass`) positive for total *C. perfringens* vegetative cells, just post-stabilization.
Post_stab_A+	The number of servings positive for type A, CPE+, vegetative cells or spores, just post-stabilization.
Post_stab_tot	The number of servings positive for total *C. perfringens* vegetative cells or spores, just post-stabilization.
Eat_A+	The number of servings positive for type A, CPE+, vegetative cells at consumption.
Eat_tot	The number of servings positive for total *C. perfringens* vegetative cells at consumption.

The subsequent entries in the file are unchanged in format and are described in the risk assessment Appendix E.3.2; however, instead of just printing information on servings that led to illnesses, in addition information is printed on all servings with `Cell_count` vegetative cells or more at consumption.

D.4.4. The case of `Instrumented=1`, `Uncertainty_loops>1`, Output file

As before (risk assessment, Appendix E.3.3) this is an ASCII text file containing tab-delimited values on each line, with each line separated by a carriage-return, line-feed pair. For each uncertainty loop and value of growth during stabilization, the 12 values described for `Uncertainty_loops=1` in Section D.4.3 are output. In this case, however, there is no additional information.

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D.5. Summary of modifications to the program code

The program code was modified to allow extraction of the values required in this demonstration. However, the unmodified case (`Instrumented=0` in the control file) is essentially identical; the main program routines were initially duplicated, and one of them modified for the instrumented case while leaving the originals largely alone; additional small routines were added to read the control file and then control selection of the main routine that is run. The principal differences are in the initial duplication of the "main" routine, so that there is also a "main_instrumented" routine; and in the initial duplication of the "next_serving" method of class TFoodServing, so there is now also a "next_serving_instrumented" method.

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