Review

Potential application of quantitative microbiological risk assessment techniques to an aseptic-UHT process in the food industry

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A B S T R A C T

Aseptic Ultra-High-Temperature (UHT)-type processed food products (e.g. milk or soup) are ready to eat products which are consumed extensively globally due to a combination of their comparative high quality and long shelf life, with no cold chain or other preservation requirements. Due to the inherent microbial vulnerability of aseptic-UHT product formulations, the safety and stability-related Performance Objectives (POs) required at the end of the manufacturing process are the most demanding found in the food industry. The key determinants to achieving sterility, and which also differentiates aseptic-UHT from in-pack sterilised products, are the challenges associated with the processes of aseptic filling and sealing. This is a complex process that has traditionally been run using deterministic or empirical process settings. Quantifying the risk of microbial contamination and recontamination along the aseptic-UHT process, using the scientifically based process Quantitative Microbial Risk Assessment (QMRA), offers the possibility to improve on the currently tolerable sterility failure rate (i.e. 1 defect per 10,000 units). In addition, benefits of applying QMRA are i) to implement process settings in a transparent and scientific manner; ii) to develop a uniform common structure whatever the production line, leading to a harmonisation of these process settings, and; iii) to bring elements of a cost-benefit analysis of the management measures.

The objective of this article is to explore how QMRA techniques and risk management metrics may be applied to aseptic-UHT-type processed food products. In particular, the aseptic-UHT process should benefit from a number of novel mathematical and statistical concepts that have been developed in the field of QMRA. Probabilistic techniques such as Monte Carlo simulation, Bayesian inference and sensitivity analysis, should help in assessing the compliance with safety and stability-related POs set at the end of the manufacturing process. The understanding of aseptic-UHT process contamination will be extended beyond the current "As-Low-As-Reasonably-Achievable" targets to a risk-based framework, through which current sterility performance and future process designs can be optimised.

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1. Introduction

Aseptic-Ultra-High-Temperature (UHT)-type processed foods are consumed extensively throughout the world due to their long shelf life (three to six months), no cold chain requirements, and their possibility to be consumed immediately. The world market for aseptic UHT-type products amounted to 123 billion litres during 2011. Drinking milk accounts for 39% of aseptic UHT-type products, beverage (e.g. fruit juice) for 24% (Warrick Research and Zenich International, 2012). Due to stability at ambient temperature, aseptic-UHT-type processed food products can be manufactured in one country and consumed in several importing countries. These products are defined as a commercially sterile product which means that the food “must be free of microorganisms capable of growing under normal non-refrigerated conditions of storage and distribution” (Codex Alimentarius Commission, 1993). A typical value for a tolerable commercial sterility failure rate is one defect per 10,000 units (Cerf and Davey, 2001). This failure could be due to pathogens (e.g. *Bacillus cereus* in acai berry juice from Sweden in December 2009) or spoilage agents (e.g. coagulation of and too high count of mesophilic bacteria in UHT milk from Slovenia in June 2012) (European Commission, 2012).

The aseptic-UHT process is relatively complex but can be defined in three main phases, based on the material and process flow (Fig. 1). The first one is a “non-sterile” phase, in which product ingredients and packaging raw materials are received and sterilised. A UHT treatment is applied to the product, and combined or separate thermal or chemical sterilisation treatments are applied to the packaging line. The second “sterile” phase occurs within a high care area, where aseptic filling and sealing is performed. The filling and sealing process steps are definitively the most challenging in terms of controlling contamination risk to the product; where biofilm formation, air recontamination, and defective sealing are significant causes of product sterility failure. The last phase encompasses storage in the distribution chain until consumption. To comply with the demand of commercial sterility, aseptically designed equipment in combination with stringent process settings is required.

Traditionally, aseptic-UHT process settings are defined and implemented in a deterministic or empirical manner. To move towards science-based process settings, risk of microbial contamination and recontamination along the whole aseptic-UHT process could be quantified. This would allow the contribution of each major process step to sterility failure to be established. That can be done by using the risk-based framework of Risk Analysis (advocated by the World Health Organisation and Food Agricultural Organisation (1995b)). It is a systematic and comprehensive methodology to estimate risk associated with a complex process. Risk analysis is split into three pillars: i) risk assessment, or more precisely in our case Microbial Risk Assessment (MRA), which consists of characterising the nature and the likelihood of hazard; ii) risk management, the evaluation of options to maintain or reduce the risk, and; iii) risk communication, the interaction between regulatory authorities, scientists, food producers and consumers. For an aseptic-UHT food process with a commercial sterility target, the four steps of MRA can be further defined as: i) hazard identification, which consists of identifying microbiological agents capable of causing adverse health effects (for pathogens) or significant sensory deterioration of the product (for spoilage agents), and; ii) hazard characterisation, the evaluation of the nature of the adverse effect of pathogens (i.e. public health concern) and spoilage agents (i.e. spoiled product); iii) exposure assessment, the quantification of contamination along different pathways through the whole process to consumption, and; iv) risk characterisation, the quantification of the risk in terms of public health (for pathogens) and the probability (“risk”) of non-compliance with a commercial sterility rate (for spoilage agents). The benefits for a food...
The objective of this article is to explore how QMRA techniques and risk management measures may be applied to aseptic-UHT-type processed food products. In the first section of this article, the risk-based framework is presented with some examples of QMRA applications in an aseptic-UHT-type food process. Then, in a second section, the mathematical models within the disciplines of predictive microbiology, food engineering and statistical science are used to describe the different potential contamination variations in an aseptic-UHT-type process. In a third section, the probabilistic and statistical methods required to estimate the risk, such as Monte Carlo simulation, Bayesian inference and sensitivity analysis are described. Finally, within the risk-based food safety management framework, the main options, to control and reduce the risk when necessary, are discussed.

2. The risk-based framework

2.1. Introduction to risk-based management

Risk analysis activities provide information for the formulation of national policy, advice on issues of public health, and aids in the transparency of decision-making and communication. Therefore, it is a significant driving force for fair commerce and safe food trade between nations. Risk analysis has been advocated by the (World Health Organisation and Food Agricultural Organisation, 1995) for more than a decade, and recently new concepts have emerged: the Appropriate Level Of Protection (ALOP) and the Food Safety Objective (FSO). ALOP is defined as an expression of a probability of an adverse health effect in terms of public health statistic (World Trade Organization, 1995). An ALOP would be articulated as a statement related to the disease burden (e.g. illness or infection incidence rates reported under surveillance programmes), associated with a particular hazard-food combination and its consumption in a country (Stringer, 2004). Whilst expression of an ALOP in terms relevant to public health serves to inform the public, it is not a useful measure in the actual implementation of food controls throughout the food chain. Instead, a measurable target for producers, manufacturers and regulatory authorities is required; this is the basis of the Food Safety Objective (FSO) concept. FSO is defined 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” (International Commission on Microbiological Specification for Foods, 2002). The FSO is described as a “bridge” between ALOP and farm-to-fork process steps (Fig. 2). For instance, Perni et al. (2009) have calculated the FSO in Ready-To-Eat (RTE) food for several microorganisms (i.e. Listeria monocytogenes $-0.33 \log_{10} (\text{CFU/g})$, Salmonella $-6.71 \log_{10} (\text{CFU/g})$, Escherichia coli O157 $-1.66 \log_{10} (\text{CFU/g})$, Campylobacter $-4.34 \log_{10} (\text{CFU/g})$, Bacillus cereus $4 \log_{10} (\text{CFU/g})$, Vibrio $-0.60 \log_{10} (\text{CFU/g})$) based upon the dose–response properties of the microorganism, severity of the disease and subjective ALOP. Other studies have investigated dose–response relationship to specific FSO values (Gkogka et al., 2013; Zwietering, 2005). However, it is important to keep in mind that ALOPs and FSOS have not yet been established for all pathogens.

On the contrary, the link between the equivalent FSO and ALOP for a spoilage hazard has not been elaborated in the literature. The task of building such a “link” is greatly aggravated by the almost infinite number of potential spoilage hazard agents capable of contaminating and growing within aseptic-UHT products. Each spoilage hazard agent has a unique profile of hazard characteristics, which differs in “severity” as a function of individual product composition (e.g. formulations and packaging) and the consumer market (e.g. demography and complaint reporting rates). These effectively contribute to determine the likelihood of consumer complaints attributable to spoilage agent, which itself can be viewed as analogous to the ALOP associated to specific pathogen hazards. The dose–response relationship which links ALOPs to FSOS for pathogen hazards has a counterpart in the consumer complaint and sterility failure rate targeted for spoilage hazards. Therefore, a targeted sterility failure rate theoretically can be related by a food company through a measure of consumer satisfaction (e.g. consumer complaint rates) to define the “link” between line performances and an acceptable quality threshold or target. This effectively could describe the current situation, where a process line, when performing within operational norms, will produce product “lots” at an acceptable level of sterility. Because of the current lack of a clear quantitative link between line

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**Fig. 2.** FSO: a bridge between ALOP and farm-to-fork process steps. Adapted from ICMSF International Commission on Microbiological Specification for Foods (2005).
performance and consumer satisfaction, the traditional subjective approach to operational management of spoilage risks in aseptic-UHT processes can be described with the “As-Low-As-Reasonably-Achievable” concept. Clearly, to advance beyond this qualitative approach for aseptic-UHT processes to one where risk management options are defined around FSOs, food company risk managers and decision-makers will need new ALOP-linked management tools that allow them to assess the impact, in terms of quality costs and benefits, when making changes to line performance or design. To our knowledge there are no publications in this area of microbial spoilage risk assessment and management. However, theoretical aspects of the relationships between consumer satisfaction, defect rates and process line performance have and continue to be studied; whether integrating quality perceptions and expectations (Linnemann et al., 2006) modelling aspects of quality deterioration (van Boekel, 2008) or formulating approaches to modelling consumer complaint rates (Peleg et al., 2011).

Within the risk-based food safety management metrics, the Performance Objective (PO) is directly related to the FSO but is set at a step before the time of consumption (e.g. at the point of product release at the end of the manufacture). The PO may be set with sufficient stringency to take into account the possible growth of the microorganism during the storage. For instance, Perni et al. (2009) have also studied the relationship between FSO and PO for Salmonella, considering the initial concentration in the RTE product, the possible growth before cooking, and the inactivation during cooking. To set a PO is a food safety management decision. To be operational, FSOs and POs must be translated into the inactivation during cooking. To set a PO is a food safety management decision. To be operational, FSOs and POs must be translated into the inactivation during cooking. To set a PO is a food safety management decision.

As-Low-As-Reasonably-Achievable (ALOP) concepts, an ILSI report (Stringer, 2004) gave an example based on the control of Clostridium botulinum in low acid canned foods:

- PrC: 3 min at 121 °C (“botulinum cook”)
- PC: 12 D reduction

A cross-disciplinary international consortium of specialists from industry, academia, and government was organized with the objective of developing a document to illustrate the FSO approach for controlling C. botulinum toxin in commercially sterile foods (Anderson et al., 2011). This paper included historical approaches to establishing commercial sterility; a perspective on the establishment of an appropriate target FSO; a discussion of control of initial levels, reduction of levels, and prevention of an increase in levels of the hazard. The initial concentration was estimated using available data, and in the case of data gaps, various options were discussed. For the reduction part of the process, microbial inactivation models were used. For the microbial increase, impact of pH, water activity, nitrite, spore injury and combination of factor on growth were discussed. The impact of Good Manufacturing Practices (GMPs) in prevention of incipient spoilage was also discussed. This last factor took into account package integrity and post-process recontamination. For each part described above, gaps are identified and some research activities were recommended to fill them. Finally, deterministic and probabilistic scenarios were used to illustrate the impact of various control measure combinations.

To apply these different risk-based food safety management concepts, first, the significant microbial pathways must be identified and quantified along the food chain. That is often done in QMRA of processed foods.

2.2. Examples of QMRA applied to processed food manufacture

QMRA in a processed food manufacture has been extensively studied (Brown and Stringer, 2002). Many examples of QMRA in food industry are listed on the FoodRisk website (Joint Institute for Food Safety and Applied Nutrition, 2012). At the time of our survey (December, 2012), the available database contained 607 articles (non-exhaustive list) but only 29 were carried out in a processed food manufacture context (e.g. chilled pasteurized foods, cold smoked salmons, RTE vacuum packed products, …etc.). Amongst these 29 studies related to a processed food context, only nine correspond to, or might be assimilated to, aseptic-UHT processes. However, three of them are highly relevant for commercial sterility purposes, they are detailed below.

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- FSO: <1 spore/10^12 g at the time of consumption.
- PO: <1 spore/10^12 g after processing
- PC: 12 D reduction

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Fig. 3. From the Food Safety Objective to the management options: from the “plate” level to the operational level. Adapted from Gorris (2005).
on the safety of several commercially sterile products within an FSO risk management framework. For instance, in shelf-stable cured luncheon meat, the authors calculated that the probability that one spore could grow and produce toxin was $10^{-7}$ at equivalent of 121.1 °C for 0.6 min, pH of 7.0, 4.5% of NaCl and 150 ppm of nitrite for an initial level in raw meat of less than 0.1 spore per kg.

In Cerf and Davey (2001), a comparison between a Single Value and a Monte Carlo assessment of the risk of non-sterile packs of UHT processed milk was made. The number of non-sterile packs obtained was compared to the numbers that are anecdotally known to be found in a UHT plant i.e. between 1 and 4 in $10^5$ production units. However, it is important to keep in mind that in their study, the authors have only modelled the thermal treatment. More generally, they concluded that the cause of the non-sterile UHT packs is traditionally attributed to leakage of pack material, or seal, and contamination either during processing or storage and distribution.

Recently, a probabilistic data analysis of an ambient stable soup product, heated in a continuous UHT line was performed by Membré and van Zuijlen (2011). The thermal process was based on actual data rather than on generic or conservative rules. The data sets came from a decade of microbiological analysis; the results were illustrated with two ingredients, garlic and milk powder. In this case, the sterilization value was estimated then to be 13 min (95th percentile) at 121 °C.

In conclusion, there are significant numbers of studies applying QMRA in processed foods but only a few in aseptic-UHT lines. In most of them, only pathogens have been considered, although risks associated with spoilage agents are often tangentially mentioned but not quantified. In QMRA studies, the lack of specific models and data is often emphasized. In all the studies outlined herein, the microbial behaviour during the thermal process step and the potential growth of post-process survival microbes are described. However, consideration or quantification of post-process recontamination is frequently only mentioned but not quantified.

3. Mathematical models describing the microbial variations at each aseptic-UHT process step

In this section, mathematical models which permit the quantification of the contamination level of the product when ready-to-be-consumed, i.e. the output of the exposure assessment part of the QMRA, are presented. Once the contamination level is determined, probability, i.e. risk, of not complying with an FSO can be estimated for various risk reduction measures can be compared quantitatively. Likewise, for commercial sterility assessment, probability of not complying with a PO chosen at the product release step can be evaluated and if required, efficiency of different reduction measures can be assessed.

3.1. General model framework

Basically, there are three phases in an aseptic-UHT process (Fig. 1), the first a “non-sterile” phase with process steps from reception of ingredients and packaging raw materials to bacterial reduction. A UHT treatment is applied to the product and combined or separate thermal or chemical sterilisation treatments are applied to the packaging line. The second “sterile” phase occurs within a high care area, where aseptic filling and sealing is performed. The last is the storage until consumption. Nonetheless, the aseptic-UHT process is complex and consequently needs to be decomposed into smaller steps to quantify any bacterial contamination pathway. Nauta (2001) introduced the “Modular Process Risk Model” concept where the food pathway is divided into several key smaller steps. This concept has been often used in the risk assessment context, for example such studies are reported in Billoir et al. (2011), Malakar et al. (2011) and Nauta et al. (2009). One way to split the process in a useful way is to identify the contamination pathway occurring at each step of the process using the following equation. The International Commission on Microbial Specification for Foods (2002) proposed an equation related to the PO and FSO concepts (Eq. (1)):

$$H_0 - \sum R + \sum I \leq PO \text{ or FSO}$$  (1)

It categorises each step from food ingredient to consumer’s fork as three main components or processes through which levels in microbial hazards may change:

- $H_0$: is the initial level of hazard, a combination of either the raw materials (for the product and the packaging) and the terminal level of hazard from the preceding step.
- $\sum R$: is the quantification of microbial inactivation in one or several of the process steps,
- $\sum I$: is any increase in the hazard due to either growth or recontamination.

This framework is a conceptual model rather than a mathematical one, it is a schematic description of the whole process. The terms in Eq. (1) are expressed in decimal logarithm which is useful for expressing log-linear inactivation or growth, but not straightforward for describing a post-process recontamination. Despite these drawbacks, this concept has been widely applied in QMRA studies, for example in Anderson et al. (2011), Membré et al. (2007), Sosa Mejia et al. (2011) and Stewart et al. (2003). Moreover, it is also used as a framework in the ILSI report on risk assessment approaches to setting thermal processes in food manufacture (Bean et al., 2012).

In the exposure assessment, our mathematical model review below covers the initial level in raw materials, the sterilisation step (for the product or the packaging), the recontamination pathways (from biofilm or from air), the storage, the mixing and partitioning process steps. In aseptic-UHT-type food processes, all contamination pathways need to be considered due to the respect of the high care area, even if the probability of occurrence is very low. Dose–response models are excluded here as they are used for modelling the relationship between FSO and ALOP, and therefore come under the hazard characterization part of the risk assessment.

3.2. Modelling initial contamination level in the product

The first step in a QMRA study is to estimate the initial contamination level in the raw materials. Ideally, quantitative data from a large number of studies on the raw material itself could be used to assess the concentration distribution of the microorganism. Prevalence of the microorganism is often more difficult to establish due to the large variety of sampling plans and microbial detection methods (Jongenburger et al., 2011). The initial concentration is often expressed in logarithm of the number of microorganism present per unit. Consequently, the concentration is often described by a normal, log-normal or some other probability distribution. In the case where data are not informative enough, the probability distribution could be completed with an expert opinion. This method has been already applied by Barker et al. (2005) on the distribution of non-protectolytic C. botulinum spores in potato flakes, coating flour and other ingredients. They used limited information from statistical samples taken by the manufacturer in combination with expert opinions.

3.3. Modelling bacterial reduction

In an UHT-aseptic process line, three types of bacterial reduction occur. The first, widely explained in the literature, is via the UHT treatment of the product, the second is from the sterilisation of the packaging, and the last is through the cleaning procedures. These two last bacterial reduction processes are essential to an aseptic-UHT food...
process, because failure of either of these often explains an increase in the microbial contamination detected. Consequently, they also must be considered as management options to control and reduce when necessary the contamination risk (e.g. a minimum efficiency in the bacterial reduction could be set as a Pc).

3.3.1. Modelling bacterial reduction in the product line

The existing models for thermal treatment have been already discussed extensively by Basset et al. (2012), Brul et al. (2007) and McKellar and Lu (2004). Consequently, in this review, we have chosen to describe only one primary model and one secondary model, and only briefly discuss the others.

In order to model the inactivation kinetics, the primary model proposed by Bigelow (1921) can be used if it is assumed that the inactivation follows a first-order kinetic in isothermal conditions:

$$\log_{10} \left( \frac{N}{N_0} \right) = - \frac{t_{HT}}{D}$$

(2)

where:
- \( N_0 \): is the bacterial concentration entering in the thermal treatment (CFU/ml),
- \( N \): is the bacterial concentration at the end of the heat treatment (CFU/ml),
- \( t_{HT} \): is the time of the heat treatment (s),
- \( D \): is the decimal reduction time (s).

This model has been widely used and long established with a logarithmic transformation (logistic reduction increase linearly with the heat treatment time) (Cerf and Davey, 2001; Malaker et al., 2011; Nauta, 2001; Sosa Mejia et al., 2011). More recently, probability distributions have been introduced in modelling bacterial survival to reflect an assumption that lethal events are probabilistic rather than deterministic and to explain that if the heat treatment is sufficiently intensive no microorganisms will survive or recover. For example a Poisson distribution has been used to describe the probability of microbial survival after heat treatment (Membré et al., 2007; Nauta, 2001).

The concept of the traditional D-value has appeared insufficient in some cases, because the decrease of the population does not always follow the Eq. (2); for instance, it might have a lag time before the decrease could be observed.

Next, the decimal reduction time (D) is described by a secondary model which takes into account the process and product characteristics. Different secondary model structures were developed to describe D-value with respect to the process (heat treatment temperature) and product formulation (pH and water activity \( a_w \)). Amongst them, it has been suggested to extend the gamma concept (Zwietering et al., 1992), widely used in microbial growth, to the inactivation area (Gaillard et al., 1998; Leguérinel et al., 2000; Mafart et al., 2010):

$$D = D^* \times 10^{-\frac{z_{aw} - z_{fl}}{z_{aw}}} \times 10^{-\left( \frac{D^* - D_10}{D^* - D_10} \right)^2} \times 10^{-\frac{z_T}{z_T}}$$

(3)

where:
- \( D \): is the decimal reduction time for conditions of temperature (\( T \)), pH and \( a_w \) (s),
- \( D^* \): is the time required at the reference temperature (\( T^* \)), reference pH (\( pH^* \)) and reference \( a_w \) (\( a_w^* \)) to reduce the bacterial concentration to 1/10th of its value (s),
- \( t_{HT} \): is the temperature of the heat treatment (\(^\circ\)C),
- \( z_{aw} \): is the difference of \( a_w \) from \( a_w^* \), which leads to a 10-fold increase of the D value,
- \( z_T \): is the distance of pH from \( pH^* \) which leads to a 10-fold reduction of D value,
- \( z_{fl} \): is the water activity of the product,
- \( z_{eq} \): is the difference of \( a_w \) from \( a_w^* \), which leads to a 10-fold increase of the D value.

For the temperature parameters, data are abundant and available in the literature, whereas for pH and \( a_w \) much less information has been published. This may explain why in the literature, the secondary model described in Eq. (3) is mostly applied with only the temperature factor in QMRA studies (Cerf and Davey, 2001; Nauta, 2001; Sosa Mejia et al., 2011).

3.3.2. Modelling bacterial reduction in the packaging line

In aseptic-UHT product manufacture, microbial contamination needs to be considered not only in the product line but also in the packaging line. For the packaging sterilisation, a review of the several types of processes has been listed by Ansari and Datta (2003).

Firstly, there are thermal processes with a sterilisation with saturated steam (moist heat) or a combination with super-heated steam and hot air or even heating by extrusion of the packaging (form-fill-seal packaging system). For this kind of sterilisation, the previous models (primary and secondary, i.e. Eqs. (2) and (3)) could be applied with the time and temperature of the treatment, as explained by Ansari and Datta (2003).

Secondly, there are radiation processes with UV rays, infrared, ionizing or pulsed light sources, or a combination of hydrogen peroxide and UV rays. In principle all the radiation processes kill bacteria in the same exponential manner as with heat (Ansari and Datta, 2003). In the WHO technical report (World Health Organisation, 1999), \( D_{re} \)-values (dose of irradiation needed to produce a 10-fold reduction in the population of microorganism) are given for some vegetative bacteria, spores, yeast and moulds in several types of food products.

Another process type is the thermal or chemical decontamination methods, such as hydrogen peroxide or ethylene oxide or peracetic acid or combinations. Hydrogen peroxide, and its combination with heat, is the most widely used for the packaging materials sterilant and consequently the effects have been most thoroughly studied as mentioned in Ansari and Datta (2003).

It seems reasonable to apply a log-linear inactivation model (Eq. (2)) for all types of packaging material sterilisation. For the secondary model, the relation between the sterilisation agent and the D-value is not always defined and may be an area of further study.

This part of the process is not generally quantified in QMRA studies, although microbial contamination through infected packaging might explain some sterility failures. The type and quantity of the packaging sterilisation agent could be set as a PrC as an option to control and reduce when necessary the contamination risk. For example, a concentration of hydrogen peroxide of 30%, a temperature of the solution of 70 °C and a time of immersion of 7 s are settings currently applied in packaging sterilisation (Tetra Pak, 2012) and could be then re-interpreted as a potential PrC.

3.3.3. Modelling bacterial reduction due to the cleaning procedure

Another contamination pathway which is not often quantified in QMRA studies, is the possible survival of bacteria after the cleaning procedure. Indeed, let us consider one batch of contaminated product as an example. If the cleaning procedure is not sufficient to remove or inactivate product residues containing bacteria, the next batch could be contaminated.

In aseptic-UHT lines, the cleaning operation could be generally described by three different phases. Cleaning-In-Place (CIP) is an application of a chemical agent, either acid or alkaline. Hot water rinsing is applied on surfaces directly in contact with products. Sterilisation-In-Place (SIP) is the last step of the cleaning procedure, aiming to re-sterilize the production line (Davey et al., 2013). The
Eqs. (2) and (3) could be applied for the SIP step with the time and temperature.

For the two first steps, a simple model (first-order reaction) assuming a process combining removal and deposition during cleaning could be applied (Lelièvre et al., 2002a):

$$\frac{d(N_f)}{dt} = k_1[N_0 - N_f] - k_2[N_0 - N_c]$$  \hspace{1cm} (4)

where:
- $N_f$: is the surface density of spores at time $t$ (CFU/cm²),
- $k_1$: is an effective deposition rate constant (/min),
- $N_c$: is the initial surface density (CFU/cm²),
- $k_2$: is an effective removal rate constant (/min),
- $N_c$: is the surface density of permanently adherent spores (CFU/cm²).

The constant deposition rate ($k_1$) depends on cleaning conditions and on the concentration of removed spores, and is thus dependent on the volume of detergent solution. The constant removal rate ($k_2$) depends on the cleaning procedure conditions and is significantly influenced by the wall shear stress applied during cleaning (Lelièvre et al., 2002b). More generally, the cleanliness depends on geometrical design of the equipment, fluid hydrodynamic and steel surface properties (Friis and Jensen, 2002; Lei, 2009). The efficiency of the complete cleaning procedure could be set as a PC to control and reduce when necessary the contamination risk.

In conclusion, modelling the effect of the heat treatment step on product contamination, and maintaining the link with the compliance to a PO or FSO has been extensively studied, for example in Anderson et al. (2011), Cerf and Davey (2001), Malakar et al. (2011), Membré et al. (2007), Nauta (2001) and Sosa Mejia et al. (2011). However, no study connecting the packaging sterilisation and the cleaning procedure to a risk-based framework has been reported in the literature, to our best knowledge. In other words, studies indicating how the CIP efficiency and the packaging sterilisation contribute quantitatively to a PO or a FSO are missing.

3.4. Modelling bacterial increase

3.4.1. Modelling growth

The growth of microorganisms can occur at several storage steps, e.g. storage in tank before the filling process or storage of the final product before consumption. Due to growth, products that are contaminated at a low level and therefore considered safe or edible may become, at a certain moment of time, either unsafe or spoilt. The first factor determining whether and when growth of the microorganisms occurs is the low level in the product surviving the heat treatment. This factor has been widely studied in the literature (Guillier and Augustin, 2006; Kutalik et al., 2005). A study has been done by Koutsoumanis (2008) on the variability in the growth limits of individual cells and its effect on the behaviour of microbial population. The author has concluded that in the case of low inoculum, the model must take into account not only the distribution of lag times of growing cells but also the variability in the growth initiation of individual cells. This variability could be included in the lag distribution of the growth model below.

In this article, we have decided to describe one primary model and one secondary model, even if many models are available (Basset et al., 2012; Brul et al., 2007; McKellar and Lu, 2004).

Most QMRA models primary models comprising an exponential growth model without a lag and stationary phase, as for example in Sosa Mejia et al. (2011). However, including the lag phase is relevant when dealing with thermally injured bacterial spores (Stringer et al., 2009). A simple model to do so is the three phase linear model (Eq. (5)) of Buchanan et al. (1997) as applied in Malakar et al. (2011) for C. botulinum.

$$\log N_{t_2} = \log N_0 \quad \text{for} \quad t_e \leq t_{lag}$$

$$\log N_{t_2} = \log N_0 + \mu \times (t_e - t_{lag}) \quad \text{for} \quad t_{lag} < t_e < t_{max}$$

$$\log N_{t_2} = \log N_{max} \quad \text{for} \quad t_e \geq t_{max}$$  \hspace{1cm} (5)

where:
- $\log N_{t_2}$: is the natural logarithm of the population concentration at time $t$ (Log CFU/ml),
- $\log N_0$: is the natural logarithm of the initial population concentration (Log CFU/ml),
- $\log N_{max}$: is the natural logarithm of the maximum population density supported by the environment (Log CFU/ml),
- $t_e$: is the storage time (h),
- $t_{lag}$: is the time when the lag phase ends (h),
- $t_{max}$: is the time when the maximum population concentration is reached (h),
- $\mu$: is the specific growth rate (/h).

To describe specific growth rate adequately by a secondary model, it must take into account several parameters such as storage temperature ($T_{st}$) and pH of the product. Several secondary models are available, amongst them the simplified gamma-type models (Ratkowsky et al., 1983; Zwietering et al., 1992) which are interesting to consider for application in sub-optimal conditions (i.e. low pH and ambient storage temperatures).

$$\mu = \mu_{opt} \times \gamma(T_{st}) \times \gamma(pH)$$  \hspace{1cm} (6)

where:
- $\mu_{opt}$: is the optimal growth rate (/h), when $T_{st} = T_{opt}$ and $pH = pH_{opt}$,
- $\gamma(T_{st})$: is the effect of the temperature calculated by the model below,

$$\gamma(T_{st}) = \begin{cases} 0 & \text{for} \quad T_{st} \leq T_{min} \\ \left(\frac{T_{st} - T_{min}}{T_{opt} - T_{min}}\right)^2 & \text{for} \quad T_{min} < T_{st} < T_{opt} \\ 1 & \text{for} \quad T_{st} \geq T_{opt} \end{cases}$$  \hspace{1cm} (7)

where:
- $T_{st}$: is the storage temperature (°C),
- $T_{min}$: is the theoretical minimum temperature below which no growth is possible (°C),
- $T_{opt}$: is the optimal temperature for growth (°C),
- $\gamma(pH)$: is the effect of the pH calculated by the model below,

$$\gamma(pH) = \begin{cases} 0 & \text{for} \quad pH \leq pH_{min} \\ \left(\frac{pH - pH_{min}}{pH_{opt} - pH_{min}}\right) & \text{for} \quad pH_{min} < pH < pH_{opt} \\ 1 & \text{for} \quad pH \geq pH_{opt} \end{cases}$$  \hspace{1cm} (8)

where:
- $pH$: is the pH of the product,
- $pH_{min}$: is the theoretical minimum pH below which no growth is possible,
- $pH_{opt}$: is the optimal pH for growth.

The growth of pathogens is often the focus of QMRA (Anderson et al., 2011; Malakar et al., 2011; Nauta, 2001), whilst the growth of spoilage agents has rarely been quantified or at least just mentioned. Few models have been proposed in the literature concerning the microbial spoilage of food. When it has been possible to focus only on one specific spoilage organism, for instance with fresh fish or minced meat, primary
and secondary predictive microbial models have been developed. These models estimate microbial log-count as a function of the storage time, for various environmental conditions such as storage temperature or packaging atmosphere (Dalgard et al., 2002; Koutsoumanis and Nychas, 2000; Vaikousi et al., 2009). In the case of post-process recontamination, it might be sensible to consider that only one, or a few, species of spoilage microorganism may be reintroduced and have the ability to grow in the product. In such a case, microbial interactions might be neglected, and consequently models developed for individual specific spoilage organisms can be applied.

In terms of management options to control the sterility failure rate, pH of the product could be set as a PdC. Likewise, the temperature and the time of storage could be set as PrC.

### 3.4.2. Modelling recontamination

Recontamination is defined as the introduction of any microorganism into the product after an inactivation step, namely post-process recontamination. A survey performed by the World Health Organisation and Food Agricultural Organisation (1995a) in Europe indicated that 25% of the food-borne outbreaks could be due to recontamination. Some epidemiological investigations (European Commission, 2009) on food-borne illness have demonstrated that the presence of vegetative pathogens, such as *Salmonella* spp. or *L. monocytogenes*, in consumed products are often due to post-process recontamination. Recontamination events in aseptic-UHT processes are a primary cause of product sterility failure, and therefore processes modelling these events would form a key part of any aseptic-UHT QMRA (Agallilo et al., 2004).

The main objectives and challenges when modelling bacterial transfer is to develop reliable mathematical models capable of predicting, firstly, whether or not transfer takes place, and secondly, what proportion of bacteria is transferred in relation to certain factors (air pressure level and cell concentration, etc.). The Campden & Chorleywood Food Research Association group (Smith, 2007) proposed a practical exposure assessment for recontamination from the environment, food contact surfaces and liquids, and is divided into three steps:

- **Step 1:** Identifying the vectors involved in the transfer of contamination.
- **Step 2:** Determining the level of contamination in/on transfer vectors.
- **Step 3:** Determining the conditions and frequency of contamination exposure.

An overview of available models has been given in Reij et al. (2004). Moreover, Den Aantrekker et al. (2003b) have built a general recontamination model framework. A contaminated source (liquid, equipment or floor) releases cells to the intermediate phase (surface, hands or air) and then cells can be transferred to the product causing recontamination. This is done with two transfer coefficients: (i) a transfer from the source to the intermediate phase, and (ii) a transfer from the intermediate phase to the product. Some QMRA studies have incorporated cross-contamination step, for example, Aziza et al. (2006) and Pérez Rodriguez et al. (2011) but none in the aseptic-UHT-type process.

#### 3.4.2.1. Modelling recontamination from air

The recontamination from air is a key issue to control for a UHT-aseptic process because the “sterile” product could be exposed to the air, e.g. in the area of the filling machine (Fig. 1).

Sedimentation of microorganisms is responsible of the post-process recontamination from air. Den Aantrekker et al. (2003a) studied the probability of recontamination via the air using Monte Carlo simulations as a function of the number of microorganisms in air and particle settling velocities. The contamination level ($I_c$) is defined as the number of CFU which contaminate a product unit, for example a $L_c$ of $10^{-3}$ means that 1 out of every 1000 products is contaminated with 1 CFU. It is calculated as follows:

$$I_c = C_{air} \times v_p \times A \times t \quad (9)$$

where:

- $I_c$: is the number of CFU which contaminate a product unit,
- $C_{air}$: is the concentration of microorganism in the air (CFU/m³),
- $v_p$: is the settling velocity (m/s),
- $A$: is the exposed area of a product unit projected on the horizontal plane (m²),
- $t$: is the exposed time (s).

A seasonal effect was found in the concentration of microbes in the air, with the summer counts higher than was found in the other seasons. Regarding the settling velocity, (Den Aantrekker et al., 2003a) conclude that there is no significant difference ($p > 0.05$) between either the product groups, or the seasons, or between bacteria, yeast and moulds, its average value was estimated to $2.70 \times 10^{-3} \text{ m/s}$. The authors have also stated that the high numbers of particles present in the air correspond to higher concentrations of microorganisms in the air. On the opposite, they have not considered the air flow movements.

For a UHT-aseptic production line, the filling machine areas are critical high care areas. In these areas, the air contaminants must be eliminated or at least their concentration ($C_{air}$ in Eq. (9)) minimized. Applications of high relative air pressure system or air conditioning system are then crucial (Brown, 2005; European Hygienic Engineering and Design Group, 2006; Hayes, 1992; Jaccens et al., 2009). Their impact on the high care area decontamination can be quantified through a mass balance. Generally speaking, in food process engineering, a mass balance equation can be written as follows (Zwietering and Hastig, 1997):

$$\text{(Flow In)} + \text{(Production rate)} = \text{(Flow Out)} + \text{(Variation over the time)} \quad (10)$$

To quantify the air contamination, parameters such as the source of the air, the frequency of air changes, the efficiency of filter and their frequency of replacement and the positive pressure applied have to be taken into account (BRC Global Standard for Food Safety, 2012; Brown, 2005; Graham, 2011; Hayes, 1992). In a risk management framework, these parameters can be set as a PC or PrC to reduce the contamination risk, e.g. the air change rate could be set between 5 and 25 air changes per hour (Brown, 2005).

#### 3.4.2.2. Modelling recontamination from biofilms

Biofilms are defined as communities of microorganisms that attach to surfaces and are a prevalent mode of growth for microorganisms in nature. Bacteria can adsorb reversibly to surfaces as result of (amongst others) van der Walls forces and electrostatic interaction. When reversibly adsorbed, the cells are still readily removed by mild rinsing. Bacteria can also adsorb irreversibly by producing extracellular products, usually polysaccharides that act as anchors between the bacteria and the surface (Hood and Zottola, 1997), and consequently are more difficult to remove. In this study, both attachment forms are considered as a biofilm.

Marchand et al. (2012) have done a complete review on biofilm formation in milk production including definition of biofilms, mechanisms of biofilm formation in dairy plants and efficacy of different cleaners and sanitizers on dairy biofilms.

In terms of modelling, the contamination through biofilm formation can be explained by a mass balance equation system (Eq. (10)) (Den Aantrekker, 2002). Basically, the number of cells in the biofilm phase depends on the number of cells adhering to the surface, the
growth of adsorbed cells and the number of cells detaching from the surface (Eqs. (11) and (12)).

\[
\text{(Accumulation)} = (\text{Adsorption}) - (\text{Desorption}) + (\text{Growth}) \quad (11)
\]

\[
\left(\frac{dN_b}{dt}\right) = (k_A \times X_L) - (k_D \times N_b^0) + (\mu_b \times N_b) \quad (12)
\]

where:

- \( N_b \) is the number of adsorbed cells (CFU/m²),
- \( k_A \) is the adsorption coefficient (m/h),
- \( X_L \) is the concentration of cells in the liquid (CFU/m³),
- \( k_D \) is the desorption coefficient (m³/CFU/m², CFU/m³),
- \( \mu_b \) is the power factor describing non-linearity for desorption,
- \( \mu_b \) is the growth rate in biofilm (/h).

The number of cells in the bulk liquid depends on the number of cells flowing into the system, the number of cells released from the surface, growth in the bulk liquid, adsorption of cells to the surface and the number of cells flowing out of the system (Eqs. (13) and (14)).

\[
(\text{Accumulation}) = (\ln) + (\text{Desorption}) - (\text{Out}) - (\text{Desorption}) + (\text{Growth}) \quad (13)
\]

\[
\left(\frac{dX_L}{dt}\right) = (D_L \times X_L) + \left(\frac{A_L}{V_L} \times k_D \times N_b^0\right) - (D_L \times X_L) - \left(\frac{A_L}{V_L} \times k_A \times X_L\right) + (\mu_L \times X_L) \quad (14)
\]

where:

- \( D_L \) is the dilution rate (/h),
- \( V_L \) is the concentration of cells entering the system (CFU/m³),
- \( A_L \) is the surface area (m²),
- \( V_L \) is the liquid volume of the system (m³),
- \( \mu_L \) is the growth rate in the liquid (/h),
- \( k_D, k_A, n \) and \( N_b \) having the same definitions as above (Eq. (12)).

Writing the mass balance equation related to biofilm contamination as mentioned above has at least three pitfalls:

- generating data or gathering information to inform the parameters \( k_A, k_D \) and \( n \) is not straightforward,
- biofilm formation is only initiated by a transfer of microorganisms from the contaminated food liquid, the possible biofilm formation due to CIP and SIP survival microorganisms is neglected,
- possible death in bulk liquid and biofilm due to sanitation are also absent from the equations above.

The adhesion and detachment kinetics of several strains of \textit{Staphylococcus aureus} were studied by Herrera et al. (2007) under three different experimental static conditions; in the absence of nutrients (i.e. clear surface), in nutrient-rich media (i.e. readily available nutrients) and dried mussel cooking juice (i.e. presence of dirt). The detachment kinetic (the detached CFU per mm²) was modelled using a logistic equation with the persistence (the number of cells that remain on the surface) and the strength of cell adhesion to the surface as parameters. The adherence kinetic was quantified by an empirical non-linear model consisting of two terms: a logistic equation that described the adhesion kinetic and a Gompertz equation that defined the detachment and death of the cells adhered. The effect of the presence of residues on biofilm was also studied, and Herrera et al. (2007) concluded that the presence of dirt (in this case, mussel cooking juice) on surfaces of processing plants would imply an additional risk of biofilm formation. The accumulation of fouling residues can be prevented by hygienic equipment design, which has been well studied since the 90s (EHEDG, 1993; Lelieveld et al., 2003). One way to control this potential biofilm contamination could be the setting of PrC associated to the complexity of the process line: number and cleanliness of pipe corners and valves, number of pipe diameter changes, etc.

3.5. Modelling other operational steps

The models above describe the inactivation, growth and introduction of microorganisms. However, additional important parts of the aseptic-UHT process are the mixing and partitioning steps. Details of these steps are described by Nauta (2005).

3.5.1. Modelling mixing step

When foods are mixed, various units \( n \) are joined together (e.g. powdered ingredients dissolved into a liquid phase). This results in microorganisms being redistributed, and consequently the prevalence is increased. When independent sized units \( i \) are mixed, the prevalence is expressed by:

\[
P_i = 1 - \prod_{i=1}^{n} (1 - P_i) \quad (15)
\]

where:

- \( P_i \) is the new prevalence in the mixed product,
- \( n \) is the number of units mixing together,
- \( P_i \) is the prevalence in the \( i \)th units before mixing.

If \( n \) is large, the central limit theorem is applied. It states that, the mean of a large number of independent random variables with finite mean and variance, will be approximately normally distributed. The mixing step was used in QMRA studies, for example in Billoir et al. (2011) and Nauta (2001).

3.5.2. Modelling partitioning step

The partitioning step occurs where a larger food unit is divided into smaller ones (e.g. at the filling process where milk is distributed into bottles). If the contamination in the large unit is low, some smaller units can contain zero microorganisms and some contain more than one microorganism. This assumption could be translated into a Poisson distribution (Nauta, 2005):

\[
N_u \sim \text{Poisson} (N \times s / S) \quad (16)
\]

with:

- \( N_u \) is the number of cells in the smaller unit (CFU/ml),
- \( N_s \) is the number of cells in the large unit (CFU/ml),
- \( s \) is the size of the smaller unit (ml),
- \( S \) is the size of the large unit (ml).

The partitioning step was used for example in Nauta (2001).

To conclude on the modelling section, there are lots of models available, and the selection of the “best” model depends on the statement of purpose, process knowledge and data availability. Moreover, in some cases, more complicated models provide the same result as a simpler one. This approach and philosophy in selection is summarized by Zwietering (2009) as “simple is not stupid and complex is not always more correct”.

The models described above are very helpful for quantifying estimates of risk, but there are many assumption and data gaps leading to uncertainty. In the section below, approaches to address this issue in a QMRA study are discussed.
4. Probabilistic risk assessment: estimation of the microbial risk

The next step after the modular model has been constructed is to compute the model. More and more often, probabilistic approaches are preferred to deterministic ones (with point estimates) for performing QMRA, this is due to the uncertainty and variability inherent to biological processes (Thompson, 2002).

4.1. Uncertainty and variability

Parameter uncertainty and model uncertainty are particularly important to consider when building a probabilistic risk assessment. Parameter uncertainty means uncertainty about the values of input variables, reflecting the lack of information available to estimate these values (e.g. the transfer rate parameters in the mass balance equation for recontamination). Model uncertainty also means uncertainty due to the approximation by a functional form of a real phenomenon (e.g. the recontamination by the biofilm formation). Variability differs from uncertainty: variability refers to natural or non-controlled heterogeneity between individuals within a population addressed by risk assessment (e.g. D and z values amongst strains of thermotolerant B. cereus). It is not always possible to separate entirely uncertainty and variability, although it is recommended to try as much as possible (Mokhtari and Frey, 2005a). In the probabilistic approach, for example in Gkogka et al. (2013), Malakar et al. (2011) and Nauta (2001), probability distributions are used to describe the uncertainty and variability associated with the inputs.

In the deterministic approach, for example in Sosa Mejia et al. (2011), a single value for each model input is selected. It should be noticed that in this case, uncertainty is not systematically neglected. In fact, it can be incorporated in the analysis as a safety margin factor, for example a 2 log10 process performance added to the peanut inactivation process may start with a screening assessment, which uses generic numbers (“defaults”) for many inputs in the deterministic approach and ultimately progress to a highly refined probabilistic analysis.

4.2. Performing probabilistic risk assessment

Probabilistic risk assessment techniques enable an incorporation of inputs as distributions of values with their associated probability of occurrence, the risk assessment output is then also a distribution of values with their associated probability of occurrence by propagation through the modelled process. Moreover, uncertainty and variability on the inputs are also propagated to the model output.

4.2.1. Monte Carlo simulation approach

Monte Carlo Simulation (MCS) approach involves the random sampling amongst probability distributions values for an input, producing simulated values, allowing the estimation of the risk. The precision of the risk estimate depends on the number of samples. Sometimes, MCS proceed by Latin hypercube sampling, which ensures values from the whole information available in the data set. In a Bayesian approach, it is important to realise that the more informative the data set is, the less the impact of the prior distribution has on the posterior distribution. Bayesian inference has been already applied in a risk assessment framework, for example in Albert et al. (2008), Membré and van Zuijlen (2011), Barker et al. (2005) and Jaloustre et al. (2011).

4.2.2. Bayesian inference

“The essence of the Bayesian approach is to provide a mathematical rule explaining how you should change your existing beliefs in the light of new evidence. In other words, it allows scientists to combine new data with their existing knowledge or expertise. The canonical example is to imagine that a precocious newborn observes his first sunset, and wonders whether the sun will rise again or not. He assigns equal prior probabilities to both possible outcomes, and represents this by placing one white and one black marble into a bag. The following day, when the sun rises, the child places another white marble in the bag. The probability that a marble plucked randomly from the bag will be white (i.e., the child’s degree of belief in future sunrises) has thus gone from a half to two-thirds. After sunrise the next day, the child adds another white marble, and the probability (and thus the degree of belief) goes from two-thirds to three-quarters. And so on. Gradually, the initial belief that the sun is just as likely as not to rise each morning is modified to become a near-certainty that the sun will always rise” (The Economist group, 2000).

In a Bayesian approach, each model parameter is considered as a random variable. This randomness includes variability and uncertainty. The variability is often modelled by a parametric distribution characterized by uncertain hyperparameters. This approach was clearly explained in Delignette-Muller et al. (2006). The example of T\(\text{min}\) (minimal growth temperature) was taken to illustrate the Bayesian approach. The variability was modelled by a normal distribution, characterized by two hyperparameters, the mean value M\(T_{\text{min}}\) and the standard deviation \(\text{ST}_{\text{min}}\). In turn, these two hyperparameters were supposed uncertain, and firstly described by a prior distribution from prior knowledge. For each hyperparameter, a posterior distribution was then calculated by the Bayes’ theorem using the prior distribution and the whole information available in the data set. In a Bayesian approach, it is important to realise that the more informative the data set is, the less the impact of the prior distribution has on the posterior distribution. Sensitivity analysis might also be seen as a prerequisite for model building, to test the robustness and the relevance of the model (Saltelli, 2002).

The added value of sensitivity analysis is highlighted in case of large and complex models. For example with a QMRA model applied to aseptic-UHT products, it might be difficult to identify which model modules, and inside each of these, which process settings, have the greatest impact on the commercial sterility failure rate. Moreover, sensitivity analysis helps in identifying and eliminating particular inputs or components of the model which are not essential, i.e. sensitivity analysis helps in simplifying complex models on a rational basis (Mokhtari and Frey, 2005a). That is particularly valuable if the QMRA

risks. If MCS is to be used and applied in food safety management, it is in all instances important to realise how an ‘event of transmission’ is defined and what the input probability distributions represent; in terms of both their variability and uncertainty.

This approach have already been used in QMRA studies, for example in Anderson et al. (2011), Afchain et al. (2008), Cassin et al. (1998), Cerf and Davey (2001) and Nauta (2001).

The model output can be as well compared with real data. In the case of an aseptic-UHT process risk assessment model, the outputs might be compared to the sterility failure rate values collected from comparable factory lines.

4.2.3. Sensitivity analysis

Sensitivity analysis is widely used in QMRA to i) identify the most influential variables on the model output, ii) provide a better understanding and interpretation of the analysis, and iii) identify data gaps and then prioritise future research. Sensitivity analysis might also be seen as a prerequisite for model building, to test the robustness and the relevance of the model (Saltelli, 2002).

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model is used at the operational level, as the model will be easier to implement (easier to inform the remaining inputs and then to run the model) and to use by the risk manager, in the decision-making process.

Frey and Patil (2002) have reported ten different sensitivity analysis methods, grouped in three approaches: mathematical, statistical and graphical methods. They did not recommend the use of one over another, but instead to combine several approaches to increase confidence in the ranking of key inputs. Likewise, a guideline for selection of sensitivity analysis methods applied to microbial food safety process risk models has been set up (Mokhtari and Frey, 2005a). Deterministic sensitivity analysis might be also of interest, at least at a first stage of model development (Zwietering and Van Gerwen, 2000).

4.2.4. Second-order Monte Carlo simulation

Due to the differences by nature and by interpretation between uncertainty and variability, it is often justified to separate them in risk assessment analysis (Nauta, 2000). Once variability and uncertainty are defined by the risk assessor, they can be propagated conditionally to each other through a two-dimensional MCS to obtain a more informative model output. By building a confidence interval around a risk estimate for different realisations of variability, it is possible to identify if further investigation on uncertainty is required before making any decision (Mokhtari and Frey, 2005b). In QMRA applied to an aseptic-UHT process, realisations of variability may correspond to different process line designs (e.g. filler head geometry, air filter efficiency) and running procedures (e.g. length of the batch, periodicity of cleaning procedure). Uncertainty may be associated to the lack of knowledge, for example when characterising the biofilm formation (e.g. lack of information on hygienic equipment design influence on potential microbial interaction and growth rate).

The uncertainty analysis for different realisations of variability has been illustrated with water consumption (Pouillot et al., 2004), the authors calculated the risk associated to Cryptosporidiosis as a function of the initial level of the hazard in a water reservoir. Membré et al. (2008) have calculated the risk of having more than 5 log_{10} of B. cereus in cooked chilled food products for different realisations of variability (e.g. pasteurisation settings, retail market) with a confidence interval capturing the uncertainty in their model. The added value of such studies is to select significant risk reduction measures despite the uncertainty in the QMRA model. Cummins et al. (2008) have built a second-order exposure assessment model for E. coli contamination of beef trimmings. Their second-order MCS helped them in identifying that the lack of sensitivity of the microbial tests was a significant contributing factor to uncertainty and had to be reduced.

Recently, Pouillot and Delignette-Muller (2010) have presented an R package “mc2d”, that is freely and publically available. It helps to build and study two dimensional (or second-order) MCS in which the estimation of variability and uncertainty in the risk estimates is separated. Overall, it should be borne in mind that second-order MCS cannot be performed in absence of a substantial amount of data. Moreover, it is not straightforward and can be time consuming; it should be fit-for-purpose and deployed only when risk assessors and managers have identified its true benefit: large and complex model, difficult choice in allocation of resources (to reduce uncertainty), difficulty in highlighting the impact of process parameters settings in presence of uncertainty... etc.

5. Risk management: options to control and reduce the microbial risk

5.1. Scenario analysis to define performance objective

Once the microbial risk is estimated (in the aseptic-UHT process, risk means the probability of non-compliance with a commercial sterility rate), risk managers have to decide if a risk reduction measure is required and in such a case, to weigh the effect of various measures. Beside sensitivity analysis and second-order MCS, scenario analysis is another powerful mathematical tool. A scenario can be defined as an outline for any proposed series of events, real or imagined. In other words, a scenario is a series of possible events. Probabilistic Scenario Analysis (PSA) is a methodology for QMRA that has been used for about sixty years in a variety of fields (Delignette-Muller et al., 2008). The analysis is designed to allow improved decision-making with a more complete consideration of outcomes and their implications. Scenarios have historically been considered deterministically. However, because of the extent of inherent variability and uncertainty, it is often difficult to identify the full range of possible outcomes of any risk management decision with just a few carefully circumscribed scenarios.

The PSA methodology of risk assessment can be split into two main processes. The first step is the process of analysing possible future events by considering alternative possible outcomes (scenarios). A scenario tree is a graphical depiction which provides a useful conceptual framework. The tree will start with one incident or aggravating event and will then fan out as more and more events happen. Once a scenario tree has been developed to depict what can go right or wrong, and how it can happen, the next step is to quantify how likely it is for the event depicted in the scenario to occur (Duffy and Schaffner, 2002; Ebel and Schlosser, 2000). The output of a PSA is often a diagram, relatively similar to a root-cause analysis tools. Root-cause analysis-like PSA tools can aid risk managers to visualize the benefit of developing a QMRA model and to have the confidence to make decisions based upon their outputs.

5.2. Setting Microbiological Criteria to assess compliance with Performance Objective

In the near future, as food safety management moves toward risk-based food management, food producers will need to provide evidence that their foods at the moment they are eaten, comply with an FSO (van Schothorst et al., 2009). For C. botulinum, an example of an FSO might be less than one product unit per 10^6 or less than one product unit per 10^7, these figures are based on the comprehensive report on commercially sterile products compiled by the National Center for Food Safety and Technology experts (Anderson et al., 2011). Setting Microbiological Criteria (MC) to assess such low occurrences is not realistic. However, MC might be valuable tools to assess the microbial quality (food spoilage) along the entire aseptic-UHT process, i.e. to assess the compliance with PO set at various critical process steps (e.g. on raw materials, mix blends pre-UHT and on product units after filling). In such a case and with a probabilistic risk assessment model, at least one key question has to be answered: what is the percentile (e.g., 99th, 99.9th, 99.99th etc.) used to verify that the PO is met? (van Schothorst et al., 2009). This question might be answered internally by a food company, or by consensus between UHT-product manufacturers.

Other important considerations for the use of MC as management tools in a risk-based framework are recommended (Codex Alimentarius Commission, 2009): they should include 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, the number of analytical units that should conform to these limits.

5.3. Setting performance criteria, process criteria and product criteria to comply with performance objective

A PC is the effect required of one or more control measure(s) working in concert to meet a PO. The effect might be inactivation (a minimum log reduction required) or an inhibition of growth (less than a
logical Criteria (including their associated sampling plans) can be reluctant to make decisions based on probabilistic risk assessment based on aspects of process compliance. For example, Advisory Committee on the Microbiological Safety of Food (2006) recommend a PrC of 70 °C for 2 min to achieve a PC of 6 reduction of E. coli O157:H7, Salmonella spp. and L. monocytogenes in meat products. Also, in the UK, to control non-proteolytic C. botulinum in cooked chilled foods, the heat-treatment has to deliver a PC of 6-log10 inactivation (Gould, 1999).

PrC and PcD are the control parameters at a step or combination of steps that can be applied to achieve a desired reduction or the desired limitation on growth and contamination, i.e. to achieve a PC. In cooked chilled foods, the heat-treatment process settings of 90 °C for 10 min, widely used by the food industry, corresponds to a PrC in risk-based food safety management, as it enables a PC of 6-log10 inactivation to be delivered. Other examples of how heat-treatment settings are articulated with FSO, PO and PC are provided in a recent review on quantitative approaches of thermal food processing (Valdr Amanda and Van Impe, 2012) and also in the ILSI report on risk assessment approaches to setting thermal processes in food manufacture (Bean et al., 2012).

In an aseptic-UHT process context, the type and quantity of the packaging sterilisation agent could be set as a PrC. For example, for the packaging sterilisation, the concentration of the hydrogen peroxide (30%), the temperature of the solution (70 °C) and also the time of immersion (7 s) might be set as a PrC (Tetra Pak, 2012). If the contamination came from the air, the efficiency of filter (commonly HEPA filter) could be translated to a PC (Brown, 2005). Moreover, for contamination by biofilm, the complexity of the line (pipe length, number of valves ...etc.) could be expressed in quantitative terms and then translated into PrCs.

Once defined, PrC and PcD can be translated as Critical Control Points or Operational Pre-requisite Plans in a HACCP plan. More generally, a risk-based framework is valuable to demonstrate equivalence of management options, for example, to demonstrate that a given PO or FSO previously achieved by heat-treatment process as sole control measure might be achieved by a combination of a milder heat-treatment and an appropriate change in formulation. An analysis within a risk-based framework can therefore provide new opportunities in food innovation.

6. Conclusions

Revisiting aseptic-UHT line process within the QMRA framework is definitively valuable since UHT products are extensively and globally consumed: 123 billion litres consumed in 2011 (Warrick Research and Zenich International, 2012). Moreover, volumes have grown by just over 5% a year since 2008, with South/South East Asia achieving the fastest rise of 22% a year, where ambient stable products are highly demanded. QMRA, applied to aseptic-UHT products, supports this global market demand; it permits a harmonisation of process settings and designs.

Another advantage of applying QMRA to aseptic-UHT products lies in the science-based approach. It enables a quantification of the sterilization failure rate and assesses various options for controlling and reducing it. Currently, the typically applied tolerable sterilization failure rate for aseptic-UHT is one defect per 10,000 units and is often obtained with empirical settings. With a modular QMRA model, improvements in sterility failure rates can be achieved in a quantitative and transparent manner. Having identified the contamination pathways, Microbiological Criteria (including their associated sampling plans) can be better targeted. Defining critical process parameters linked to PcD and PrC through QMRA could also allow an eventual move from product release decisions based on analytical results, to immediate decisions based on aspects of process compliance.

However, it is important to keep in mind that risk managers might be reluctant to make their decisions based on probabilistic risk assessment model outputs. Indeed, there is still very limited guidance and supporting documentation to explain how risk assessors should communicate the QMRA outputs to risk managers. In addition, often risk managers do not have the background in probabilistic theory to understand the QMRA model outputs. These factors may hinder or jeopardize the deployment of a risk-based food safety management system, and emphasize the importance of education and training, and the need for risk communication tools in this area. Non-governmental institutes, such as ILSI Europe or Institute for Food Safety and Health, could act as a forum to promote discussion in this area between the relevant aseptic-UHT process scientists and specialists from academia, governmental bodies and food industries. It is the collaboration of risk assessors and risk managers from industry and academia within the risk-based framework of QMRA that can lead to a greater understanding of aseptic-UHT process contamination, and so extend the current “As-Low-As-Reasonably-Achievable” sterility failure rate to ones where sterility performance can be optimized around defined process settings and designs.

References


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